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Thèse pour l'obtention de DOCTORAT

Etude de bactéries solubilisant le phosphate isolées des rhizosphères de Papilionacées au Maroc : Identification, caractérisation et application agronomique

Préparée par : Mr Walid Janati

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Nom et Prénom	Grade	Etablissement	Qualité
EL HADRAMI El Mestafa	PES	Faculté des Sciences et Techniques de Fès	Président
IBIJBIJEN Jamal	PES	Faculté des Sciences de Meknès	Rapporteur
EL GHACHTOULI Naima	PES	Faculté des Sciences et Techniques de Fès	Rapporteur
EL KHALFI Bouchra	PH	Faculté des Sciences Ain chock de Casablanca	Rapporteur
El Ghadraoui Lahsen	PES	Faculté des Sciences et Techniques de Fès	Examinateur
SOUKRI Abdelaziz	PES	Faculté des Sciences Ain chock de Casablanca	Examinateur
ZINEDINE Abdellah	PH	Faculté des Sciences El Jadida	Examinateur
ERRACHIDI Faouzi	PH	Faculté des Sciences et Techniques de Fès	Directeur de Thèse
Karima MIKOU	PES	Faculté des Sciences et Techniques de Fès	Co-Directeur de Thèse

Résumé

Le phosphore est majoritairement présent dans le sol sous des formes insolubles et non disponible pour la plante. Le développement d'inoculant microbiens contenant des bactéries solubilisant le phosphate (PSB) est une solution biologique pour améliorer la disponibilité du phosphate (P) dans la rhizosphère. L'objectif de cette thèse est d'isoler, d'identifier et de caractériser les PSB de la rhizosphère de légumineuses au niveau de quatre sites de la région Fès-Meknès, en mettant en évidence la symbiose plante-PSB et en expliquant la contribution de ces rhizobactéries à réduire la dépendance aux engrais phosphatés et à stimuler la croissance des plantes.

Les conditions environnementales des sites étudiés exercent une forte pression sélective sur les communautés de PSB et sur leurs propriétés, tels que la capacité de solubiliser le P. L'identification classique des souches isolées a révélé neuf genres bactériens, dont *Bacillus spp*., *Pseudomonas spp*. et *Rhizobium spp*. La solubilisation moyenne du P rocheux et du P tricalcique par ces PSB atteint 40,43 $mg.L^{-1}$ et 94,54 $mg.L^{-1}$, respectivement.

Les souches isolées améliorent l'activité de solubilisation du P dans un milieu NBRIP avec une combinaison (RP/TCP) et augmentent ça solubilisation de 20,58% par rapport au témoin, où la souche WJEF63 montre la concentration de P soluble la plus efficace (102,69 $mg.L^{-1}$). Parmi toutes les souches isolées, douze présentent une résistance significative aux conditions stressantes (Température, pH, sécheresse et salinité) associés à une activité antifongique allant jusqu'à 49,15% d'inhibition de la croissance mycélienne d'*Aspergillus niger*, *Fusarium culmorum* et *Rhizopus stolonifer*.

L'inoculation des graines de *Vicia faba* et de *Pisum sativum* par ces PSB dans des conditions contrôlées et supplémentées du RP augmentent la teneur en N, en P, le poids frais et le poids secs des plantes testées de 67,50 %, 23,11 %, 56,6% et 42,28%, respectivement. La phosphatase alcaline, la respiration basale du sol et l'activité β-glucosidase ont augmenté de 45.46%, 67.5% et 66.12%, respectivement. En outre, les souches de PSB utilisées présentent plusieurs activités dont la fixation de N, la production d'auxines et des sidérophores.

L'application de ces PSB sous forme de consortium stimule le développement de *Vicia faba* et de *Pisum sativum*, améliore la qualité de sol et augment le rendement de plantes étudiées de 20.85% et 13.41%, respectivement, dans des conditions de stress hydrique et salin. De plus, ces souches sont capables de former un biofilm, présentent une activité antioxydant et tolèrent la salinité et la sécheresse. L'identification moléculaire (ARNr 16S) a permis d'identifier ces souches comme étant des *Pseudomonas spp*. et des *Bacillus spp*.

Les PSB étudiées montrent un potentiel bénéfique en tant que rhizobactéries promotrices de la croissance. Les retombés de ce projet de thèse fournissent une méthode pratique pour augmenter la mobilisation du P dans le sol et la croissance des plantes hôtes.

Mots clés : Maroc ; Phosphate ; PSB ; Légumineuses ; Agronomie ; Biotechnologie ; Développement durable.

Abstract

Phosphorus is mainly present in the soil in insoluble forms and is not available to plants. The development of microbial inoculants containing phosphate-solubilizing bacteria (PSB) is a biological solution for improving phosphate (P) availability in the rhizosphere. The aim of this thesis is to isolate, identify and characterise PSB from the rhizosphere of leguminous plants at four sites in the Fès-Meknès region, highlighting the plant-PSB symbiosis and explaining the contribution of these rhizobacteria to reduce dependence on phosphate fertilizers and stimulating plant growth.

The environmental conditions at the sites studied exerted strong selective pressure on the PSB communities and on their properties, such as the ability to solubilize P. Classical identification of the strains isolated revealed nine bacterial genera, including *Bacillus spp., Pseudomonas spp., and Rhizobium spp*. The average solubilization of rock P and tricalcium P by these PSBs reached 40.43 $mg.L^{-1}$ and 94.54 $mg.L^{-1}$, respectively.

The isolated strains improved P solubilization activity in NBRIP medium with a combination (RP/TCP) and increased solubilisation by 20.58% compared with the control, where strain WJEF63 showed the most effective soluble P concentration (102.69 $mg.L^{-1}$). Of all the strains isolated, twelve showed significant resistance to stressful conditions (temperature, pH, drought and salinity) associated with antifungal activity of up to 49.15% inhibition of the mycelial growth of *Aspergillus niger, Fusarium culmorum,* and *Rhizopus stolonifer*.

Inoculation of *Vicia faba* and *Pisum sativum* seeds with these PSBs under controlled conditions and supplemented with RP increased the N, P, fresh weight and dry weight of the plants tested by 67.50%, 23.11%, 56.6% and 42.28%, respectively. Alkaline phosphatase, basal soil respiration and β-glucosidase activity increased by 45.46%, 67.5% and 66.12%, respectively. In addition, the PSB strains used exhibited several activities including N-fixation, auxins production and siderophores.

Application of these PSBs in consortium form stimulated the development of *Vicia faba* and *Pisum sativum,* improved soil quality and increased the yield of the plants studied by 20.85% and 13.41%, respectively, under water and salt stress conditions. In addition, these strains are able to form a biofilm, exhibit antioxidant activity and are tolerant to salinity and drought. Molecular identification (16S rRNA) identified these strains as *Pseudomonas spp*. and *Bacillus spp*.

The PSBs studied showed beneficial potential as growth-promoting rhizobacteria. The results of this thesis project provide a practical method for increasing soil P mobilisation and host plant growth.

Keywords: Morocco; Phosphate; PSB; Legumes; Agronomy; Biotechnology; Sustainable development.

ملخص

يوجد الفوسفور بشكل رئيسي في التربة بأشكال لا يمكن الوصول إليها وغير متاحة للنباتات. يمثل تطوير اللقاحات الميكروبية التي تحتوي على بكتيريا مذيبة للفوسفاط حلاً بيولوجيًا ناشئًا لتحسين توافر الفوسفاط في منطقة الجذور. في هذا السياق، تم إجراء هذه الأطروحة بهدف استكشاف بالتفصيل البكتيريا المذيبة للفوسفاط المعزولة من منطقة الجذور في أربعة مواقع لجهة فاس مكناس من خلال تسليط الضوء على الآليات الكامنة وراء التعايش بين النبات وبكتيريا مذيبة للفوسفاط، وشرح مساهمة هذه البكتيريا الجذرية في تقليل الاعتماد على الأسمدة الفوسفاتية وتحفيز نمو النبات.

مارست الظروف البيئية في المواقع التي تمت دراستها ضغطًا انتقائيًا قويًا على مجتمعات PSB وعلى خصائصها، مثل القدرة على إذابة P. وكشف التحديد الكلاسيكي للسلالات المعزولة عن تسعة أجناس بكتيرية، بما في ذلك *Bacillus spp* و*Pseudomonas spp* و*Rhizobium spp*. يصل متوسط ذوبان صخرة الفوسفاط والفوسفاط ثلاثي الكالسيوم إلى 40,43 $mg.L^{-1}$ و94,54 $mg.L^{-1}$ على التوالي.

أدت السلالات المعزولة إلى تحسين نشاط ذوبان الفسفور في وسط NBRIP مع مزيج (RP/TCP) وزيادة الذوبان بنسبة 20.58%، حيث أظهرت السلالة WJEF63 تركيز الفسفور القابل للذوبان الأكثر فعالية (102.69 ملغم.لتر-1). من بين جميع السلالات المعزولة، أظهرت اثنتا عشرة سلالة مقاومة كبيرة للظروف العصيبة (درجة الحرارة، ودرجة الحموضة، والجفاف، والملوحة) المرتبطة بالنشاط المضاد للفطريات بنسبة تصل إلى 49.15٪ تثبيط نمو الفطريات في *Aspergillus niger*، و*Fusarium culmorum*، و*Rizopus stolonifer*.

أدى تلقيح بذور *Vicia faba* و*Pisum sativum* بهذه الـPSBs تحت ظروف خاضعة للرقابة ومكملة بـ RP إلى زيادة N وP والوزن الطازج والوزن الجاف للنباتات التي تم اختبارها بنسبة 67.50% و23.11% و56.6% و42.28% على التوالي. زاد الفوسفاتيز القلوي وتنفس التربة القاعدي ونشاط بيتا جلوكوزيداز بنسبة 45.46% و67.5% و66.12% على التوالي. بالإضافة إلى ذلك، أظهرت سلالات PSB المستخدمة العديد من الأنشطة بما في ذلك تثبيت N، وإنتاج الأوكسينات و siderophores.

استخدام PSBs في شكل اتحاد إلى تحفيز تطوير *Vicia faba* و*Pisum sativum*، وتحسين جودة التربة وزيادة إنتاجية النباتات التي تمت دراستها بنسبة 20.85% و13.41%، على التوالي، تحت ظروف الإجهاد المائي والملحي. وبالإضافة إلى ذلك، فإن هذه السلالات قادرة على تكوين غشاء حيوي، وتظهر نشاطًا مضادًا للأكسدة وتتحمل الملوحة والجفاف. حدد التحديد الجزيئي (16S rRNA) هذه السلالات على أنها *Pseudomonas spp*. و*Bacillus spp* .

أظهرت PSBs التي تمت دراستها إمكانات مفيدة مثل البكتيريا الجذرية المعززة للنمو. توفر نتائج مشروع الأطروحة طريقة عملية لزيادة تعبئة التربة ونمو النبات المضيف.

الكلمات المفتاحية: المغرب; الفوسفاط؛ البكتيريا المذيبة للفوسفاط؛ البقوليات؛ الهندسة الزراعية؛ التكنولوجيا الحيوية؛ التنمية المستدامة.

بسم الله الرحمن الرحيم

« قالوا سبحانك لا علم لنا إلا ما علمتنا إنك أنت العليم الحكيم »

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List of abbreviations

ANOVA	**:**	Analysis of variance
ACC	**:**	Aminocyclopropane-1-carboxylic acid
BNF	**:**	Biological Nitrogen Fixation
CFU	**:**	Colony-forming unit
IAA	**:**	Indole Acetic Acid
LB:	**:**	Luria-Bertani
N_2	**:**	Dinitrogen
NBRIP	**:**	National Botanical Research Institut's Phosphate
OD	**:**	Optical density
P	**:**	Phosphorus
PCA	**:**	Principal component analysis
PGPR	**:**	Plant Growth Promoting Rhizobacteria
pNP	**:**	Para-Nitrophenyl
pNPP	**:**	Para-4-Nitrophenylphosphate
PSB	**:**	Phosphate Solubilizing Bacteria
PSM	**:**	Phosphate-Solubilizing Microorganisms
PUE	**:**	Phosphorus Use Efficiency
RP	**:**	Rock Phosphate
TCP	**:**	Tricalcium Phosphate

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Appendixes I

Introduction générale

Le sol constitue l'une des ressources naturelles les plus précieuses de notre planète ; son rôle essentiel dans la production alimentaire mondiale ne saurait être sous-estimé. Cependant, la disponibilité limitée des nutriments, en particulier du phosphate (P), demeure un défi majeur pour l'agriculture durable. Le phosphate, en tant que nutriment essentiel pour les plantes, joue un rôle central dans la croissance végétale, le développement des cultures et la production alimentaire mondiale. Néanmoins, sa disponibilité limitée dans le sol est un problème majeur qui entrave l'agriculture durable. Les agriculteurs ont souvent recours aux engrais phosphatés pour répondre aux besoins des cultures. Cependant, l'utilisation excessive d'engrais phosphatés a des conséquences environnementales néfastes. Par conséquent, la gestion efficace de ces nutriments est cruciale pour répondre à la demande croissante en produits agricoles tout en préservant la qualité des sols et en réduisant l'impact environnemental. C'est dans ce contexte que les bactéries solubilisant le phosphate (PSB) entrent en scène.

Les PSB sont un composant crucial de l'écosystème du sol et jouent un rôle primordial dans le recyclage et la redistribution du P, contribuant ainsi à maintenir l'équilibre des nutriments dans les écosystèmes terrestres. Leur activité est particulièrement vitale dans les sols dégradés ou appauvris en P, où elles peuvent aider à restaurer la fertilité du sol sans recourir à des apports massifs d'engrais chimiques. Ces microorganismes, ubiquitaires dans le sol, possèdent la capacité unique de solubiliser les formes minérales du P et la mise à disposition de la forme inorganique. La solubilisation du P est généralement réalisée par la libération d'acides organiques, tels que l'acide citrique et l'acide oxalique, favorisant ainsi la mise à disposition du P pour les plantes.

Les légumineuses sont des cultures cruciales dans l'agriculture mondiale avec une capacité particulièrement importantes à former des nodosités symbiotiques avec des bactéries rhizobiennes fixatrices d'azote atmosphérique. Ce statut est d'autant plus significatif qu'elles sont reconnues comme des cultures multifonctionnelles offrant de nombreux avantages à la fois pour les systèmes agricoles et pour l'environnement. Cependant, elles dépendent également d'un approvisionnement adéquat en P pour leur croissance et leur développement optimaux. Compte tenu de ce fait, le lien entre les PSB et les légumineuses est d'une importance capitale.

A la lumière de ce qui précède, l'objectif global de ce projet de thèse est, d'une part, isoler, identifier et caractériser les PSB de la rhizosphère de légumineuses au niveau de quatre sites de la région Fès-Meknès, créer une banque de souches compétentes suites à des examens

biochimique et moléculaire, de plus, étudier les facteurs abiotiques qui influencent l'activité de ces bactéries. D'autre part, mettre en évidence la symbiose plante-PSB et expliquer la contribution de ces rhizobactéries à réduire la dépendance aux engrais phosphatés et tester leurs effets sur le développement des plantes.

Dans le cadre de la réalisation de ce travail multidisciplinaire, nous avons mis en place une synthèse bibliographique indiquant l'état de l'art de cette thématique. Par la suite, des bactéries solubilisant le phosphate ont été isolées et caractérisées en évaluant les effets climatiques et pédologiques de leurs sources d'origines influençant ainsi leurs potentialités. En outre, la méthode des plans d'expériences a permis d'optimiser la solubilisation de P par ces bactéries par une modélisation mathématique des experiences. De plus, nous avons évalué l'activité d'inoculation bactérienne sur la germination et la croissance de deux légumineuses, *in vitro* et *in vivo*, sous des conditions conventionnelles et stressantes (sécheresse et salinité). Dans la dernière partie expérimentale nous avons évalué le potentiel d'un consortium bactérien sur le développement et le rendement des deux légumineuses testés. Les résultats de ce travail ont été renforcés et étayés par des analyses statistiques approfondies qui ont apporté des preuves substantielles et de la crédibilité aux conclusions tirées. Afin de réaliser ceci, nous avons adopté une démarche qui a subdivisé notre thèse en trois parties descriptives et référentielles. Une introduction générale pour situer la problématique étudiée et le choix des approches scientifiques et techniques, la deuxième partie est clivée en cinq chapitres :

Le premier chapitre intitulé " Les biofertilisants bactériens solubilisant le phosphate : Source et technologies vertes pour le développement agricole futur " est une revue bibliographique qui présente la composition et la diversité fonctionnelle des PSB, les mécanismes de solubilisation/minéralisation du P, la manière dont les PSB induisent la croissance des plantes dans des conditions conventionnelles et stressantes, et leur rôle potentiel en tant que bio-engrais phosphaté dans la production de cultures vivrières. Cette partie décrit la manière dont les taxons microbiens contribuent à l'acquisition du P par les plantes et comment manipuler efficacement ces microorganismes pour augmenter la disponibilité et l'absorption du P. Au terme de ce chapitre introductif de l'état de l'art antérieur on a mis l'accent sur les consortiums de PSB, après nous avons illuminé la façon dont le P et les biofertilisants PSB interagissent est mentionnée comme un pas en avant dans la formulation d'un inoculum microbien efficace.

Le deuxième chapitre intitulé " Bactéries solubilisant le phosphate issues de sols aux conditions environnementales variées : Occurrence et fonction " est concentré sur les différents effets climatiques et pédologiques qui affectent l'abondance, la diversité et le potentiel des PSB

dans la rhizosphère des légumineuses dans quatre stations bioclimatiques dissemblables de la région Fès - Meknès, au Maroc. Les objectifs spécifiques de ce chapitre sont centrés sur :

- L'étude des effets abiotiques sur la capacité de solubilisation du P et les caractères de promotion de la croissance des plantes (PGP),
- La détermination de l'efficacité de solubilisation du P, la production d'auxine et des sidérophores, la solubilisation de zinc et l'activité de l'ACC désaminase des isolats,
- L'évaluation de l'impact et la corrélation des conditions environnementales de chaque site étudié sur l'abondance, la diversité et les compétences des souches de PSB isolées et caractérisées.

Le troisième chapitre intitulé " Isolement et caractérisation des bactéries solubilisant le phosphate et colonisant naturellement la rhizosphère des légumineuses au Maroc " vise à explorer et caractériser les souches PSB isolées de la rhizosphère de diverses légumineuses afin d'améliorer l'efficacité des bioinoculants. Les objectifs spécifiques de ce chapitre sont canalisés sur :

- l'évaluation de la capacité de ces souches à améliorer l'activité de solubilisation du P dans un milieu NBRIP avec du P tricalcique (Ca_3 $(PO_4)_2$) (TCP) et du RP séparément, ainsi que leur combinaison (RP/TCP) selon un plan d'expérience à deux niveaux ($2^2 = 4$ expériences),
- La caractérisation des PSB isolées et détecter leur tolérance aux différentes conditions de croissance et de stress (salinité, pH, sécheresse et température).
- L'étude de l'efficacité des PSB à inhiber la croissance des champignons grâce à leur activité antifongique.

Le quatrième chapitre intitulé " Stimulation de la croissance de deux légumineuses (*Vicia faba* et *Pisum sativum*) par inoculation de bactéries solubilisant le phosphate " vise à évaluer l'impact de cinq souches PSB isolées de la rhizosphère de légumineuses, sur la croissance des deux légumineuses et sur certaines propriétés du sol. Les objectifs de ce chapitre sont :

- L'évaluation des effets de l'inoculation par des PSB, in vitro et *in vivo*, en conditions contrôlées supplémentées du P naturel (RP), sur les propriétés chimiques et les caractéristiques biologiques du sol,
- L'évaluation de l'effet des différents paramètres sur la croissance tels que le taux de germination, la teneur en nutriments, la biomasse végétale, la longueur des tiges et de racines, et le nombre de nodules par racines de la fève et de petit pois.

Le cinquième chapitre intitulé " Amélioration du rendement de *Vicia faba* et *Pisum sativum* dans des conditions de stress par l'inoculation des bactéries solubilisant le phosphate en

association (consortium) et la fertilisation au phosphate naturel " vise à mettre en évidence le rôle des PSB dont l'amélioration de l'adaptation au stress hydrique et salin chez la fève et le petit pois, en tenant compte des propriétés morphologiques et biochimiques des plantes testées. Ce chapitre mire les objectifs suivants :

- L'évaluation de la capacité des souches testées à tolérer le sel et la sécheresse, leur activité antioxydante et leur pouvoir à former un biofilm,
- L'identification des souches compétentes à l'aide de la technique moléculaire ARNr16S,
- L'étude des fonctions et les mécanismes potentiels des PSB isolées en tant que consortium pour améliorer les propriétés physicochimiques du sol, l'induction de la nodulation, la croissance des plantes, l'absorption des nutriments et le rendement dans des conditions drastiques.

En dernière partie, une conclusion générale rassemblant les résultats 'résumant' les différents chapitres est élaborée, suivie par des perspectives qui ouvrent de nouvelles voies de recherche prometteuses dans cette thématique qui forme une priorité nationale et internationale.

Etude bibliographique

Etude bibliographique

1. Généralités sur le Phosphore

Le phosphore (P) est un élément nutritif nécessaire et irremplaçable pour les besoins vitaux des plantes. En effet, il s'agit d'un élément essentiel du métabolisme énergétique de toutes les formes de vie (H. Malhotra et *al*., 2018). Le P est indispensable pour les cellules vivantes vu qu'il est un composant principal de l'ADN, de l'ARN, de l'ATP et des phospholipides. Il est conséquemment impliqué dans la division cellulaire, la transmission de l'information génétique, la transformation d'énergie et aussi dans le système photosynthétique (Masrahi et *al*., 2023). Toutes ces propriétés font de cet élément l'un des trois macronutriments nécessaires pour la croissance et le développement des plantes avec l'azote et le potassium. Pour comprendre l'interaction entre le P, les processus biogéochimiques et d'autres distributions élémentaires, il est nécessaire de comprendre la distribution du P sur la surface terrestre et les processus contrôlant sa distribution.

De nos jours, l'agriculture dépend essentiellement de l'apport régulier d'engrais phosphaté. Cet apport indispensable en P fait de cette ressource non renouvelable un produit irremplaçable (Harika et *al*., 2023). D'après l'institut d'études géologiques des États-Unis (USGS), les ressources telluriques en P seront complètement épuisées dans 50 à 100 ans et la production mondiale du P connaîtra un pic dans les années à venir (approximativement en 2030). Le Maroc, la Chine et les États-Unis, respectivement, sont les trois principaux producteurs de P dans le monde (Hakkou et *al*., 2016). Ils détiennent plus que 80 % des gisements exploitables constitués de réserves et ressources potentielles à durée de vie limitée. Ces gisements se répartissent en deux groupes suivant leur origine géologique : les gisements ignés qui résultent de l'intrusion du magma dans les roches cristallines (dépôts volcaniques) et ceux sédimentaires qui tiennent leur origine des innombrables débris d'animaux marins (Cordell et *al*., 2009).

L'exploitation minière produit un P brut qui ne convient qu'exceptionnellement à un emploi immédiat comme engrais phosphaté (Schipanski & Bennett, 2021). Généralement, pour obtenir un produit marchand ou P naturel (Pn), des procédés d'épierrage, criblage, concassage, broyage, séchage, flottation et calcination s'avèrent indispensables. Cependant, le Pn procure une source moins chère de fertilisant phosphaté pour la production des cultures et peut être aussi appliqué directement comme engrais phosphaté mais ça dépend de divers facteurs et de leurs interactions (Yu et *al*., 2022). Les facteurs principaux comprennent : les propriétés physiques et chimiques des Pn, les facteurs pédologiques et climatiques, les espèces de plantes cultivées, les systèmes

de culture et les procédures de gestion de la production (Johan et *al*., 2021). Toutefois, l'application du Pn augmente le P total avec un grand potentiel de réapprovisionnement en P labile disponible pour les plantes.

La flore microbienne du sol a un rôle essentiel dans le cycle du P, car elle établit un lien entre le réservoir de P dans l'environnement vivant et non vivant. Certains microorganismes facilitent, en effet, l'altération, la minéralisation, et la solubilisation des différentes formes de P, rendant l'orthophosphate à la disposition des communautés microbiennes et végétales. Cependant, ces microorganismes contribuent également à l'immobilisation du P, un processus qui diminue la biodisponibilité du P en convertissant les formes solubles réactives du P en formes insolubles. Le mécanisme de participation microbienne dans ces processus varie d'un mécanisme passif à un mécanisme très actif. Le cycle du P englobe de nombreux réservoirs environnementaux vivants ou non vivants ainsi que différentes voies de transport. En suivant le mouvement du P dans l'environnement, l'interaction entre le processus physique et biologique devient apparente. En effet, en plus d'agir comme des réservoirs de P dans l'environnement, les microorganismes contribuent à la transformation du P dans les autres réservoirs, comme dans le sol ou dans les environnements aquatiques environnants.

Dans les solutions du sol, le P est généralement disponible sous sa forme triacide (l'acide phosphorique (H_3PO_4)). Le pH du sol est l'un des paramètres qui joue un rôle clé dans la disponibilité du P. La forme de P prédominante dans un sol acide (pH inférieur à 6.8) est le mono-phosphate, un anion monovalent ($H_2PO_4^-$) qui est facilement absorbé par les racines des plantes (Khan et *al*., 2022). Pour des pH neutre (entre 6.9 et 7.2), la forme qui domine est le HPO_4^{2-}, qui est absorbée plus difficilement. Dans les sols de nature alcaline (pH > 7.2), la forme prédominante est celle de l'ion trivalent PO_4^{3-}, que les plantes ne peuvent pratiquement pas absorber. Cependant, la plante ne peut utiliser que le pool de P inorganique (Pi) libre dans la solution du sol à partir duquel elle absorbe les ions ortho-phosphates ($H_2PO_4^-$ / HPO_4^{2-}) (Ducousso-Détrez et *al*., 2022). Dans les sols alcalins, le P est précipité par les complexes calciques et magnésiens tandis qu'à un pH neutre, le P tend à former les complexes insolubles avec l'aluminium et le fer (Rfaki et *al*., 2014). Le phosphate sous ses formes organiques (Po) (phytate, phosphate tricalcique, …) qui ne sont pas assimilées par les plantes, semble avoir des qualités consistantes (Goswami et *al*., 2019). Pour être absorbé, le Po doit tout d'abord être converti en Pi sous l'action des microorganismes du sol, et par conséquent il sera disponible pour la plante. Une bonne exploration du sol permet d'augmenter l'accès au pool du Pi libre,

sauf que, dans le sol, le pool de Pi libre représente une infime fraction du P total du sol. Ce dernier est alimenté par le pool de P minéral plus ou moins facilement disponible via les phénomènes de désorption et/ou de solubilisation (Johan et *al*., 2021). Il peut aussi être alimenté par la minéralisation du P complexé au carbone via des liaisons mono-ester (R-O-P) ou diester (R-O-P-O-R') constituant le Po du sol (Huang et *al*., 2017).

2. Mobilisation du phosphore dans le sol

Bien que le contenu en P total (inorganique et organique) des sols ne dépasse généralement pas les 0.12%, seulement 0.1% de ce P total existe sous la forme inorganique soluble facilement assimilable par les plantes (Goldstein 1994). Quant au P organique, sa contribution au P total, peut dépasser les 50% dans certains sols. Il peut exister sous plusieurs formes comme les phytates et polyphosphates faiblement biodisponibles et formant des complexes avec des cations, à l'origine de certaines limitations en minéraux importants pour la nutrition des plantes (Dalal 1977). La déficience en P soluble limite la productivité végétale de plusieurs sols agricoles de manière universelle (Arcand and Schneider 2006). Cependant la protection de l'environnement exige l'utilisation de pratiques durables de gestion, faisant usage de peu d'intrants chimiques. Par ailleurs, l'application de fertilisants s'effectue toujours sans tenir compte des mycorhizes et de la microflore présente au niveau de la mycorhizosphère, ce qui conduit à des applications excessives et souvent néfastes. Ainsi, une énorme quantité de P est immobilisée dans les sols, ce qui présente un risque potentiel pour l'environnement si ce sol est transféré par érosion dans les cours d'eau.

Dans cette partie nous nous intéresserons à détailler les différents procédés induits de transformation des phosphates comme la désagrégation, la solubilisation, la minéralisation et l'immobilisation. La composition chimique des minéraux phosphatés dépend de l'ion ou des ions présents au moment de la transformation.

La désagrégation

Dans la nature, certaines roches phosphatées s'altèrent suite à de nombreux processus écologiques. Les processus d'altération sont classés en deux principales catégories : la désagrégation mécanique et chimique. Dans la désagrégation mécanique, des procédés physiques (incluant l'expansion thermique, la pression, l'action hydraulique, la formation de cristaux de sel, le gel et le dégel…) peuvent causer une détérioration ou encore une fragmentation du matériel rocheux sans modifier sa composition chimique. En revanche, la désagrégation chimique (incluant divers produits chimiques) cause l'altération de la roche phosphatée en modifiant la structure chimique des minéraux à partir desquels la roche

phosphatée est faite. Le processus de l'altération chimique comprend la dissolution, l'hydrolyse, l'hydratation et l'oxydoréduction (Mackey and Paytan 2009).

La solubilisation

La solubilisation microbienne du phosphate joue un rôle important dans la conversion du P insoluble en P soluble. En effet, il a été démontré que certains microorganismes du sol sont impliqués dans la solubilisation des phosphates insolubles. Ces microorganismes bénéficient directement du P biodisponible nécessaire pour leur croissance. De même, d'autres organismes sont en mesure de profiter du P solubilisé, tels que les champignons et les plantes supérieures. La solubilisation du Pn est généralement produite par des microorganismes selon deux mécanismes primaires ; l'excrétion des protons (H^+) et la production d'acide organique. L'excrétion de H^+ par les microorganismes due à l'assimilation de NH_4^+ est une alternative métabolique pour la solubilisation du Pn (Ducousso-Détrez et *al*., 2022). Certains microorganismes solubilisent le Pn par sécrétion des acides organiques à travers une diminution du pH de la solution du sol et aussi par la réaction de chélation en formant des complexes stables avec les cations tel que : Ca^{2+}, Fe^{2+}, Al^{3+} (Kalayu, 2019). Les acides organiques produits par ces microorganismes sont généralement les acides gluconique, oxalique et citrique (Silva et *al*., 2021). Notons que ces microorganismes produisent des acides organiques et relâchent des protons, qui à travers leurs groupements carboxyliques, chélatent les cations fixés aux phosphates insolubles ce qui permet de les convertir en formes solubles (mono et dibasiques) (Mackey and Paytan 2009).

La minéralisation

Dans le sol, les plantes et les détritus animaux constituent un énorme réservoir de P organique qui est généralement indisponible pour la plupart des organismes vivants. Pour devenir biodisponible, le P contenu dans la matière organique doit tout d'abord être minéralisé en phosphate. Le processus de minéralisation, au cours duquel les complexes de P organique sont convertis en minéraux phosphatés, est un processus modulé grâce à l'activité de certaines enzymes microbiennes, notamment les phosphatases qui sont classées en fonction du type des groupements carbonés liés aux phosphates qu'elles clivent. Les phosphatases ont des exigences spécifiques vis-à-vis de certains substrats. Les catégories les plus courantes des phosphatases microbiennes contribuant à la minéralisation du P comprennent les phosphomonoestérases, les phosphodiestérases, les nucléases, et les nucléotidases, ainsi que les phytases (Mackey and Paytan 2009).

L’immobilisation

Lors du processus de l’immobilisation, le P labile est séquestré et retiré de l’environnement pour une période de temps. Les procédés d’immobilisation peuvent être regroupés en deux catégories : La première catégorie, l’immobilisation transitoire ou l’assimilation cellulaire, comprend tous les processus de séquestration du P dans les cellules vivantes microbiennes et est rapidement réversible à la mort cellulaire. La deuxième catégorie appelée la formation de minéraux phosphatés, englobe les processus de minéralisation influencé par l’activité microbienne qui génèrent des minéraux contenant du P : il s’agit de la phosphogénèse (Mackey and Paytan 2009). Les bactéries, champignons, micro-algues, protozoaires représentent la majorité de la biomasse vivante sur Terre. La plupart d’entre eux sont organisés en biofilms et sont très utiles à l’équilibre de notre planète.

3. Rhizosphère

Le concept de la rhizosphère mis par le chercheur allemand Hartmann et *al*. (2008), correspond au volume du sol autour des poils absorbants des plantes supérieures où prolifèrent des organismes vivants (Edaphon). Cette zone d'interaction s'étend de quelques micromètres à plus de 2 mm en dehors de la surface racinaire. De même, cette zone se caractérise par une importante diversité microbienne comparativement au sol environnant (non rhizosphérique) (Gamalero et *al*., 2020). Par rapport aux racines, la rhizosphère peut être divisée en trois zones distinctes :

- l’endo-rhizosphère : comprend des parties du cortex et de l'endoderme dans lesquelles les microbes et les cations peuvent occuper l’espace libre entre les cellules (espace apoplastique).
- le rhizoplan : situé au voisinage immédiat de la racine, y compris l'épiderme racinaire et le mucilage (Y. Li et *al*., 2023).
- l’ecto-rhizosphère : représente la zone extérieure qui se trouve directement après le rhizoplan.

La rhizosphère héberge en effet une grande diversité d’organismes (microorganismes, animaux et végétaux) dont la plupart sont responsables de processus de biotransformation et de transfert des éléments ou des composés (A. Yadav & Yadav, 2022b). Cette diversité biologique est associée à une importante diversité fonctionnelle et à une grande complexité des interactions écologiques. Ainsi de nombreux processus se déroulant dans le sol sont assurés par des organismes très variés (bactéries, champignons, protozoaires, faune). Les organismes du sol affectent la productivité végétale, que ce soit de manière directe ou indirecte (modifications des cycles du carbone et des nutriments, de la structure du sol, interactions trophiques et contrôle de certains parasites et pathogènes) (J. Li et *al*., 2021). La distribution dans le temps et dans

l’espace des processus biologiques n’est ni aléatoire, ni homogène. Les activités des organismes du sol sont concentrées dans des sites généralement associés à la disponibilité en substrats carbonés (source d’énergie). C’est ainsi que les habitats microbiens sont associés aux fractions organiques, aux agrégats, à la litière en décomposition, au sol influencé par les vers de terre et au contact étroit des racines en rhizosphère.

La rhizosphère joue un rôle actif dans la régulation des interactions plantes microorganismes. Elle est la zone des échanges entre sol, racines, microorganismes et faune associés. Ces échanges, intenses, se traduisent par des flux bidirectionnels d’eau et de nutriments. Dans la rhizosphère jusqu'à 30 % des composés photo-synthétisés par la plante sont remis à la disposition des micro-organismes qui y vivent par le biais d’exsudats racinaire (Mavrodi et *al*., 2021). Ces derniers incluent une grande quantité d'acides organiques et de sucres ainsi que des composés organiques complexes. C’est une niche écologique qui éveille et stimule diverses activités microbiennes, en participant ainsi, au fonctionnement des cycles biogéochimiques comme le carbone, l’azote et le phosphore. La densité de la microflore associée aux racines est significativement plus élevée dans la rhizosphère que dans le sol nu. La richesse de la rhizosphère en sucres, en aminoacides, en acides organiques, en isoflavonoides, en régulateurs de croissance et en enzymes libérées par la plante, rend ce microenvironnement un site d'une remarquable activité biologique et d’une richesse naturelle en microorganismes (Zhou et *al*., 2020). Par ailleurs, la communauté microbienne joue un rôle notable dans l’amélioration et la stabilisation de la structure du sol et l’état de santé des plantes (Janati et *al*., 2021) . De nombreuses interactions bénéfiques, tels que la symbiose, sont observées entre plantes bactéries et champignons. Parmi les interactions bénéfiques aux plantes, on peut citer celles avec les bactéries fixatrices d'azote moléculaire, les associations avec les bactéries promotrices de croissance ou de santé, ou les interactions avec les champignons mycorhizogènes (Gouda et *al*., 2018). Ainsi, cet environnement particulier incluant autant des micro-organismes bénéfiques que pathogènes exerce une influence importante sur la croissance et le rendement des cultures végétales.

La diversité et la prédominance de la population microbienne de la rhizosphère dépendent d'un certain nombre de facteurs biotiques et abiotiques prévalant dans une niche écologique particulière (Z. Wang et *al*., 2022). En effet, les plantes, grâce à l’émission des signaux spécifiques, exercent une pression sélective qui tend généralement à réduire la diversité microbienne et à favoriser des espèces ou des souches particulières. En outre, la compétition entre les microorganismes pour les nutriments, la colonisation des sites, la production des

antibiotiques et des bactériocines contribuent à cette dynamique microbienne (Chamkhi et *al*., 2020). Dans la rhizosphère il existe différentes interactions entre les populations microbiennes qui peuvent être reconnues comme des interactions négatives (compétition, amensalisme), positives (commensalisme, synergie et mutualisme), ou positives pour l'un et négatives pour l'autre population (parasitisme ou prédation). Les interactions plantes-microorganismes dans la rhizosphère se résument par la libération d'exsudats racinaires, ces exsudats favorisent des populations microbiennes particulières au sein de la communauté tellurique (Ducousso-Détrez et *a*l., 2022). En retour, certaines de ces populations favorisent la croissance (exemple : nutrition en P) et la protection (exemple : antagonisme microbien) de la plante-hôte ; ces effets bénéfiques compensent le coût représenté par les exsudats racinaires pour la plante.

Diversité microbienne du sol

Dans le sol, les microorganismes représentent la majorité des organismes vivants et constituent une importante part de la diversité écologique de la planète. Il a été estimé qu'un gramme de sol contient de 10^{10} à 10^{11} de bactéries (Claire Horner-Devine *et al*., 2003), de 6000 à 50000 espèces bactériennes (Curtis *et al*., 2002). Ces microorganismes jouent un rôle clé dans un grand nombre de processus dans les différents écosystèmes incluant l'acquisition d'éléments nutritifs pour les plantes (Sprent, 2001 ; Smith et Read, 2010). La microflore tellurique est naturellement constituée de procaryotes et d'eucaryotes (Cardon et Gage, 2006). Certains sont sans effets sur le développement des végétaux (microorganismes commensaux), d'autres encore sont favorables (mutualistes) alors que d'autres, ont des effets délétères (parasites et phytopathogènes) (Whipps, 2001). De ce fait, ila été suggéré que la plante favorise celles qui lui sont bénéfiques (Cook *et al*., 1995; Lynch et Leij, 2012).

4. Rhizobactéries

Les bactéries sont les organismes les plus nombreux d'un sol et représentent en moyenne 6.10^8 cellules par gramme de sol et un poids de 10 $t.ha^{-1}$ équivalant à 5% du poids sec des composés organiques du sol (Zhao et *al*., 2021). On définit alors les bactéries associées aux racines des plantes comme les rhizobactéries. Celles-ci sont généralement des souches très compétitives capables de coloniser le système racinaire riche en éléments nutritifs, tout au long du cycle de développement de la plante (Thomas & Singh, 2019). Si la plante libère des composés organiques, à l'inverse elle prélève de l'eau et des éléments minéraux indispensables à son métabolisme. Les échanges entre la plante et le sol sont influencés par les rhizobactéries et ce d'autant plus que leur densité et leur activité sont élevées. Les rhizobactéries sont des hétérotrophes typiques, elles nécessitent donc des composés organiques comme source

d'énergie. Leurs besoins sont entièrement comblés à l'intérieur même de la rhizosphère. Les rhizobactéries utilisent en effet de nombreux substrats provenant de la plante : les cellules corticales et épidermales des racines qui se détachent, les polysaccharides du mucilage racinaire, les sucres et les acides aminés et organiques des exsudats racinaires (Neumann & Ludewig, 2023). L'abondance des bactéries dans le sol s'explique par leur multiplication rapide et leur capacité à utiliser une grande variété de substrats comme sources d'énergie et d'éléments nutritifs. Les microorganismes rhizosphériques incluent les symbiotes (*Rhizobium*, actinobactéries et champignons mycorhiziens) et les saprophytes libres (Margalef et *al.*, 2017). Par ailleurs, l'association des bactéries avec les racines a des influences importantes sur la santé de la plante, la productivité et la qualité du sol. La colonisation des racines par les bactéries est observée depuis longtemps, mais seulement dernièrement, son importance en vue de croissance et de développement des plantes est devenue claire. Ces bactéries sont souvent mentionnées comme des rhizobactéries promotrices de la croissance des plantes **(**PGPR) (Pérez-Montaño et *al*., 2014).

5. Rhizobactéries promotrices de la croissance des plantes

Les rhizobactéries connues sous le terme PGPR stimulent directement la croissance des plantes en augmentant la disponibilité des éléments nutritifs du sol, en produisant des régulateurs de croissance végétale et en activant les mécanismes de résistance induite chez les végétaux. Elles stimulent indirectement la croissance des végétaux par leur effet antagoniste sur la microflore néfaste, en transformant les métabolites toxiques et en stimulant la nodulation des légumineuses (V. Gupta & Buch, 2019 ; C. Wang et *al*., 2021). Les PGPR ont la capacité de coloniser les racines de façon intense. Toutefois, les activités bactériennes résultent de la synthèse de métabolites inclus les antibiotiques, sidérophores, substances de croissance, acide cyanhydrique, lipopolysaccharides (Elhaissoufi et *al*., 2020 ; Khourchi et *al*., 2022). Cette influence se manifeste par une modification de la croissance de la plante et de la fréquence des infections fongiques de la racine. De plus, de nombreuses études sur la relation PGPR/amélioration de l'absorption des nutriments ont conclu que l'application des inoculations bactériennes sous forme de PGPR améliore considérablement l'absorption du N, P, et K (Timofeeva et *al*., 2022). Les bactéries non symbiotiques répondant à cette définition appartiennent à différents genres et espèces dont les plus étudiés sont : *Agrobacterium, Azospirillum, Bacillus* et *Pseudomonas* (Batista et *al*., 2018 ; Mishra et *al.*, 2014). En outre, le processus d'inoculation avec *Azospirillum* et *Bacillus* a montré une nette accumulation des minéraux dans les tissus de la plante (J. Wang et *al*., 2020).

6. Mécanismes d'actions des PGPR

La présence des microorganismes dans le sol, dont les PGPR, a des effets importants sur la croissance. Ils exercent une grande influence sur plusieurs mécanismes responsables de la plante et sa physiologie (Jiang et *al*., 2019). Ils règlent souvent la réponse de la plante au stress environnemental et contribuent au développement des racines et de la biomasse. Les effets bénéfiques des rhizobactéries sur la croissance végétale résultent de différents mécanismes exercés par les PGPR dont les modes d'action sont directs ou indirects, bien que la différence entre les deux ne soit pas toujours évidente (Bargaz et *al*., 2017). Les mécanismes indirects sont, en général, ceux qui se produisent en dehors de la plante, tandis que les mécanismes directs sont ceux qui se produisent à l'intérieur de la plante et affectent directement leur métabolisme. Le mécanisme direct comprend la fixation biologique d'azote, la production des phytohormones, la solubilisation des minéraux comme le phosphore, le fer, le potassium, le zinc, la production de sidérophores et l'induction de la résistance systémique (Janati et *al*., 2021; Song et *al*., 2022). Ces mécanismes directs facilitent l'absorption des nutriments en augmentant leurs disponibilités. De même, l'utilisation des PGPR a montré de nombreuses efficacités à travers des divers mécanismes indirects tels que : La production de l'acide cyanhydrique (HCN), la production des antibiotiques, qui est considérée comme l'un des mécanismes de bio-contrôle les plus puissants des PGPR contre les phyto-pathogènes, la production des siderophores et des enzymes hydrolytiques (Chaiharn et *al*., 2020 ; Orozco-Mosqueda et *al*., 2020). En plus, les mécanismes d'action directs des PGPR sur la survie des espèces végétales englobent la production d'hormones telles les auxines, les cytokines, les gibbérellines ainsi que l'abaissement du taux d'éthylène chez la plante (A. Yadav & Yadav, 2022b).

Les PGPR jouent également un rôle primordial dans la lutte biologique contre les agents pathogènes des plantes. Elles peuvent éliminer un large spectre de maladies bactériennes, fongiques et parasitaires via plusieurs mécanismes tels que la production des agents antimicrobiens. De même, certaine isolats de lutte biologique peuvent produire des enzymes telles que chitinase, glucanase, protéase et lipase pouvant lyser les cellules fongiques (Sahu, et *al*., 2022). Les PGPR sont considérées comme une constituante pour le maintien de la nutrition adéquate des plantes en favorisant l'absorption des nutriments ; ils réduisent ainsi la nécessité de l'apport d'engrais et anticiper l'accumulation de nitrates et du P dans les sols agricoles.

6.1. Production des siderophores

Le fer est un oligoélément essentiel pour la croissance et le développement des plantes qui y est nécessaire en tant que cofacteur pour les protéines impliquées dans le métabolisme végétal.

Il est aussi l'un des oligoéléments le plus important pour la croissance microbienne. Chez les microorganismes, il agit comme un régulateur global pour de nombreux processus cellulaires, métaboliques et biosynthétiques. Malheureusement, une énorme quantité de fer est sous la forme d'ions ferriques (Fe^{3+}) très peu assimilable par les organismes vivants. Pour pallier cette difficulté et fournir du fer à la plante, les PGPR ont développé des diverses stratégies d'absorption du fer, des stratégies de survie et d'adaptation à leur environnement. Une de ces stratégies est la production de siderophores (Boubekri et *al*., 2021). Ces derniers sont des molécules biologiques de petite taille produites par des microorganismes dans des conditions de carence en fer, ce qui améliorent son absorption (Zhou and Boyd, 2016). Ce sont des métabolites secondaires qui ont pour rôle d'extraire le fer de nombreux complexes minéraux ou organiques, de le solubiliser et de le chélater, le rendant ainsi disponible aux organismes. Les plantes ont développé des techniques actives d'acquisition du fer complexé aux siderophores microbiens au moyen de différents mécanismes, par exemple, la chélation et libération de fer, l'absorption directe des complexes siderophores-Fe, ou par une réaction d'échange de ligand (J. Zhang et *al*., 2017). Cette complexassion du fer par les siderophores permet de libérer éventuellement le P complexé au fer, ce qui contribue ainsi significativement à la nutrition des plantes en ces éléments essentiels pour leur croissance et développement (Cavite et *al*., 2021). De plus, étant donné que les siderophores ne soient pas principalement spécifiques du fer, ils peuvent aussi complexer d'autres métaux lourds pour atténuer leur toxicité ou posséder d'autres fonctions biologiques (Boubekri et *al*., 2021).

6.2.Production de l'AIA

Généralement, la majorité (> 80%) des bactéries du sol sont capables de sécréter les auxines surtout l'acide indole acétique, l'acide indole butyrique ou d'autres composés similaires via le métabolisme du tryptophane (Ramos-Solano *et al.,* 2010). La synthèse de l'AIA par des microbes implique une de ces trois voies :

(1) La formation d'acide indole acétique par l'intermédiaire de l'acide indole-3-pyruvique et de acide-3-indole-acétaldéhyde aété rencontrée chez la majorité des bactéries comme *Erwinia herbicola*, les espèces saprophytes du genre *Agrobacterium* et *Pseudomonas*; et certains représentants de *Bradyrhizobium, Rhizobium, Azospirillum, Klebsiella* et *Enterobacter*.

(2) La conversion du tryptophane en acide-3- indole-acétaldéhyde peut constituer une voie alternative dans laquelle la tryptamine est formée. Cette voie est censée fonctionner chez Pseudomonades et Azospirilla (Khan *et al.,* 2014).

(3) La biosynthèse de l'AIA par la formation de l'indole-3-acétamide est signalée chez certaines

bactéries phytopathogènes comme *Agrobacterium tumefaciens, Pseudomonas syringae* et *Erwinia herbicola* et des Pseudomonades saprophytes telles que *Pseudomonas putida et P. fluorescens* (Khan *et al.,* 2014). Parmi les phytohormones, les auxines sont les principaux régulateurs de la croissance des plantes produites par MSP et présentent de nombreuses fonctions physiologiques telles que l'élongation des racines primaires, l'extension et la division cellulaire, la différenciation des tissus, la formation des pigments, la stimulation de la fixation de l'azote et la résistance aux différents facteurs de stress (Ahmad *etal.,* 2008b; Oves *et al.,* 2013).

6.3. Production de l'acide cyanhydrique

Un autre métabolite secondaire produit par certains PGPR est l'acide cyanhydrique (HCN). Bien que le cyanure soit un inhibiteur métabolique général, il est synthétisé, excrété et métabolisé par les PGPR suivant la voie de décarboxylation oxydative en utilisant la glycine, le glutamate ou la méthionine comme précurseurs (Chaiharn et *al*., 2020). Son rôle est d'éviter la prédation ou la compétition. L'HCN joue un rôle dans la lutte biologique contre les mauvaises herbes et sa production est une activité commune des *Bacillus* dans le sol rhizosphérique (Mahmud et *al*., 2021).

6.4. Fixation d'azote

L'azote est le principal élément nutritif limitant pour la croissance des plantes. C'est un constituant essentiel des nucléotides, lipides membranaires et acides aminés. La majeure partie de cet élément est sous forme gazeuse (N_2) inaccessible aux animaux et aux plantes. Les bactéries fixatrices d'azote (BFN) sont une source majeure d'azote pour les plantes en tant qu'élément de l'environnement dans les pratiques agricoles (Janati et *al*., 2021). La majeure partie de contribution d'azote (83%) provient d'associations symbiotiques, alors que le reste est fournie par des systèmes de vie libre ou associatifs (Timofeeva et *al*., 2022). Les PGPR ont la capacité de fixer l'azote atmosphérique et de le rendre disponible aux plantes. La fixation d'azote symbiotique est une relation mutualiste entre les bactéries et la plante. Les bactéries symbiotiques qui agissent en tant que PGPR sont généralement du genre *Rhizobium, Bradyrhizobium, Sinorhizobium* et *Mesorhizobium* avec les légumineuses et *Frankia* avec des arbres et des arbustes non légumineux (Forni et *al*., 2017 ; M. Khan et *al*., 2022). D'autre part, la fixation d'azote non symbiotique est réalisée par des diazotrophes libres. Ils stimulent la croissance des plantes non légumineuses comme le radis et le riz. Les bactéries rhizosphériques fixatrices d'azote non symbiotiques appartiennent aux genres *Azoarcus, Azotobacter, Acetobacter, Azospirillum, Burkholderia, Diazotrophicus, Enterobacter, Gluconacetobacter,*

Pseudomonas et *cyanobactéries* (Satyaprakash et *al*., 2017 ; Tagele et *al*., 2018 ; A. Sharma et *al*., 2019; Rizvi et *al*., 2021).

6.5. Solubilisation du zinc

Le Zn est le seul micronutriment pertinent, il est le constituant des classes d'enzymes présentes dans les systèmes biologiques et près de 2800 protéines ont besoin de Zn pour leur intégrité structurelle et leur activité (Ramesh et *al*., 2014). En effet, la carence en Zn est considérée comme le stress abiotique le plus répandu dans plusieurs pays d'Asie, quelques pays d'Afrique, ainsi que de nombreuses régions d'Europe et aux Etats-Unis. Cependant, cette carence en millions de hectares de terres agricoles a non seulement réduit les rendements mais aussi a gravement entravé la qualité nutritionnelle des cultures, causant un problème nutritionnel et sanitaire critique dans un tiers des pays du monde (Myers et al., 2015). Le contenu total de zinc dans les sols est sensiblement élevé et existe sous des formes fixes telles que smithsonite ($ZnCO_3$), sphalérite (ZnS), zincite (ZnO), franklinite ($ZnFe_2O_4$), de la wellemite (Zn_2SiO_4) et de l'espavite ($Zn_3(PO_4)^{2-}$, $4H_2O$), qui ne sont que peu solubles (Yasmin et *al*., 2021). Par conséquent, la capacité innée de certaines bactéries à solubiliser ces formes insolubles de Zn en formes solubles pour une disponibilité accrue et une absorption ultérieure pour les plantes est une alternative réalisable. En effet, des études in vitro sur quelques genres de bactéries telles que *Pseudomonas, Gluconacetobacter, Thiobacillus, Bacillus, Acinetobacter* ont montré la capacité de solubiliser le Zn insoluble (S. K. Sharma, 2012).

6.6. Solubilisation du potassium

Le potassium (K) est un élément essentiel à la croissance des plantes après le N et le P. En raison du développement rapide de l'agriculture dans le monde, le niveau de K disponible dans les sols a considérablement diminué à cause du lessivage, du ruissellement et de l'érosion. Les réserves totales de K dans le sol sont généralement larges, mais la disponibilité biologique de ce dernier dépend du type des minéraux contenant le K présents. La libération de K échangeable doit être réapprovisionnée en permanence par l'apport de K non échangeable par l'altération des réserves de K ou l'ajout d'engrais K, qui aboutit à une croissance maximale de la culture (Gamalero et *al*., 2020). Beaucoup de microorganismes dans le sol sont capables de solubiliser les formes indisponibles des minéraux porteurs de K dans le sol, tels que les micas, le feldspaths, l'illite et orthoclases, en excrétant des acides organiques qui peuvent dissoudre directement la roche K ou chélater des ions de silicium. La production des acides carboxyliques, tels que les acides citriques, tartriques et oxaliques, et des capsulaires des polysaccharides ont été associées à la solubilisation du feldspath par les *Bacillus mucilaginosus* et *Bacillus*

edaphicus (Liu et *al*., 2018). Ceci fait de l'application de micro-organismes solubilisant le K une technique prometteuse pour accroître la disponibilité du K dans le sol.

6.7. Activité de biocontrôle

Depuis 1945 jusqu'à nos jours, l'essor de l'utilisation des produits phytosanitaires pour la protection des cultures a permis d'obtenir des rendements très importants. Cet essor a donné naissance à des problèmes de plus en plus fréquents de résistance des pathogènes à ces molécules actives, et d'augmentation de risques écologique par l'utilisation massive et intempestive de celles-ci. Le control biologique peut être une solution alternative pour résoudre certains des problèmes de pollutions chimiques. Ce moyen de biocontrôle se propose de prendre en compte le rôle des PGPR. Ce groupe de microorganismes a été retenu comme le meilleur modèle utilisable. Parmi les mécanismes d'action des PGPR : (1) La compétition pour l'espace et les nutriments, (2) la production de sidérophores et de composés allelochimiques inhibiteurs et (3) l'induction de la résistance systémique (RSI) dans les plantes hôtes à un large spectre d'agents pathogènes (Ryu *et al.,* 2004; Haas et Défago, 2005).

6.8. Capacité antibiose

Depuis plusieurs décennies, diverses espèces bactériennes colonisant les racines de plantes se sont révélées être de puissants agents de lutte biologique contre divers agents phytopathogènes en produisant un ensemble de composés chimiques hétérogènes de faible poids moléculaire (Thomashow *et al.,* 1997; Duffy *et al.,* 2003). La production des antibiotiques par les microbes est le mécanisme clé de protection contre les maladies méditéespar les bactéries solubilisant le phosphate chez les plantes et en particulier celles transmises par le sol et les semences (Glick *et al.,* 2007a; Mazurier *et al.,* 2009). Les bactéries solubilisant le phosphate telles que les bactéries à Gram négatif, *Pseudomonas fluorescens*, *P. aeruginosa* et *Chromobacterium violaceum* sécrètent également des antibiotiques (Taurian *et al.,* 2010) et assurent la protection des plantes contre les pathogènes transmis par le sol (Vassilev *et al.,* 2006). Les antibiotiques sont aussi connus pour leurs activités antiviraux, antimicrobiennes, antiappétantes contre les insectes et les mammifères (Niranjana et Hariprasad, 2014). Bien que l'HCN soit un agent phytotoxique capable de perturber l'activité enzymatique impliquée dans les processus métaboliques majeurs, son rôle en tant que substance de biocontrôle est important. L'HCN est hautement toxique pour tous les microorganismes aérobies à des concentrations de picomolaires ; il peut bloquer la voie de la cytochrome oxydase (Voisard *et al.,* 1989; Devi *et al.,* 2007).

7. Bactéries solubilisant le phosphate

Pour satisfaire les besoins nutritionnels des cultures agricoles en phosphate, ce dernier est

généralement ajouté au sol comme engrais chimique, mais la synthèse des engrais à base de phosphore est un processus à forte intensité d'énergie (Sharma *et al.,* 2007). Cette pratique a généralement un impact négatif à long terme sur l'environnement en termes d'eutrophisation, de réduction de la fertilité des sols et d'empreinte de carbone. En outre, les plantes peuvent utiliser seulement une petite quantité du phosphore alors que 75 à 90% de phosphore ajouté est précipité par des cations de complexes métalliques et devient rapidementfixé dans le sol. Ces préoccupations environnementales ont conduit les scientifiques à la recherche d'une alternative plus respectueuse de l'environnement et d'une manière durable pour la nutrition des cultures agricoles (Sharma *et al.,* 2013).

Dans ce contexte, les microorganismes solubilisant le phosphate (MSP) ont été considérés comme les meilleurs candidats écologiques pour la nutrition des plantes par le phosphore (Sharma et *al*., 2007). Ces organismes possèdent une capacité de solubilisation du phosphate et peuvent également convertir les composés phosphatés insolubles présent dans le sol à des formes solubles et les rendre disponibles pour les plantes (Pradhan et Sukla, 2006). Les bactéries solubilisant les phosphates (BSP) appartiennent au groupe hétérogène de PGPB (Plant Growth Promoting Bacteria). Elles sont caractérisées par leur capacité à solubiliser facilement et efficacement des formes minérales de phosphore inorganique (Anand *et al.,* 2016). Le rôle des organismes rhizosphériques dans la solubilisation du phosphate minéral était connu dès 1903 (Khan *et al.,* 2007). Depuis cette année, de nombreuses études ont été réalisées sur la solubilisation du phosphate minéral par les microorganismes naturellement abondant dans la rhizosphère. Un nombre important d'espèces microbiennes ayant la capacité de solubilisation du phosphate a été isolé et identifié ; celles-ci comprennent des bactéries, deschampignons, des actinomycètes et même des algues microscopiques. En plus de *Pseudomonas* et de *Bacillus*, d'autres bactéries se sont identifiées comme solubilisant le phosphate, il s'agit de : *Rhodococcus, Arthrobacter, Serratia, Chryseobacterium, Gordonia, Phyllobacterium, Delftia* sp. (Wani *et al.,* 2005; Chen *et al.,* 2006), *Azotobacter* (Kumar *et al.,*2001), *Xanthomonas* (De Freitas *et al.,* 1997), *Enterobacter, Pantoea,* et *Klebsiella* (Chung *et al.,* 2005). Alors que la flore fongique solubilisant le phosphate, est représentée essentiellement par les genres, *Aspergillus* et *Penicillium* (Seshadri *et al.,* 2004).

Les principaux mécanismes de solubilisation du phosphate employées par les microorganismes du sol comprennent: (1) la libération des complexants ou composés dissolvant les minéraux par exemple des anions d'acides organiques, des sidérophores, des protons, des ions hydroxyle et le CO_2; (2) la libération d'enzymes extracellulaires (minéralisation biochimique du P) et (3) la

libération de phosphore lors de la dégradation du substrat (minéralisation biologique du P) (Figure 1) (McGill et Cole, 1981).

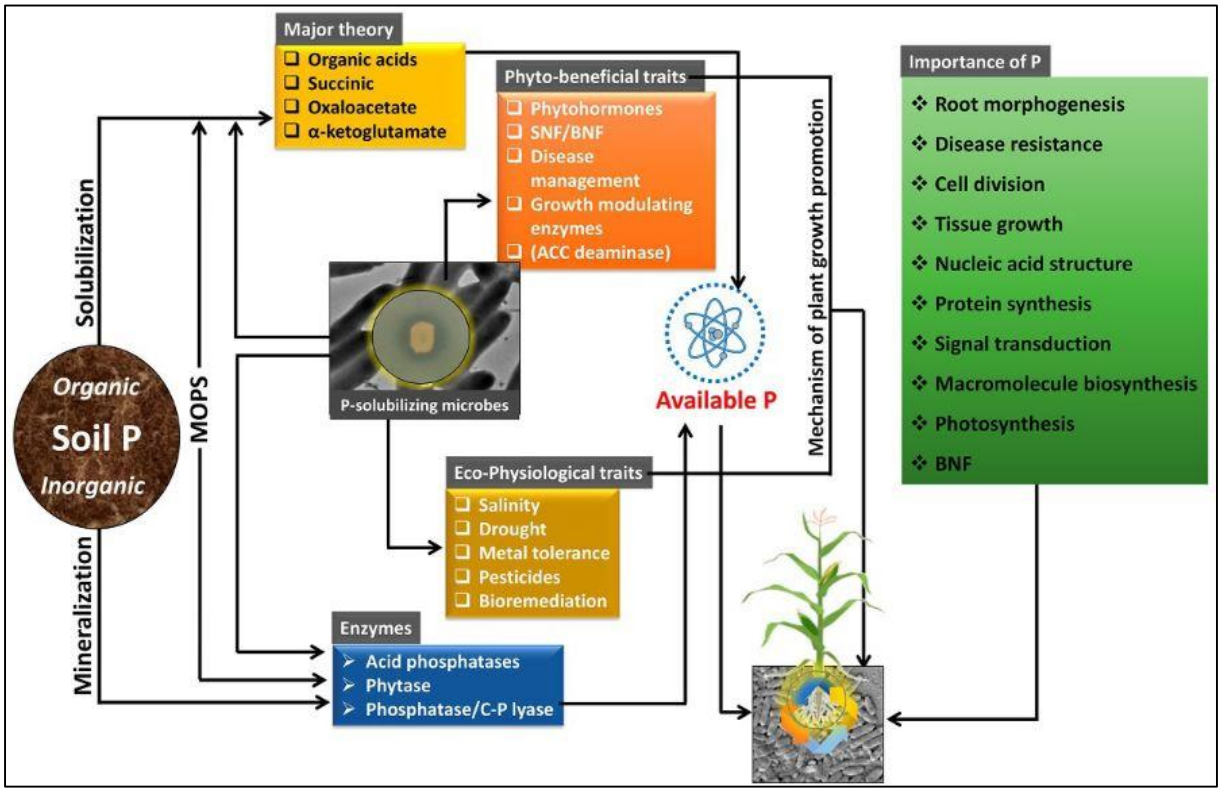


Figure 1. Description de l'importance des microorganismes dans l'augmentation de la production végétale dans différents agrosystèmes ; mécanisme de solubilisation du phosphate, fixation symbiotique de l'azote, fixation biologique de l'azote. Modifié à partir (M. Khan et *al.*, 2022).

Solubilisation du phosphore organique (minéralisation)

La solubilisation du phosphore organique est également appelée la minéralisation du phosphore organique. La minéralisation du phosphore organique du sol (Po) joue un rôle essentiel dans le cycle du phosphore dans un système d'exploitation agricole et il peut constituer de 4 à 90% du phosphore total du sol (Khan *et al.*, 2009). Ce phosphore peut être libéré à partir de composés organiques dans le sol par des enzymes de phosphatases suivantes:

(i)- Phosphatases acides non spécifiques (NSAP), qui déphosphorylent les liaisons phospho-ester ou phosphoanhydride de la matière organique (Sharma *et al.*, 2013). Parmi la variété de classes d'enzymes de phosphatase libérées par MSP, les phosphomonoesterases (souvent appelées phosphatases). Elles sont les plus abondantes et les mieux étudiées (Nannipieri *et al.*, 2011). En fonction de leur pH optimal, ces enzymes sont divisées en phosphomonoesterases acides et alcalines et les deux peuvent être produites par le MSP en fonction des conditions extérieures (Jorquera *et al.*, 2008). Certains éléments de preuve indiquent que les phosphatases

d'origine microbienne possèdent une plus grande affinité pour les composés de phosphore organique que ceux qui sont dérivés à partir de racines de plantes (Tarafdar *et al.,* 2001).

(ii)- Phytases, qui provoquent spécifiquement la libération de phosphore de la dégradation des phytates. Dans sa forme de base, la phytate est la principale source de l'inositol et la principale forme de phosphore stockée dans les graines et le pollen de plantes,et elle est une composante majeure de phosphore organique dans le sol (Richardson, 1994). Par conséquent, les microorganismes sont en fait un facteur clé dans la régulation de la minéralisation des phytates dans le sol et leur présence dans la rhizosphère peut compenser une incapacité des plantes à acquérir autrement le phosphore directement à partir de phytate (Richardson et Simpson, 2011).

(iii)- les phosphatases et les C-P-lyases, qui clivent la liaison C-P organophosphorés (Rodriguez *et al.,* 2007).

Solubilisation du phosphore inorganique

La solubilisation du phosphore inorganique par des microorganismes se fait principalement par la production d'acides organiques, soit par: (i) l'abaissement du pH, ou (ii)en augmentant la chélation des cations liés au phosphore (iii) en concurrence avec le phosphore pour les sites d'adsorption sur le sol (iv) en formant des complexes solubles avec les ions métalliques associés aux phosphore insolubles (Ca, Al, Fe) et donc le phosphore est libéré (Sharma *et al.,* 2013).

Production des acides organiques

L'abaissement du pH du milieu suggère la libération des acides organiques par les microorganismes solubilisant le phosphate (Maliha *et al.,* 2004) par l'intermédiaire de la voie d'oxydation directe qui se produit sur la face externe de la membrane cytoplasmique (Zaidi *et al.,* 2009a). Ces acides sont le produit du métabolisme microbien, principalement par la respiration oxydative ou par fermentation de sources de carbone organique (par exemple, le glucose) (Trolove *et al.,* 2003). Ces acides organiques peuvent directement dissoudre le phosphore minéral comme résultat d'un échange d'anions de phosphate par un anion de l'acide soit par chélation les ions de Fe, Al et Ca associés avec le P (Omar, 1998). Différents acides organiques tels que l'acide gluconique, l'acide 2-cétogluconique, l'acide lactique, l'acide isovalérique, l'acide isobutyrique, l'acide acétique, l'acide oxalique, et l'acide citrique ont été rapportés être produits par des rhizobactéries (Omar, 1998; Rodriguez et Fraga, 1999) et connus pour leur activité de solubilisation des phosphates insolubles.

Autres mécanismes de solubilisation

Cependant, l'acidification ne semble pas être le seul mécanisme de solubilisation, caril s'est avéré que la capacité à réduire le pH dans certains cas, n'est pas corrélée avec l'aptitude à solubiliser les phosphores minéraux (Subba Rao, 1982). D'autres mécanismes de solubilisation

de phosphore ont été suggérés comme l'emploi des acides inorganiques (par exemple l'HCl), bien que l'HCl a été capable de solubiliser une faible quantité de phosphore à partir de l'hydroxyapatite comparativement à l'acide citrique ou l'acide oxalique au même pH(Kim *et al.,* 1997). D'après la théorie de l'évier, les organismes solubilisant le phosphore assimilent le phosphore à partir du liquide et par conséquent activent la dissolution indirecte des composés de phosphate de calcium par élimination constante de phosphore à partir du milieu de culture liquide. Un autre mécanisme est la production d'H_2S, qui réagit avec le phosphate de fer pour produire du sulfate ferreux avec libération concomitante de phosphate (Swaby et Sperber, 1958). Il a été suggéré que l'activité de solubilisation du phosphate par les microorganismes se produit en conséquence de l'oxydation microbienne du soufre, la production de nitrate et de la formation de CO_2. Ces processus conduisent à la formation d'acides inorganiques tels que l'acide sulfurique (Sperber, 1958). Toutefois, leur efficacité a été moins accepté que le concept d'implication des acides organiques dans la solubilisation (Kim *et al.,* 1997).

L'excrétion de H^+ provenant de l'assimilation de $NH4^+$ proposée par Parks *et al.,* (1990) pourraient être les mécanismes alternatifs de solubilisation du phosphore. Krishnaraj *etal.,* (1998) ont proposé un modèle mettant en évidence l'importance des protons qui sont pompés hors de la cellule d'être le principal facteur responsable de la solubilisation du phosphate et pour certains microorganismes, les protons libérés de NH_4^+ semble être le seul mécanisme pour promouvoir la solubilisation du phosphate. Goldstein (1995) et Song *et al.,* (2008) ont suggéré que l'oxydation extracellulaire par voie d'oxydation directe peut jouer un rôle essentiel dans les sols où les phosphates de calcium fournissent une source importante de phosphore minéral indisponible. Des études ont rapporté la production des sidérophores et les exopolysaccharides (EPS) par les MSP (Vassilev *et al.,* 2006; Caballero-Mellado *et al.,*2007; Yi *et al.,* 2008), cependant, cette production n'a pas été largement impliquée dans le mécanisme de solubilisation de phosphate.

8. Biofilms associés aux plantes en base de BSP

Les biofilms constituent un ensemble de microorganismes (plus que 90 %) capables de s'attacher à certaines surfaces et former des communautés microbiennes plus ou moins complexes et symbiotiques (Ghosh et al., 2019). Ces microorganismes, qui adhérent entre eux et à une surface, sont marqués par la sécrétion d'une matrice adhésive, protectrice, extracellulaire et composée de polymères. Dans la nature, chaque niche de biofilm est colonisée par un microbiote complexe adapté aux conditions de son micro-habitat local (pH, température, oxygène et nutriments) (Saeed et al., 2021). Il s'agit en effet, d'un système ouvert et dynamique,

au niveau duquel des échanges et des migrations de populations affectent continuellement son organisation. La formation des biofilms est un perpétuel recommencement depuis l'adhésion des bactéries pionnières à une surface, la construction du biofilm, l'édification de sa structure tridimensionnelle (ou maturation) jusqu'à la dispersion et la libération de cellules spécialisées de la dissolution du biofilm (B. Sharma et al., 2023). Les observations microscopiques effectuées sur des biofilms matures en utilisant la microscopie électronique à balayage montrent que ces derniers possèdent une structure hétérogène de cellules microbiennes organisées en monocouches à la surface (Banerjee et al., 2019).

Certains PSB vivent en symbiose étroite avec les racines des plantes, allant jusqu'à former des biofilms, dont lequel ils permettent d'agglutiner diverses substances (fibres végétales, cristaux de roche et débris d'animaux) et de former par conséquent des microcavités assurant la protection des bactéries ainsi que le transport de l'eau et des nutriments (P. Kumar, 2023). Dans la mycorhizosphère, les biofilms possèdent diverses particularités intéressantes en agriculture à savoir l'amélioration des échanges sol/plante, la synergie avec d'autres microorganismes du sol, la protection contre les pathogènes. Les caractéristiques métaboliques des PSB au sein d'une communauté de biofilms sont distinctes de ceux de leurs homologues planctoniques (Balasundararajan & Dananjeyan, 2019). Il est à noter que l'architecture complexe des biofilms prévoit la possibilité d'une coopération métabolique entre les différentes bactéries, qui sont exposées à un éventail de différents signaux environnementaux. En effet, les canaux dispersés à travers les biofilms dans les zones entourant les micro-colonies des PSB constituent un moyen efficace d'échange de nutriments et de métabolites avec la phase aqueuse, d'amélioration de la disponibilité des nutriments ainsi que de suppression de métabolites potentiellement toxiques. Des études menées dans le développement de biofilms de PSB ont montré qu'ils peuvent augmenter l'activité biofertilisant avec un effet positif sur la croissance et le rendement des cultures (Sionov & Steinberg, 2022).

Habituellement, on trouve les biofilms sur des substrats solides immergés ou exposés à une solution aqueuse. La matrice du biofilm contient des éléments synthétisés par des organismes qu'elle abrite (protéines, lipides, ADN, ARN, ...), elle est également constituée de polysaccharides et d'une importante proportion d'eau (Meryem et *al*., 2022). Dans un biofilm mature, les cellules bactériennes produisent des messagers chimiques sous la forme de petites molécules médiatrices appelées aussi auto-inducteurs. Ces molécules diffusibles activent l'expression de certains gènes codant pour une panoplie de fonctions essentielles, et ce en fonction de leur concentration elle-même reflétée par la densité de la population bactérienne du

biofilm (Altaf et *al*., 2017). Il s'agit en fait d'un mode de signalisation bactérien nommé le « Quorum Sensing » définit comme étant un processus très complexe faisant partie d'un réseau global de régulation de gènes intégrant divers signaux externes en vue de favoriser l'adaptation des bactéries aux conditions environnementales (Sionov & Steinberg, 2022). Ceci implique que les PSB formant le biofilm sont capables de percevoir les différents paramètres du milieu et exprimer les fonctions régulées adéquatement au type d'environnement rencontré. Les études ont montré que les PSB colonisent le mycélium pour former des biofilms. Ceci entraine des taux plus élevés de fixation biologique de l'azote d'une part et une production d'acides organiques d'autre part avec disponibilité importante de nutriments. Des expériences en champs ont été réalisées afin de s'assurer de l'effet bénéfique de ces formes bactériennes sur *Oryza sativa*, *Camellia sinensis*, *Triticum aestivum*, et *Anthurium andraeanum* (Seneviratne et *al*., 2009). Pour le riz et le thé, les résultats montrent qu'il faut réduire d'environ 50% l'ajout d'engrais chimique tout en appliquant ces biofertilisant.

La composition et la structure des constituants des sols représentent une véritable mine énergétique et structurante ce qui confère aux sols une meilleure disponibilité en nutriments facilement assimilables. Ces constituants représentent un stock de matières carbonées favorisant ainsi la stabilité de la structure du sol et le développement à long terme de l'activité microbiologique. Les microorganismes, dont la quasi-totalité sont hétérotrophes, ont développé des mécanismes pour en tirer profit (Davey and O'Toole 2000). Par exemple, certains microorganismes bénéfiques du sol vivent en symbiose étroite avec les racines des plantes, allant jusqu'à former des structures spécialisées et complexes comme les mycorhizes, les nodules et les biofilms. Ces modes de vie permettront à la fois d'améliorer la croissance microbienne et végétale.

Les biofilms peuvent aussi augmenter l'activité biofertilisant des organismes qui en font partie assurant ainsi un effet positif sur la croissance et le rendement des cultures (Seneviratne et al. 2009). Ces biofilms peuvent contenir une seule espèce microbienne ou plusieurs. Seneviratne et al. (2009) ont décrit les études menées dans ce domaine essentiellement dans le développement de biofilms de bactéries fixatrices d'azote et de champignons solubilisant les phosphates (Seneviratne et al. 2009). Cette synthèse de la littérature nous indique que ce domaine de recherche est encore à ses débuts, des expériences à l'échelle de laboratoire et sur le terrain sont nécessaires pour explorer pleinement le potentiel de cette nouvelle approche biotechnologique à l'avenir et pour chercher d'autres activités importantes réalisées par les PSB.

9. Facteurs influençant la survie des PSB

Les micro-organismes de la rhizosphère du sol créent des communautés complexes sur le plan de la composition ; mais plusieurs variables influencent leur diversité et leur abondance. Même si les PSB sont largement distribués avec une variété de populations et de formes dans divers sols, la densité de population des PSB peut être significativement influencée par des facteurs géographiques, climatiques et environnementaux (Crowther et *al*., 2019 ; J. Li et al., 2021). Selon Alori et *al*. (2017), l'écologie du sol, les espèces végétales, les techniques agronomiques et les caractéristiques physico-chimiques du sol, comme la matière organique et le pH, peuvent influencer la prévalence de la solubilisation microbienne du phosphore. Il est largement connu que la compréhension de l'impact des facteurs environnementaux est essentielle pour améliorer la qualité et la fiabilité des PSB. La prévalence, l'abondance, la diversité, la bioactivité et les taux de survie des PSB varient d'un sol à l'autre. Cette variation est le résultat des diverses caractéristiques du sol. Outre les conditions physicochimiques du sol et les conditions nutritionnelles agissent égalemeent. Ainsi, la capacité d'un PSB introduit à coloniser les racines et à exercer son activité est influencée par les nutriments facilement disponibles (Malgioglio et *al*., 2022).

10. Facteurs influençant la productivité des cultures

De nombreux facteurs entraînent une baisse de la productivité des cultures conduisant à l'insécurité alimentaire en particulier dans les pays en voie de développement. Parmi ces facteurs, la disponibilité des terres agricoles, les ressources en eau douce, une faible activité économique dans le secteur agricole et l'incessante croissance des stress biotiques et abiotiques (Van Zelm et *al*., 2020). Il est généralement admis que les stress abiotiques sont considérés comme la principale cause de la chute du rendement agricole. Les pertes potentielles de rendement sont estimées à 17% dues à la sécheresse, 20% à la salinité, 40% à la température élevée, 15% à la basse température et 8% à d'autres facteurs) (Deiss et *al*., 2018). La sécheresse et la salinité sont deux stress abiotiques majeurs qui influent sur différents aspects de la vie humaine d'une population dont la santé humaine et la productivité agricole. L'augmentation de la sécheresse et la salinité entraîne une baisse de la productivité agricole et cause la dégradation des terres arables (Thaker et *al*., 2021). Selon les rapports de l'ONU, les changements climatiques ont augmenté la fréquence et l'intensité de la pénurie d'eau dans les zones subtropicales d'Asie et d'Afrique. Il est attendu, si la situation persiste, que d'ici 2025, 1,8 milliard de personnes vivront dans des régions où la pénurie d'eau est absolue et donc des

circonstances non souhaitable menace l'agriculture. En outre, sur 230 millions d'hectares de terres irriguées, 45 millions d'hectares (environ 20%) en sont affectés (Latif et *al*., 2023).

La salinité du sol inhibe la croissance et le développement des plantes et aboutit finalement à entraver l'économie agricole des pays en voie de développement. Les effets néfastes les plus communs de la salinité sont le stress osmotique, la toxicité par Na et Cl, la production d'éthylène et la plasmolyse, une réduction de la croissance, une sénescence précoce, une diminution de la capacité photosynthétique suite à la fermeture partielle des stomates, des changements respiratoires, une perte de l'intégrité cellulaire, une nécrose des tissus et même la mort de la plante (Slimani et *al*., 2023). De même, les plantes souffrant de stress hydrique présentent des altérations de leur homéostasie, principalement en raison de la réduction du potentiel osmotique et ionique et une distribution inadéquate provoquant un déséquilibre nutritionnel. La concentration en sel dans les feuilles âgées entraîne la sénescence précoce des feuilles et un retard de croissance en augmentant la production de l'acide abscissique (ABA). Le taux optimum ou seuil de tolérance de la salinité de la plupart des cultures agricoles a été jugée à 7.2 $g.L^{-1}$ (pour les glycophytes), mais cette tolérance pourrait être augmentée par l'emploi de méthodes biologiques (Latif et *al*., 2023). L'utilisation des microorganismes comme inocula dans l'agriculture est l'approche la plus prometteuse afin d'améliorer la production et le rendement dans les régions avec des conditions extrêmes (De Vasconcelos & Chaves, 2019). La grande opportunité pour la recherche sur la tolérance aux stresses est la capacité d'utiliser les bactéries comme inoculant et d'attirer l'attention sur les stratégies futures de la recherche pour le développement d'un meilleur développement des plantes en utilisant différentes bioformulations. Les microorganismes bénéfiques feront la plus grande contribution à l'économie agricole en tant qu'une approche prometteuse, si peu coûteuse et facile à utiliser.

11. Tendances actuelles et pratiques des biofertilisants sous forme de BSP

Les rhizobactéries qui encouragent la croissance des plantes colonisent les racines des plantes et favorisent la croissance des plantes dans la rhizosphère ; elles sont donc utilisées comme biopesticides, agents de biocontrôle, phytostimulants et biofertilisants. Les inoculant microbiens, également appelés biofertilisants, sont des solutions contenant des bactéries, des champignons ou des algues vivants, individuellement ou collectivement, qui favorisent la croissance des plantes et augmentent le rendement des cultures (Thomas & Singh, 2019). Face aux divers stress environnementaux et comme moyen de répondre à la question de l'alimentation d'une population mondiale croissante, les biofertilisants devraient constituer un système alternatif viable pour une culture agricole durable. L'utilisation de microbes bénéfiques

comme biofertilisants est devenue extrêmement importante dans l'industrie agricole en raison de leur contribution potentielle à la sécurité alimentaire et à la production de cultures durables avec une variété d'applications (Itelima et *al*., 2018) comme inoculants pour les semences ou le sol. Grâce à la fixation de N_2, à l'activité cellulolytique ou à la solubilisation du P, les microbes bénéfiques des biofertilisants améliorent les propriétés chimiques et biologiques du sol. Ces techniques respectueuses de l'environnement présentent également les avantages suivants : durée de conservation plus longue sans nuire à l'écosystème, maintien d'un environnement pédologique riche en tous types de micro- et macronutriments grâce à la fixation de l'azote, à la solubilisation ou à la minéralisation du P et du K, libération de composés qui contrôlent la croissance des plantes, production d'antibiotiques et décomposition de la matière organique dans le sol (Itelima et *al*., 2018). Pour ces raisons, les céréales, les légumes, les oléagineux et les légumineuses sont les cultures les plus testées avec les biofertilisants PSB (M. Khan et *al*., 2022).

Les techniques d'application des biofertilisants PSB les plus populaires sont le traitement des semences, le trempage des semis et l'application au sol. Cependant, la méthode d'application la plus populaire et la plus simple dans le passé a été l'inoculation de la surface des semences (M. Khan et *al*., 2022). D'une manière générale, les biofertilisants considérés comme des PSB et disponibles dans le commerce pour les agriculteurs à ce jour comprennent : *Rhizobium, Azotobacter, Azospirillum brasilense, Azotobacter chroococcum, Acetobacter diazotrophicus, Bacillus licheniformis, B. megaterium, B. muococcum, Bacillus licheniformis. megaterium, B. mucilaginous, B. edaphicus, B. subtilis, Actinomyces, Herbaspirillum seropedicae,* et *Rhizobium phaseoli* (Seenivasagan & Babalola, 2021). Il est important de se rappeler que l'efficacité des biofertilisants PSB peut varier en fonction d'éléments tels que la souche bactérienne utilisée, les caractéristiques du sol et les espèces cultivées. À la lumière des besoins uniques des cultures ciblent et des conditions actuelles du sol, il est conseillé de choisir le biofertilisant PSB approprié. En raison de sa capacité à résister à la chaleur et à la dessiccation, le genre Bacillus a une durée de conservation plus longue dans les formulations commerciales et est un genre privilégié dans l'industrie des biofertilisants (Yadav & Yadav, 2022b).

Les avantages des biofertilisants à base de PSB doivent cependant être compris afin de les intégrer dans les pratiques agricoles contemporaines. Par conséquent, le contrôle de la qualité des biofertilisants sera nécessaire à mesure que l'utilisation des biofertilisants à base de microbes augmentera à l'avenir (Harahap et *al*., 2022). La création de nouvelles méthodes pour l'utilisation des PSB et leur mise en œuvre appropriée dans l'agriculture sont essentielles au

succès du domaine des biofertilisants (Figure 2). En outre, l'une des rares raisons pour lesquelles les écologistes et les agronomes ignorent encore l'existence de nombreuses PSB utiles est le manque de connaissances sur les protocoles pratiques d'application d'engrais sur le terrain.

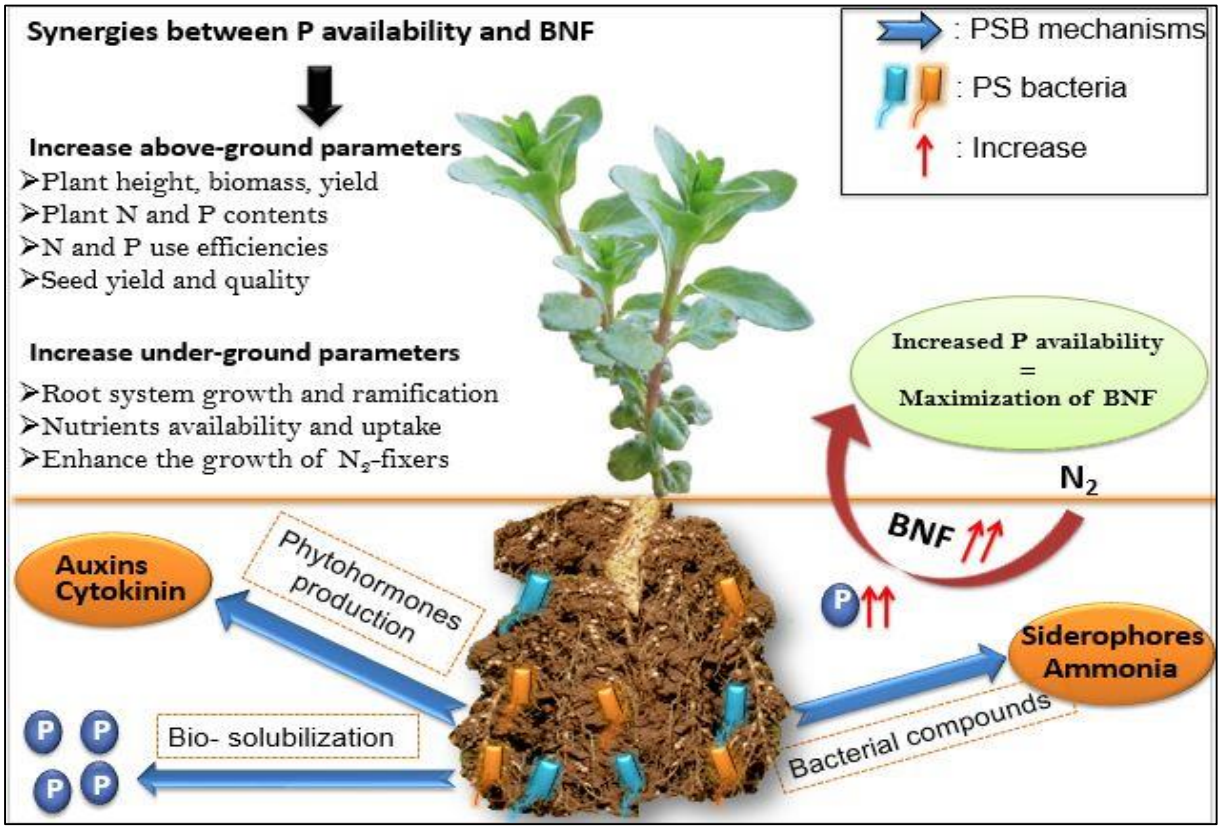


Figure 2. Interactions entre les PSB et les racines des plantes et leurs effets sur la disponibilité des nutriments et les paramètres globaux de la croissance. Modifié à partir (Janati et *al*., 2021).

12. Consortiums microbiens à base de BSP

Les consortiums microbiens (CM), qui visent à établir les limites entre deux groupes de produits microbiens, sont devenus plus populaires ces dernières années que les produits microbiens basés sur des souches uniques. Les biofertilisants à large spectre comprennent les algues bleues (BGA), le rhizobium et l'azolla, ainsi que les bioinoculant spécifiques aux cultures, tels que l'azospirillum, l'azotobacter, les bactéries solubilisant le phosphore (PSB), les mycorhizes vésiculaire-arbusculaire (VAM) et l'anabaena, en tant que cyanobactéries fixatrices d'azote (Seenivasagan & Babalola, 2021). Selon Ramasamy et *al*. (2020), l'utilisation de ces biofertilisants sur les MC est un outil agricole moderne, crucial pour la gestion intégrée des nutriments, la réduction de l'utilisation de produits chimiques nocifs, la rentabilité et la source d'énergie renouvelable pour les plantes. Une stratégie a récemment été proposée pour accroître l'activité et améliorer la viabilité des PGPM, le consortium microbien pour les PGP (Kour et *al*., 2020). Pour se faire, les biofertilisants issus de la même famille de micro-organismes ou de

familles différentes peuvent être utilisés en colonie pure ou en consortium. Selon Deveau et ses collaborateurs (2018), les caractéristiques comportementales de l'un ou des deux partenaires en interaction modulent intrinsèquement l'interaction bactéries-champignons. Le développement de l'interdépendance bactérie-champignon résulte généralement d'associations biophysiques et métaboliques intimes qui se produisent au cours de leur coexistence (Mekonnen et *al*., 2023). Une étude réalisée par Saxena et *al*. (2015) évalue les effets de la co-inoculation de *Bacillus sp*. RM-2 et d'*Aspergillus niger* S-36 avec le TCP sur la croissance et le rendement des plants de pois chiches, comparés à ceux de leur inoculation unique ou individuelle. La co-inoculation de PSB et de souches fongiques s'est avérée supérieure à leurs inoculations individuelles ou uniques, comme le montre les teneurs en chlorophylle significativement plus élevées dans les feuilles, des pousses et des racines plus longues, et des pousses et des racines plus lourdes calculés sur la base du poids frais et sec.

En outre, Abdullahi et ses collaborateurs (2016) ont indiqué que le traitement des semences avec un mélange de *Pseudomonas fluorescens* et de *Trichoderma viride*, ainsi qu'entre *Bacillus thuringiensis* et *Trichoderma viride*, peut faire partie de la gestion intégrée de la maladie du mildiou de la pomme de terre et d'intérêt agronomique. Étant donné que le manque d'activités d'un microbe supplémentaire peut être comblé par l'action de l'autre, l'utilisation d'un mélange de deux ou plusieurs microorganismes compatibles d'espèces (ou de souches) différentes peut faciliter l'obtention de résultats additifs ou synergiques (Nadarajah, 2019). Par conséquent, l'ajout de consortiums microbiens peut restructurer et stimuler les mécanismes qui soutiennent la croissance des plantes à la fois dans des circonstances idéales et en réponse à divers stress biotiques et abiotiques (Woo & Pepe, 2018). Dans certaines circonstances, une combinaison de diverses souches de la même espèce peut présenter des comportements améliorés et être désignée comme un consortium.

Il est rapporté que le quorum sensing est un facteur clé de la compatibilité bactérienne dans les formulations de consortium et constitue un signal moléculaire important pour la communication du consortium bactérien (Mekonnen et *al*., 2023). Kapadia et *al*. (2022) ont examiné les effets du consortium composé de *Bacillus sp., Delftia sp., Enterobacter sp*. et *Achromobacter sp.* sur la croissance de la tomate (*Solanum Lycopersicum L*.) et l'absorption des minéraux en cas de stress salin et dans des conditions de sol typiques. En comparaison avec le contrôle, les plantules bactérisées semées dans un sol salin ont significativement augmenté le poids sec des feuilles (105,17%), des pousses (105,62%), des racines (109,06%) et les teneurs en chlorophylles (61,49%), le nombre de feuilles (75,68%), la longueur des pousses (92,95%), la longueur des

racines (146,14%) et les racines secondaires (91,23%). Selon Kapadia et *al*. (2022), les consortiums microbiens peuvent être la méthode la plus efficace, la plus durable et la plus rentable pour gérer la salinité du sol et améliorer la croissance des plantes en cas de stress salin. De même, Barra et *al*. (2016) ont testé le MC en utilisant des bactéries halotolérantes des genres Enterobacter, Serratia, Microbacterium, Pseudomonas et Achromobacter qui produisent de l'IAA et de l'ACCD.

L'effet du stress salin sur l'émergence, la croissance et la biomasse des plantules de blé exposées au stress salin en chambre de culture et en serre a été significativement réduit par l'inoculation des semences. En outre, la co-inoculation de trois PSB *Enterobacter, Montrichardia arborescens et Serratia marcescens* s'est avérée la plus efficace pour favoriser la croissance, le rendement et l'absorption de nutriments (N, P, Cu, Zn, Mn et Fe) par le blé. Selon Kumar et *al*. (2017), la co-inoculation d'*Enterobacter* avec *Serratia marcescens* et *Montrichardia arborescens* peut être utilisée pour créer une formulation puissante de consortium PGP pour une production de blé durable et respectueuse de l'environnement. En outre, Yadav & Yadav (2022) ont étudié la capacité des plants de cumin, traités avec des consortiums de PSB à base de talc ($Mg_3Si_4O_{10}(OH)_2$) et de glycérol, à promouvoir la croissance. Le traitement avec le rendement le plus élevé de 0,42 g de graines de cumin par plante avec des inoculations de PSB à base de talc contenait une combinaison de *Klebsiella pneumoniae* MF351845, *Erwinia sp*. MF351846, et *Pseudomonas nitritireducens* MF351919. Dans l'ensemble, les combinaisons de PSB à base de talc ont amélioré la hauteur, le poids sec, le poids de 100 graines et le rendement de tous les plants de cumin. De même, Admassiea et al. (2017) ont constaté que la technique de double culture sur les isolats de *Colletotrichum* du poivre a montré que les isolats de *Bacillus* et de *Trichoderma* en combinaison ont donné la plus grande réduction de la maladie de l'anthracnose du poivre par rapport aux plantes inoculées par l'agent pathogène. C'est pourquoi l'intérêt de la création de consortiums synthétiques destinés à être utilisés en biotechnologie industrielle est suscité par les caractéristiques puissantes des consortiums naturels.

13. Commercialisation des BSP

Le succès et la commercialisation des souches rhizobactériennes favorisant la croissance des plantes et des bactéries solubilisant le phosphate dépendent des liens entre les organisations scientifiques et les industries (Gupta *et al.,* 2015). De nombreux travaux ont montré les différentes étapes du processus de commercialisation, y compris l'isolement des souches antagonistes, le dépistage, les méthodes de fermentation, la production de masse, la viabilité de la formulation, la toxicologie, les liens industriels, le contrôle de la qualité et l'efficacité du

champ (Nandakumar *et al.,* 2001). En effet la commercialisation d'un produit biologique présente des défis difficiles et cette difficulté est plus importante quand il s'agit d'un produit qui vise à améliorer la nutrition du phosphore. L'inoculant au phosphate a été homologué pour le blé en 1990 et est maintenant inscrit sur la plupart des grandes cultures dans l'Ouest canadien. D'un petit nombre d'hectares inoculés en première année, à 1,9 million d'hectares qui ont été inoculés en 2003 (Leggett *et al.,* 2007).

Les progrès récents de la biologie moléculaire et de la biotechnologie dans la compréhension des interactions rhizobactériennes avec les nodules des plantes cultivées encourageront une zone de recherche appropriée dans les mécanismes PGPR relatifs à la colonisation des rhizosphères (Bhattacharyya et Jha, 2012). 60-75% de coton, de cacahuètes, de soja, de maïs, de légumes cultivées aux États-Unis sont maintenant traités avec un produit commercial de *Bacillus subtilis,* qui entrent en vigueur contre les agents pathogènes du sol (Nakkeeran *et al.,* 2005). En outre, *Bacillus* spp., Certaines autres souches de PGPR appartenant aux genres tels que *Agrobacterium, Azospirillum, Bulkholderia, Pseudomonas* et *Streptomyces* sont également utilisées pour la production de plusieurs produits commerciaux qui sont généralement appliquées contre plusieurs agents pathogènes cibles (Nakkeeran *et al.,* 2005). Le développement d'un inoculant commercial de phosphate ou de PGPR a été un processus stimulant et satisfaisant pour l'agriculture. Les défis ne se terminent pas avec le processus de commercialisation, mais continuent à apparaître au fur et à mesure que l'amélioration des produits et des processus, en suivant les nouveaux développements dans l'agriculture en élargissant le marché mondiale (Leggett *et al.,* 2007).

14. Les légumineuses : diversité et intérêts

Les plantes capables de former des associations symbiotiques avec les rhizobia appartiennent à la famille des légumineuses à l'exception des *Parasponia* (Sprent et al. 1987). En terme du nombre d'espèces connues, les Fabaceae représentent l'une des familles de plantes à fleurs les plus importantes avec les Orchidaceae et les Asterraceae. La famille des légumineuses a une origine monophylétique qui en se basant sur les caractères morphologiques des fleurs était, traditionnellement, répartie en trois sous-familles, les Caesalpinioideae, les Mimosoideae et les Papilionoideae (Lewis 2005). Contrairement au groupe paraphylétique des Caesalpinioideae, les sous-familles des Mimosoideae et des Papilionoideae sont monophylétiques (Bruneau et al. 2008). En 2017, l'utilisation des séquences du gène *matK* a permis de reconsidérer la classification reconnue jusqu'à lors en considérant les six sous familles suivantes : Caesalpinioideae, Cercidoideae, Detarioideae, Dialioideae, Duparquetioideae et

Papilionoideae. L'ancienne sous-famille des Mimosoideae a été réassignée comme appartenant à la sous-famille des Caesalpinioideae qui se compose de 148 genres regroupant environ 4'400 espèces (Azani et al., 2017).

Les Papilionoideae représente la plus grande sous-famille des Fabaceae avec environ 14'000 espèces regroupées dans 503 genres (Azani et al., 2017). La capacité de la plupart des légumineuses à former des symbioses fixatrices avec les rhizobia et par conséquent à se développer sur des sols pauvres en azote assimilable où d'autres plantes ne peuvent survivre, leur confère un intérêt agronomique et écologique majeur. De nombreuses légumineuses à graines qui sont cultivées pour l'alimentation humaine (soja, haricot, pois chiche…) ou animale (luzerne, trèfle, fenugrec…) appartiennent aux Papilionoideae, illustrant l'importance économique des plantes appartenant à cette sous-famille. Ces légumineuses figurent aussi parmi celles qui sont les plus nodulées par des rhizobia ce qui en fait un groupe particulièrement étudié. Afin de mieux comprendre les mécanismes moléculaires impliqués dans la symbiose rhizobialégumineuses, quelques espèces de Papilionoideae ont par conséquent été sélectionnées comme plantes modèles par la communauté scientifique. Initialement ce sont les deux espèces *Lotus japonicus* et *Medicago truncatula* qui ont été sélectionnées du fait de leur taille réduite, leur caractère autogame, la courte durée de leurs cycles et aussi la taille de leur génome qui ne dépasse pas 500 Mb (Journet et al. 2001; Stougaard 2001). Par la suite et principalement grâce aux progrès réalisés dans le séquençage des génomes végétaux d'autres légumineuses d'intérêt agronomique ont fait l'objet de recherches plus intenses : il s'agit principalement du haricot (*Phaseolus vulgaris*) (Schmutz et al. 2014), du pois d'Angole (*Cajanus cajan*) (Varshney et al. 2012), du pois chiche (*Cicer arietinum*) (Varshney et al. 2013) mais aussi du soja (*Glycine max*) (Schmutz et al. 2010).

Intérêt agronomique, et environnemental

Les légumineuses jouant un tel rôle sur les plans nutritionnel, agronomique et écologique, l'ONU désigna l'année 2016 comme Année Internationale des Légumineuses. En effet, avec une population mondiale en constante augmentation la production de denrées alimentaires suffisantes grâce à une agriculture plus durable est devenue un enjeu sociétal majeur. Au contraire des cultures céréalières qui nécessite d'importantes quantités d'intrants azotés, les légumineuses d'intérêt agricole sont en principe capables de prospérer sans addition de fertilisants azotés coûteux. Ceci implique toutefois que les plantes cultivées soient en mesure de fixer l'azote atmosphérique à l'aide de souches de rhizobium soit déjà présentes dans le sol soit inoculées. De ce fait, le pois chiche, le pois, le haricot, la fève et les lentilles sont cultivées

dans de nombreuses régions pauvres et représentent un des piliers de l'alimentation humaine. Pour plusieurs populations, les légumineuses représentent une source essentielle de protéines d'origine végétale pour l'alimentation humaine, mais sont aussi utilisées comme fourrage ou pour la production de bois. De plus, en contribuant à améliorer la structure et la qualité des sols au niveau des teneurs en azote et matière organique, les légumineuses permettent à moindre coûts d'assurer une productivité durable de l'agrosystème (Köpke et Nemecek 2010; Chianu et al. 2011). Les légumineuses sont aussi capables d'établir des symbioses racinaires avec les champignons mycorhiziens qui leur fournissent du phosphate. Ainsi la co-inoculation de rhizobium et de champignons mycorhiziens dans un sol pauvre en azote et en phosphore a permis d'améliorer le rendement de la plante hôte et/ou de la plante associée (en rotation ou en association) dans les systèmes de cultures céréalières (Wahbi et al. 2012). Ainsi, la fixation symbiotique de l'azote permet de réduire les apports d'azote sous la forme d'engrais chimiques tout en gardant un niveau de fertilité azoté suffisant pour garantir une productivité acceptable et avec des méthodes agricoles durables (Dwivedi et al. 2015). L'azote provenant de la fixation symbiotique étant directement incorporé dans la matière organique des plantes hôtes (Cellier et al. 2015), il n'y a pas ou peu de perte ce qui en fait une source d'azote stable, peu volatile et moins susceptible d'engendrer des effets négatifs dans l'environnement que les engrais d'origine synthétique. D'ailleurs, selon Jensen et al. (2012), la quantité de N2O émise par les cultures de légumineuses est trois fois inférieure à celles émises par les cultures céréalières. En outre, les légumineuses peuvent également participer à la phytoremédiation par le piégeage et la dégradation de contaminants du sol tels des pesticides, métaux lourds et des hydrocarbures (Afzal et al. 2014).

15. Atténuation du stress salin dans les plantes par les PGPR

Les plantes sont souvent confrontées à des conditions environnementales défavorables qu'on peut dénommer « stress » et qui ont pour conséquence une diminution de la croissance. Tous les stress impliquent des réactions de signalisations capables d'aboutir à la mise en place de défense ou de déclencher une mort cellulaire programmée. On distingue deux grandes catégories de stress : **Biotique** : imposé par d'autres organismes (insectes, herbivores…). **Abiotique** : provoqué par un défaut ou excès de l'environnement physico-chimique comme la sécheresse, les températures extrêmes, la salinité…

Les stress abiotiques ou environnementaux affectent la croissance et le rendement des plantes, contrairement aux animaux qui peuvent se déplacer lorsque les conditions de vie ne leur sont plus favorables. Les plantes ont développé des stratégies d'adaptation pour répondre aux chocs

chimiques ou physiques, engendrés par l'environnement en contrôlant et en ajustant leur système métabolique (Levitt, 1980 ; Zhu, 2002 ; Vincent, 2006). On peut considérer que la notion de stress implique, d'une part, une déviation plus ou moins brusque par rapport aux conditions normales de la plante ou de l'animal ; et d'autre part une réaction sensible de l'individu dans les différents aspects de sa physiologie, avec soit une adaptation à la nouvelle situation, soit à la limite dégradation menant à une issue fatale (Leclerc, 1999). Pour survivre, la plante doit échapper ou éviter le stress. Nous citerons le cas des géophytes et des hémicryptophytes en hiver, grâce à leurs parties souterraines ou très proches du sol, également les thérophytes printanières qui évitent de pousser pendant la saison froide et la saison chaude (Leclerc, 1999). Dans le cas du stress salin, la présence de fortes concentrations de sels dans le milieu crée une pression osmotique élevée dans l'environnement racinaire, réduisant la disponibilité de l'eau du sol pour la plante. A ce déficit hydrique s'associe un stress ionique dont l'ampleur dépend de la perméabilité des membranes végétales vis-à-vis des ions, et du niveau de toxicité de ces ions pour l'espèce végétale considérée (hamza, 1980). Le maintien des processus vitaux dans ces conditions de forte salinité passe donc par une résistance de la plante à la déshydratation, par une adaptation de son potentiel osmotique afin de rétablir les relations hydriques et d'assurer une alimentation en eau convenable, ainsi que par un contrôle efficace des flux ioniques intracellulaires (Chretien, 1992).

Différentes études ont suggéré que les rhizobactéries peuvent également développer un certain degré de tolérance chez les plantes donnant ainsi une résistance à ces dernières. La tolérance peut être définie comme la propriété intrinsèque du microbe de combattre des conditions stressantes, alors que la résistance est la capacité du microorganisme à résister à des conditions stressantes par certains mécanismes. Des données expérimentales indiquent que les microorganismes avec des capacités de tolérance/ résistance peuvent aider les plantes à s'adapter avec succès à différentes situations stressantes (Khan *et al.,* 2010; Milosevic *et al.,* 2012). Certains mécanismes par lesquels les PGPR améliorent les situations de stress sont présentés dans la section ci-dessous.

Synthèse des auxines et de composés similaires

Les auxines microbiennes peuvent affecter celles de la plante gouvernant un processus de développement tels que le développement des racines, y compris la longueur, la superficie et le nombre de pointes de ces racines. Ce développement racinaire permet en outre l'absorption des nutriments par les plantes, améliorant ainsi leur santé en présence de composés inhibiteurs dans des conditions de stress (Egamberdieva et Kucharova, 2009). La production d'acide indole-

acétique est un trait relativement commun de PGPR, et de telles bactéries sont censées d'atténuer le stress salin dans les plantes. Dans la même optique, Sadeghi *et al.,* (2012) ont démontré qu'un isolat de *Streptomyces* a augmenté la croissance de la plante du blé en produisant de l'acide indole acétique en présence de sel.

Sécrétion des Exopolysaccharides (EPS)

Les microorganismes appartenant à différents groupes fonctionnels, par exemple les rhizobactéries, sécrètent des exopolysaccharides (EPS), qui fournissent une résistance aux cellules contre différents facteurs de stress et protègent ainsi le microorganisme du stress. Les EPS améliorent également la structure du sol en formant des macroagrégats avec le sol, ce qui augmente encore la capacité de rétention d'eau du sol (Alami *et al.,* 2000). Les macroagrégats maintiennent l'équilibre dans les conditions aérobies et anaérobies dans le sol et déterminent également l'absorption progressive des nutriments du sol. En cas de stress salin, ces agrégats aident à lier des cations (Na^+) en les rendant inaccessibles aux plantes (Ashraf *et al.,* 2004). Les rhizobactéries ont la capacité de former des biofilms en sécrétant des polysaccharides et des protéines, la matrice ainsi formée limite la diffusion de composés tels que les hormonesde croissance végétale et les nutriments du voisinage de la plante, favorisant ainsi la croissance des plantes en atténuant les conditions de stress (Timmusk *et al.,* 2013).

Accumulation des osmolytes

L'une des réponses de stress les plus courantes dans les plantes est la surproduction de différents types de solutés organiques compatibles tels que la proline, la glycine bétaine et le glutamate (Serraj et Sinclair, 2002). L'accumulation de la proline est un indice physiologique sensible de la réponse des plantes au sel et aux autres stress pour maintenir un potentiel hydrique plus élevé pendant le stress (Peng *et al.,* 2008). Il a été démontré que les PGPR améliorent la tolérance au stress des plantes en contribuant à l'accumulation d'osmolyte dans les plantes. Zarea *et al.,* (2012) ont également signalé une augmentation de l'accumulation de proline dans le blé lors de la colonisation des racines avec les PGPR. La proline aide également la cellule végétale en stabilisant les structures subcellulaires telles que les membranes et les protéines, en éliminant les radicaux libres et en tamponnant le potentiel oxydatif cellulaire sous stress salin pour atténuer ce stress (Ashraf et Foolad, 2007; Kohler *et al.,* 2009). Selon Upadhyay *et al.,* (2012), une augmentation de la proline et du sucre soluble total dans les plants de blé traités par les PGPR ont contribué de façon significative à leur osmotolérance. De même, le métabolisme du tréhalose chez les espèces de *Rhizobium* semble également être important pour améliorer la croissance et l'adaptation au stress abiotique des légumineuses (Suarez *et al.,* 2008).

Modulation de l'enzyme 1-aminocyclopropane-1-carboxylate désaminase

Certains travaux ont démontré que chez les plantes cultivées dans les sols sous stress salin, les concentrations en 1-aminocyclopropane-1-carboxylate (ACC) augmentent, ce qui entraîne une forte concentration en éthylène et augmente les dommages causés aux plantes (Botella *et al.,* 2000). Les rhizobactéries ont été largement signalées pour leur hydrolysation de l'ACC (Saleem *et al.,* 2007) en ammoniac et en α-cétobutyrate en abaissant ainsi le taux d'éthylène dans les plantes stressées. En présence de bactéries productrices de l'ACC désaminase, l'ACC végétal est séquestré et dégradé par des cellules bactériennes pour fournir de l'azote et de l'énergie (Mayak *et al.,* 2004), facilitant la croissance des plantes sous le stress salin (Nadeem *et al.,* 2010). En outre, en éliminant l'ACC, la bactérie réduit l'effet délétère de l'éthylène, diminue le stress des plantes et favorise la croissance des plantes (Glick *et al.,* 2007).

L'enzyme 1-aminocyclopropane-1-carboxylate désaminase a été mis en évidence dans une large gamme de rhizobactéries et en particulier les genres : *Achromobacter, Acidovorax, Alcaligenes, Enterobacter, Klebsiella, Methylobacterium, Pseudomonas, Rhizobium et Variovorax* (Esquivel-Cote *et al.,* 2010).

Enzymes antioxydants

La formation des dérivés réactifs de l'oxygène suite à un stress salin dans les plantes entraîne des dommages aux lipides, aux protéines et aux acides nucléiques. La production des dérivés réactifs de l'oxygène est favorisée par la réduction excessive de la chaîne d'électrons photosynthétiques par la limitation de la photosynthèse sous salinité (Johnson *et al.,* 2003). Les activités des enzymes antioxydantes telles que la catalase (CAT), la peroxydase (POX) et la superoxydedismutase (SOD) augmentent dans les plantes sous stress salin et une corrélation existe entre ces niveaux enzymatiques et la tolérance au sel (Mittova et al., 2002 et 2003).

Des recherches sur l'application des PGPR chez les plantes ont montré une augmentation significative de plusieurs enzymes liées à la défense végétale comme la superoxyde dismutase, la peroxydase, la catalase, la polyphénol oxydase, la phénylalanine ammonia- lyase, la lipoxygénase et les composés phénoliques (Nautiyal et al., 2008; Chakraborty et al., 2013). Ces enzymes antioxydantes induites par les PGPR contribuent à la tolérance au stress salin dans les plantes aussi en éliminant le peroxyde d'hydrogène des racines ciblés par le sel (Kim et al., 2005).

Tolérance systémique induite

A l'inverse de la résistance systémique induite impliquée pour les facteurs biotiques, un autre terme de tolérance systémique induite (TSI) avait été proposé pour définir l'atténuation des

stress abiotiques par les PGPR. TSI est défini comme étant des changements physiques et chimiques provoquées par les PGPR en réponse aux stress abiotiques tels que le stress salin, le stress dû à la sécheresse, le stress thermique, le stress aux métaux lourds, ou d'une déficience nutritionnelle (Yang et al., 2009). Ces communautés microbiennes suivent différents mécanismes, tels que la production de : (i) molécules volatiles pour moduler l'homéostasie de Na+ sous stress salin (Farag et al., 2013), (ii) production de l'acide abscissique, provoquant la fermeture des stomates, ce qui permis d'empêcher la perte d'eau dans le stress hydrique, (iii) production des enzymes comme la superoxyde dismutase et la catalase, qui dégradent les dérivés réactives de l'oxygène pour atténuer les dommages cellulaires (Selvakumar et al., 2012). Certaines bactéries appartenant au groupe des PGPR ont été signalé au sujet de la TSI, il s'agit des genres : B. cereus, B. subtilis, Serratia sp. (Wang et al., 2012), Paenibacillus polymyxa (Timmusk et Wagner, 1999), Achromobacter piechaudii (Mayak et al., 2004).

Chapitre I

Les biofertilisants bactériens solubilisant le phosphate : Source et technologies vertes pour le développement agricole futur

Les biofertilisants bactériens solubilisant le phosphate : Source et technologies vertes pour le développement agricole futur

Synthèse

La nutrition minérale est essentielle pour maintenir la viabilité du sol en tant que ressource agricole et pour aider les cultures à atteindre leur plein potentiel. Le phosphore (P) est l'un des minéraux les plus importants qui ont un impact significatif sur le métabolisme, la croissance et la production des plantes. Le phosphore est essentiel à la conversion d'énergie pour les processus métaboliques, il est nécessaire en tant que composant de l'adénosine triphosphate (ATP) qui est impliqué dans l'organisation de plusieurs voies membranaires chez les plantes ainsi que dans les acides nucléiques et les phospholipides. Les plantes, par l'intermédiaire des transporteurs racinaires du sol, peuvent absorber directement des formes spécifiques de P, mais leur capacité à le faire est entravée par les faibles niveaux de P disponibles dans la rhizosphère. L'agriculture est limitée par la biodisponibilité du P et les agriculteurs sont actuellement confrontés à une diminution du rendement des cultures agricoles en raison de l'infertilité des sols et de l'inadéquation des cultures. La plante dépend donc de ses relations microbiennes rhizosphériques comme l'un des mécanismes conçus pour faciliter l'absorption du P. Les microorganismes colonisant la rhizosphère jouent un rôle crucial dans le contrôle de la croissance, la santé et les performances des plantes, en plus d'être essentiels au cycle du P. Il existe des taxons spécifiques de microorganismes du sol qui transforment les composés organiques et inorganiques en P disponible pour les plantes, tel que les bactéries solubilisant le P (PSB), un type de rhizobactérie favorisant la croissance des plantes (PGPR) par la libération de phytohormones, la fixation de N_2, la solubilisation du potassium et du zinc et la réduction du stress abiotique. En outre, les PSB ont aussi une activité 1-aminocyclopropane-1-carboxylate désaminase (ACCd) qui favorise la croissance des plantes et limite l'effet de divers stress.

Les bactéries solubilisant le P agissent comme biofertilisants et/ou biopesticides en colonisant les racines des plantes favorisant ainsi leur croissance. Les biofertilisants en tant que PSB sont bien impliquées dans la disponibilité de certains macro- et micronutriments, la décomposition de matière organique et la minéralisation de matière inorganique, ce qui facilite la mobilité des nutriments dans les sols et leur absorption par les plantes. L'utilisation des PSB en résulte d'une grande quantité de P dans le sol facilement accessible, ce qui encourage le développement des racines et produit un effet de nodulation favorable (pour les cultures de légumineuses) avec une activité plus élevée. L'inoculation de semences ou de sol avec des PSB seules ou en combinaison, est une stratégie prometteuse pour améliorer l'absorption de P par les plantes et,

par conséquent, réduire l'utilisation d'engrais chimiques polluants. Les biofertilisants tels que les PSB représentent une voie alternative rentable aux engrais chimiques et préviennent la pollution de l'atmosphère en tant que source d'énergie renouvelable, ce qui rend leur utilisation inévitable pour augmenter la productivité de la planète.

La promotion de la croissance causée par les PSB dans une variété de plantes est bien documentée. Différentes études menées dans des serres et des champs agricoles ont évalué la stimulation de la croissance des plantes et le rendement des cultures par les PSB, afin de réduire l'utilisation de produits agrochimiques ou la pollution causée par ces derniers. Cependant, en tant qu'agent de biocontrôle, solubilisent le P, tolèrent le sel et résistent aux métaux lourd, les PSB du genre *Pseudomonas* et *Bacillus* sont l'un des biofertilisants les plus efficaces. Par conséquent, les bio-engrais sont considérés comme un outil utile pour améliorer la croissance des cultures, l'absorption des nutriments et le rendement, en particulier dans des circonstances stressantes.

Les PSB colonisent avec succès la rhizosphère lorsqu'ils sont utilisés en consortiums, ce qui se traduit par une amélioration de la croissance des plantes. L'inoculation de consortiums microbiens entraîne la production d'un plus grand nombre de racines secondaires, qui stockent et transportent davantage de minéraux vers les différentes parties de la plante, expliquant ainsi une biomasse et une croissance plus élevées. Toutefois, en raison de leur interaction grâce au quorum sensing et la dynamique de leur population, la recherche sur le quorum sensing est cruciale pour comprendre les interactions biologiques entre les espèces bactériennes présentes dans la rhizosphère et pour voir comment elles affectent l'environnement. Les consortiums microbiens favorisant la croissance des plantes semblent être une option viable pour promouvoir la croissance tout en préservant la fertilité du sol et en garantissant leur survie.

En outre, les applications de la biotechnologie et de la nanotechnologie dans la création de bio- et nano-engrais représentent des méthodes modernes au profit de l'humanité. Pour faire face aux pénuries alimentaires, à la dégradation des terres agricoles, au changement climatique et à de nombreux autres défis auxquels nous sommes confrontés, les applications des nano- et bio-engrais devraient permettre d'augmenter la production agricole pour suivre l'augmentation rapide de la population humaine. La biotechnologie et les nano-engrais, en tant qu'approches biologiques respectueuses de l'environnement, jouent un rôle important et constituent des méthodes prometteuses pour accroître l'efficacité des diverses ressources tout en réduisant la pollution de l'environnement, ce qui en fait l'un des outils les plus importants de l’agriculture moderne. Les nano- et bio-engrais représentent une alternative aux engrais conventionnels en

raison de leurs potentiels à améliorer la croissance et la productivité des plantes, augmenter leur tolérance au stress environnementaux et renforcer leur résistance aux maladies. Ils améliorent également les propriétés du sol, ralentissent son érosion et augmentent sa fertilité.

Cette étude présente une vue d'ensemble de la composition et de la diversité fonctionnelle des PSB, les mécanismes de solubilisation/minéralisation du P, la manière dont les PSB induisent la croissance des plantes dans des conditions favorables et stressantes, et leur rôle potentiel en tant que bio-engrais P dans la production de cultures vivrières. Cette revue prend en compte la manière dont certains taxons microbiens spécifiques contribuent à l'acquisition de P par les plantes et la manière d'utiliser efficacement ces microorganismes pour augmenter la disponibilité et l'absorption de P. Dans ce contexte, nous avons tout d'abord examiné le rôle des taxons microbiens dans la rhizosphère. Nous avons collecté les informations récentes sur les forces qui façonnent la spéciation du P dans le sol et la dynamique de ses formes. Nous présentons ensuite un ensemble de données sur les interactions entre le P et la rhizosphère. Après, nous parlons en revue des recherches récentes sur la manière dont les PSB influencent la réponse des communautés microbiennes et végétales. Ultérieurement, en mettant l'accent sur les consortiums de PSB, une compréhension plus approfondie de la façon dont le P et les biofertilisants PSB interagissent est mentionnée comme un pas en avant dans la formulation d'un inoculum microbien efficace à grande échelle. Enfin, nous nous sommes concentrés sur les découvertes et la comparaison des nano- et bio-engrais. Les similitudes et les différences entre les bio- et nano-engrais, ainsi que les avancées dans les sciences des plantes et des sols sont abordées dans cette revue, afin de comprendre les interactions entre les bio- et nano-engrais issus de PSB.

Cette revue bibliographique peut nous aider à comprendre l'importance des PSB dans l'agriculture et à formuler ces microbes en tant que nano- ou biofertilisants pour la production de cultures à long terme. Toutefois, jusqu'à 99 % ou plus de la vie microbienne est encore incapable d'être cultivée en laboratoire, ce qui rend impossible son étude et sa compréhension de la manière dont les écologistes microbiens se sont habitués à le faire au cours du siècle dernier. Par conséquent, pour promouvoir le développement économique de l'agriculture durable, la recherche doit se concentrer davantage sur le repérage et le choix des meilleures cultures et souches de PSB capables de fixer l'azote, de solubiliser le phosphore et le potassium dans une variété de conditions agroclimatiques. De plus, pour créer de meilleurs consortiums avec une durée de conservation plus longue et de protéger les biofertilisants sous forme de nanoparticules après leur application sur le terrain, des recherches supplémentaires sont

nécessaires pour comprendre la capacité de survie des PSB, la dynamique des populations et les interactions dans les sols de la rhizosphère. Les cultures de biofertilisants devraient être échangées entre les pays ayant des conditions climatiques similaires, d'être évaluées pour leur performance afin de trouver des meilleures souches pour une culture particulière. Il en va de même pour la création de formulations de substitution appropriées, telles que des inoculant liquides ou des formulations granulaires pour tous les bioinoculant, ainsi que pour la normalisation du milieu et de la méthode d'inoculation pour les nouvelles formulations. Il est donc impératif de créer des processus sûrs, efficaces, fiables, propres, non toxiques et respectueux de l'environnement pour la production de nanoparticules et d'inoculant microbiens. Les recherches futures devront également s'attacher à comprendre comment les bio- et nano-engrais dérivés de bactéries interagissent avec les nanoparticules, qui sont également des micronutriments bénéfiques pour la vie microbienne et les plantes.

Phosphate Solubilizing Bacteria Biofertilizers: Source and Green Technologies for Future Agricultural Development

This part of the work was the subject of an article submitted for publication.

Abstract

Farmers are currently facing a decrease in agricultural crop yield because of infertile soils and inadequate cultivation. Agriculture is frequently restricted by the bioavailability of phosphorus (P), a significant macronutrient. The plant may depend on its rhizospheric microbial partners as one of the mechanisms designed to make P absorption easier. The P-solubilizing bacteria (PSB) promote plant growth through several different mechanisms and act as biofertilizers or biopesticides by colonizing plant roots and promoting plant growth in the rhizosphere, which suggested the use of PSBs inoculants to increase soil P fertility in agriculture land. Inoculating seed/seedling/soil with PSBs, either alone or in combination is thus a promising strategy for improving P absorption by plants and, as a result, reducing the use of toxic chemical P fertilizers. Biofertilizer such as PSBs, represent a cost-effective alternative to chemical fertilizer and prevents pollution of the atmosphere as a renewable energy source, which make their use unavoidable to increase the earth's food productivity. Furthermore, biology and nano-fertilizers as environmentally friendly biological approaches play a significant role and promising methods to increase the efficiency of various water and land resources while reducing environmental pollution, making them one of the most important tools in modern agricultural technology, which could provide alternatives to nano chemical fertilizers. For these reasons, this review will concentrate on discoveries and a comparison of nano- and bio-fertilizers. We intend to present a current view on the composition and functional diversity of PSB, mechanisms of P solubilization/mineralization, how PSB induce plant growth under conventional and stressful conditions, and their potential role as P-biofertilizer in food crop production. This review's findings can help us to understand the importance of PSBs in agriculture and to formulate these microbes as nano- or biofertilizers for long-term crop production.

Keywords: Crop Productivity; Inoculant Nano-Technology; Phosphorus; PSB-biofertilizers; Soil Fertility; Sustainable Agriculture.

1. Introduction

Phosphorus (P) is one of the most important minerals that have a significant impact on the growth and metabolism of plants. P is essential for the storage and transfer of energy for metabolic processes, which affects growth and biological production (H. Malhotra et al., 2018). It is necessary as a component of adenosine triphosphate (ATP), which is involved in organizing several metabolic pathways in plants, nucleic acids, and phospholipids (Masrahi et al., 2023). Plants through root transporters from the soil can directly absorb specific P forms, but the ability to do so is hampered by low levels of available P in the rhizosphere (Ducousso-Détrez et al., 2022). Rhizosphere-colonizing microorganisms play crucial roles in regulating plant growth, health, and performance in addition to being essential to soil P cycling. Through soil P hydrolysis, specific taxa in the soil microbiome can transform organic compounds into P that are available to plants (Zheng et al., 2022). The agricultural and rangeland soils have the highest populations of the P-solubilizing bacteria (PSB), a plant growth-promoting rhizobacteria (PGPR) type that promotes plant growth by saturating insoluble soil P. Most frequently, PSBs include *Pseudomonas fluorescens* and *Bacillus subtilis*, which promote plant growth through the release of phytohormones, N_2 fixation, P and Zn solubilization, reduction of salinity stress, and other processes. Additionally, it has been suggested that PSBs with ACC deaminase activity (1-aminocyclopropane-1-carboxylate) promote plant growth by counteracting negative effects (Zhao et al., 2021).

Mineral nutrition is essential for maintaining the soil's viability as an agricultural resource in addition to assisting crops in reaching their full potential (Harika et al., 2023). Biofertilizers like PSBs create these plant development ingredients in the soil. The PSBs inoculation is described as a producer of hormones and antioxidants, as well as being significant in N_2-fixation, the validity of some macro- and micronutrients, decomposition of organic and inorganic materials, and mineralization and conversion from organic materials to inorganic, which facilitates its movement in the soils and absorption by plants (Kalayu, 2019; Harahap et al., 2022). After PSBs inoculation, more P is easily accessible in the soil, which encourages root development and produces a favourable nodulation effect (for legume crops) with higher activity, and boosts P utilization productivity (Gamalero et al., 2020). In the scientific literature, the growth promotion caused by PSBs in a variety of plants is well documented. The potential of *Pantoea dispersa* MF351847 as a biocontrol agent against *Xanthomonas albilineans* and *Fusarium udum* on sugarcane and pigeon pea, in addition to P solubilization, salt tolerance, and arsenic resistance was reported (Jiang et al., 2019). The same goes for Endophytic *K.*

pneumoniae with biocontrol abilities (A. Yadav & Yadav, 2022a). Likewise, rhizobium fixes atmospheric nitrogen with the aid of legumes, making it one of the various bio-fertilizers of utmost significance. The Klebsiella genus is also recognized for its capacity to dissolve P (Musa & Ikhajiagbe, 2020). As a result, bio-fertilizers are thought to be a useful tool for enhancing crop growth, nutrient uptake, and yield, especially under stressful circumstances.

On the other hand, because of the various environmental factors, PSBs frequently do not survive. However, the PSBs successfully colonize the rhizosphere when used in consortia, which is evident in the form of improved growth (Bargaz et al., 2017). The inoculation of microbial consortia resulted in the production of more secondary roots, which store and transport more minerals to the various parts of the plant, resulting in higher biomass and growth. However, to understand the biological interactions among the bacterial species found in the rhizosphere, and to see how they affect the environment, quorum sensing research is crucial (Kumar, 2023). Because of their quorum sensing interaction and population dynamics, plant growth-promoting microbial consortia appear to be a viable option for promoting plant growth while preserving soil fertility and guaranteeing their survivability (Meryem et al., 2022). In addition, applications of biotechnology and nanotechnology in the creation of bio- and nano-fertilizers represent contemporary methods that can be created soon to humanity's benefit. To deal with food shortages, the degradation of agricultural land, climate change, and many other challenges that we face, nano- and bio-fertilizer applications should increase agricultural production to keep up with the rapid increase in the human population (Sahu et al., 2022). The nano- and bio-fertilizers are an alternative to conventional fertilizers because they improve plant growth and productivity, increase plant tolerance to environmental stress, and increase plant disease resistance. They also enhance soil properties, slow down soil erosion, and increase soil fertility (A. Yadav & Yadav, 2022b).

This review has taken into account how specific microbial taxa contribute to plant P acquisition in the rhizosphere and how to manipulate effectively these microorganisms to increase P availability and uptake. In this context, we first go over recent information on the forces that shape soil P speciation and the dynamics of P forms. Second, we present a growing body of recent research looking into how PSB affects the plant-microbial community's response. Then, with a focus on PSBs consortia, a deeper understanding of how P and the PSB biofertilizers interact is mentioned as a step forward in formulating a widely effective microbial-based inoculum. Finally, we solicit how understanding the interactions between bio- and nano-fertilizers from bacteria and nanoparticles is currently helping humanity in the agricultural

sector, with comprehension of how bacteria-derived bio- and nano-fertilizers interact with nanoparticles.

2. The importance of P and how microorganisms affect agricultural systems

2.1. Current status of P in soil

Phosphorus (P) is one of the six elements required by all organisms on Earth; a large amount of P is necessary to sustain Earth's life (J. Li et al., 2021). On a geological timescale, the primary supply of P to the biota is the P-rock weathering (Schipanski & Bennett, 2021). However, the world reserves of rock P (RP) (the only significant global resource of P) are declining consistently due to its unregulated use (Yu et al., 2022), resulting in a massive increase in P fertilizers cost. Furthermore, as P is a finite resource and based on its current rate of use, it has been estimated that the world's known reserves of high-quality RP may be depleted within the current century (Yu et al., 2022). Likewise, the P mines could be depleted by 2060, while the amount of P in agricultural soils is sufficient to sustain maximum crop yields worldwide for only about 100 years (J. Zhu et al., 2018). Under such conditions, researchers investigate new techniques for indigenous low-grade RP utilization by converting it into a potential P resource for direct application to the soil. On the other hand, P is second only to nitrogen (N) among mineral nutrients most commonly limiting growth crops (Elhaissoufi et al., 2021) and makes up about 0.2% of a plant’s dry weight (Maharajan et al., 2018). It is one of the less-abundant elements in the lithosphere (0.1% of the total) (Campos et al., 2018) 95-99% of its presence in the soil is insoluble and cannot be utilized by plants (Nacoon et al., 2020). Globally, it is estimated that 5.7 billion hectares of the world's land do not have enough available P to support crop production (Solangi et al., 2023). In addition, the concentrations of available P in the soil solution are typically low (0.05–0.30 μg P mL^{-1}) (J. Zhu et al., 2018) due to the comparatively low content of P in the parent material, but also due to the high reactivity of P inorganic that results in strong retention by the soil’s mineral matrix. Many soils have high reserves of total P, accounting for approximately 0.05% (w/w) of soil dry weight, but only a small proportion (1- 5%) of total soil P is available in a soluble form used by plants (H. Wang et al., 2015) and only 0.1% of this P is available for plant use (Goswami et al., 2019). In addition, only 1% of the total soil P (400–4000 $kg.ha^{-1}$ in the top 30 cm) is incorporated into living plant biomass during each growing season (10–30 $kg.ha^{-1}$), reflecting the P biogeochemistry characteristic and its low availability for plant uptake (Quiquampoix & Mousain, 2005). These are rather low compared with the adequate P concentrations for optimal plant growth, which can reach values of several mM or ten μM for the most demanding crop species (Lambers, 2022).

2.2. Diversity of P forms, P-dynamic in soils, and its challenges

Phosphorus is abundant in soils in both inorganic and organic forms, inorganic P (Pi) occurs in soil mostly in insoluble mineral complexes, some of them appearing after frequent application of chemical fertilizers. The inorganic pool usually accounts for 35% to 70% of total P in soil (Ducousso-Détrez et al., 2022), mainly depending on soil age. The apatite is one of the most common forms of Pi, forming about 95% of the Earth's crust's P. Indeed, primary apatite minerals exhibit a hexagonal crystal structure with long open channels, in its pure form, F^-, OH^- or Cl^- occupies sites along these channels to form fluorapatite, hydroxyapatite, or chlorapatite, respectively (Ducousso-Détrez et al., 2022). Ultimately, P occurs naturally in more than 300 different minerals varying widely in their chemistry and structure (Paytan & McLaughlin, 2007). It is reported that Pi can also be present in trace on the surface of particles such as clays, calcium carbonate, and ferric oxyhydroxides, or in the crystal lattices of other minerals. Generally, P is not observed as a free elemental form due to its high reactivity to combine rapidly with other elements. For instance, in acidic soils, P is associated with aluminium and iron compounds, whereas calcium P is the predominant form of Pi in calcareous soils (Johan et al., 2021). Notably, P has many degrees of oxidation (from -3 to +5), in natural systems, it is almost exclusively present in the (+5) oxidation as H_3PO_4 which is a very polar molecule makes it highly soluble in water and the dissociation successively produces dihydrogen P anions ($H_2PO_4^-$), hydrogen P anions (HPO_4^{2-}) and the tetrahedral oxy-anion orthophosphate (PO_4^{3-}) (Ducousso-Détrez et al., 2022). On the other hand, organic matter is also an important reservoir of immobilized P that accounts for 20–80% of P in soils (Goswami et al., 2019). Organic P (Po) forms are characterized by the presence of carbon-hydrogen bonds and enter the soil during the decay of natural vegetation, dead animals, and excretions (Anand et al., 2016). According to (J. Zhu et al., 2018) Po may also make up a large fraction of soluble P, as much as 50% in soils with high organic matter content. In general, Po consists of a large variety of compounds which are classified into four groups: monoester P, diesters P, phosphonates, and organic polyphosphate (Huang et al., 2017). The monoesters P are the predominant form of Po in soils under aerobic conditions, which occur mainly as inositol P (Huang et al., 2017). In addition, soil P content results also from immobilized P in biomass, such as the microbial biomass can contain up to 26% of the total soil P (Raymond et al., 2021) depending on seasonal conditions and the turnover time. The release of P from the microbial biomass can be significant; it, therefore, represents an important potential pool of bioavailable P (Spohn et al., 2018).

Additionally, physicochemical and biological processes characterize soil P dynamics; simply exposing it to air will stimulate a chemical reaction with oxygen. Although, to date, Po has received less attention in most research than the biological manipulation of Pi availability in soil. According to a recent review by Jindo et al. (2023), the major processes of the soil P cycle that affect soil P concentrations are (1) dissolution–precipitation (mineral equilibria), (2) sorption–desorption (interactions between P in solution and soil solid surfaces), and (3) mineralization–immobilization. In most cases, the poor mobility and low levels of soil Pi solution are due to the large reactivity of P ions relative to numerous soil constituents. Therefore, only a marginal proportion of soil P is present as P ions in the soil solution due to the peculiar mineralogy and ambient geochemistry of soils that favour strong retention of P ions (Johan et al., 2021). These include the tropical and subtropical soils that are predominantly acidic, rich in iron, and often extremely P-deficient with high P sorption capacities of Fe and Al, resulting a complex P speciation (Pereira et al., 2019), also the Mediterranean basin regions that are largely dominated by calcareous and alkaline soils (Wahba et al., 2019). However, because of their quick fixation rates (reactions that remove available P from the soil solution into the soil solid phase); the majority of P forms continue to be inaccessible to plants. There are two types of reactions; P sorption on the surface of soil minerals and P precipitation in the soil solution (Johan et al., 2021). However, soils that exhibit the highest P fixation capacity occupy 1,018 million hectares in the tropics (S. B. Sharma, 2017). It is for this reason that available P levels have to be supplemented on most agricultural soils by adding chemical P fertilizers, which not only represent a major cost of agricultural production but also impose adverse environmental impacts on overall soil degradation, microbial health, freshwater, and marine resources. Moreover, the efficiency of applied P fertilizers in a chemical form rarely exceeds 30% due to their rapid fixation, either in iron/aluminium P forms in acidic soils or in calcium P forms in neutral to alkaline soils (Devi et al., 2022).

2.3. Importance of P in agro-systems and plant responses

In most soil conditions, P is by far the least mobile and available to plants when compared to the other major nutrients. It is one of the necessary elements for plant nutrition, development, and growth. The main characteristics associated with adequate P nutrition in all major metabolic processes in the plant include; photosynthesis, energy transfer, and storage; which is used for growth and reproduction, as well as membrane formation, legume N_2 fixation, crop quality, and resistance to plant diseases (Janati et al., 2021). At cellular levels, P participates in cell division, growth of new tissues and nucleic acid structure, protein synthesis regulation, respiration, signal

transduction, macromolecular biosynthesis, phospholipids, carbon metabolism, and a wide range of enzymes (Khan et al., 2022). Furthermore, P forms a part of the genetic memory "DNA" of all living organisms (Ingle & Padole, 2017). Also, it plays an important role in root elongation and proliferation, it easily translocate within the plants, moving from older to younger tissues as the plant forms cells and develops roots, stems, and leaves (Anand et al., 2016). Phosphorus, a light-bearing, non-metallic plant nutrient, is absorbed from the soil solution as monovalent ($H_2PO_4^-$) and divalent (HPO_4^{2-}) orthophosphate anions in proportions relative to the soil pH. For instance, when the soil pH is less than 7.0, $H_2PO_4^-$ is the dominant form in the soil, facilitating root morphogenesis such as development, root anatomy modifications, and root hair density, resulting in increased crop yield and plant resistance to multiple diseases (Khan et al., 2022). On the other hand, plants P-deficient exhibit retarded growth with reduced cell and leaf expansion, respiration, and photosynthesis, often characterized by symptoms such as reddish leaves (enhanced anthocyanin formation), and necrosis in old leaf tips and dark green colour (higher chlorophyll concentration) (J. L. M. Young et al., 2018). Furthermore, phytate accumulates grains as a large portion of the P absorbed by the plant, and its deficiency harms root architecture, seed development, grain yield, and normal crop maturity (Anand et al., 2016; A. Kumar, Singh, Raigond, et al., 2021). Therefore, to counterbalance the low P availability and to allow plants to grow and function normally, P-fertilizers are frequently used on a large scale to maintain soil fertility in crop cultivation practices. Among synthetic fertilizers, approximately 52.3 billion tons of P-based fertilizers are applied annually worldwide to maintain available P levels in soil-plant systems (FAO, 2017). Of these, only 0.2% of applied P is used by plants (Khan et al., 2022), and the remaining is lost due to complex formation in the soils, needing frequent P enrichment. However, excessive use of Pi-fertilizers can cause environmental issues such as groundwater contamination and waterway eutrophication (Alori et al., 2017). Since the indiscriminate and excessive application of chemical fertilizers has led to health and environmental hazards, agronomists are desperate to find alternative strategies that can ensure competitive yields while protecting soil health.

2.4. Mechanisms of P solubilization and mineralization through soil microorganisms

Soil microorganisms with different mechanisms have great potential in regulating soil-fixed P transformation and increasing P availability to plants. Convert insoluble organic and inorganic P to plants accessible forms, like orthophosphate, is an important trait for PGPR, such as *Bacillus*, *Burkholderia*, *Pseudomonas*, and *Rhizobium* genera (M. Verma et al., 2019). There

are Pi-solubilizing and Po-mineralizing prokaryotes, through either exudation of organic acids or the synthesis of functional enzymes to solubilize Pi or mineralize Po by the production of acid phosphatases (Alori et al., 2017) (Figure 1). It is known that the production of organic acids by P-solubilizing bacteria (PSB) is the main mechanism for solubilizing Pi and the amount of soluble P released depends on the potency and type of organic acid produced (Bhowmik & Das, 2018). These organic acids are derived from microbial metabolisms mainly by oxidative respiration or by fermentation of sources such as glucose (Goswami et al., 2019). The production of organic acids lowers the pH and helps convert non-assimilable divalent and trivalent forms of P into assimilable monovalent forms, hence, P ions are released by substitution of H^+ for Ca^{2+} (Kalayu, 2019). Janati et al. (2022) and co-workers indicate that solubilization of TCP and RP led to acidification of the medium which could be due to organic acids production and chelating substances. Furthermore, it should be noted that organic acids act as chelating agents through their hydroxyl (-OH) and carboxyl (-COOH) groups which dissociate cations bound to P (Al^+, Fe^{2+}, Ca^{2+}), thus releasing assimilable forms of P (Kalayu, 2019). Gluconic acid is considered to be the most common agent for the solubilization of Pi and is the main organic acid produced by PSBs including *Erwinia herbicola*, *Pseudomonas cepacia*, and *Burkholderia cepacia* (Behera et al., 2017; Satyaprakash et al., 2017), in addition to the 2-keto gluconic acid which is made by PGPR strains (Silva et al., 2021). Likewise, Abdelgalil et al. (2022) reported that *Bacillus liqueniformis* and *Bacillus amyloliquefaciens* strains produce mixtures of lactic acids and acetic acids.

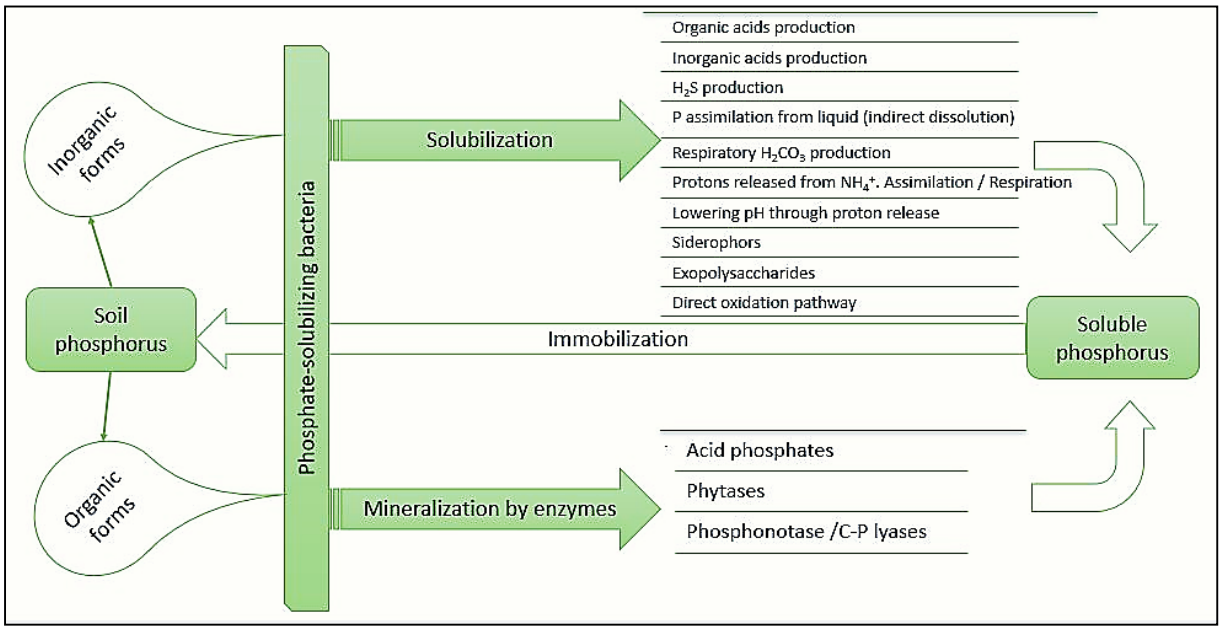


Figure 1. The mechanism of soil P solubilization/mineralization and immobilization by PSBs.

Other organic acids have also been identified, such as oxalic, malic, malonic, fumaric, Isovaléric, propionic, Isobutyric, and fulvic acids (Table 1) (Bhowmik & Das, 2018). Likewise, some bacterial species can produce inorganic acids such as sulphuric, nitric, and carbonic acid which lower the pH, but their efficiency to release assimilable P in soils appears to be low and inefficient compared to organic acids (Anand et al., 2016). On the other hand, P can be released from soil organic compounds by three groups of enzymes (1) phosphatases, which affect the phosphoanhydride bonds. (2) Phytases, which specifically cause the release of P from phytic acid. (3) Phosphonate and C-P lyases, which affect the cleavage of C-P bonds in organophosphonates (Behera et al., 2014). According to Behera et al. (2014), many PSBs such as *Emericella rugulosa, Serratia marcenscens, Chaetomium globosa, P. fluorescens, Proteus mirabilis, Burkholderia cepacia, Enterobacter aerogenes,* and *Freund Citrobacter freundi* have developed phosphatase enzyme that releases Pi from the organophosphorus complex. These enzymes are classified as alkaline (pH > 7) or acidic (pH < 6) phosphatases depending on their optimal pH activity. It catalyzes the P hydrolysis of many macromolecules (primary, secondary, and cyclic alcohols, phenols, and amines). As well, PSBs phytases make possible the release of assimilable P from phytates, the main organic forms of P in the soil (Billah et al., 2019).

Table 1. Overview of P solubilization mechanisms using organic acids.

Organic acids	Bacteria producers	References
Gluconic	*O. pseudogrignonense, P. fluorescens, B. cereus, Arrhrobacter, B. megaterium, B. subtilis*	(Anand et al., 2016; Chawngthu et al., 2020; Silva et al., 2021)
2-keto gluconic	*K. variicola, P. ananatis, Pseudomonas sp.*	(Silva et al., 2021)
Acetic and Citric	*Enterobacter sp.* *Pseudomonas sp., Serratia quinivorans*	(Rfaki et al., 2020)
Malic	*Enterobacter sps, Arrhrobacter*	(Anand et al., 2016)
Propionic	*Bacillus licheniformis*	(Rawat et al., 2022)
Lactic, Isovaléric, and Isobutyric	*Bacillus amyloliquefaciens* *Bacillus liqueniformis,*	(Behera et al., 2014)

However, the degradability of organophosphorus compounds (mineralization) depends mainly on the physicochemical and biochemical potential of the soil. For example, nucleic acids, phospholipids, and sugar P are easily broken down, however, phytic acid, polyphosphates, and phosphonates decompose more slowly (Dipta et al., 2019). Despite the importance of microbial-mediated P cycling to terrestrial ecosystem functioning and services, little has been known about the genetic and metabolic potential of microorganisms involved in P-solubilization. Among the microbial enzymes involved in the soil phosphorus cycle, the pyrroloquinoline quinone (PQQ) and Glucose dehydrogenase (GDH) holoenzyme are the major determinant of RP and hydroxyapatite solubilization by gluconic acid production (Shrivastava et al., 2018). Likewise, soil microbes containing appA, phoA, and phoC genes, coding for alkaline phosphatase have high capacities to solubilize Pi and mineralize Po compounds (Dai et al., 2020). Similarly, exopolyphosphatase (PPX), encoded by the ppx gene, was shown to play an important role in the degradation of inorganic polyphosphate (H. Song et al., 2020). The genes encoding the high-affinity; P-specific transporter (pst), and low-affinity; Pi transporter (pit) are essential to facilitate microbial P uptake under phosphorus-depleted or phosphorus-rich conditions (Oliverio et al., 2020). Recently, the ecological network method has been increasingly used to explore possible associations between soil microbial taxa in various environments, which could provide new information on the interactions between important soil microbial groups involved in P-cycling.

3. Comprehensive research to uncover the P-solubilizing bacteria's secret

3.1. P-solubilizing bacteria benefice

Plant growth-promoting rhizobacteria are a group of bacteria that enhance plant growth by varied mechanisms. These bacteria colonize plant roots and deliver plant growth-promoting effects in the rhizosphere. Beneficial microbes improve soil chemical and biological characteristics by fixing nitrogen (N), cellulolytic activity, or nutrient availability (Figure 2). A complex network of signalling between plants and microbes in the rhizosphere reveals extensive links between the environment, microbes and crop nutrition. They are applied to seeds, plant surfaces, roots, or soil; they inhabit the rhizosphere, and through their biological activity, promote plant growth and increase the soil microflora. The main group of PGPRs are PSBs with their potential to solubilize insoluble soil P, such as tricalcium phosphate ($Ca_3(PO_4)_2$), aluminium phosphate ($AlPO_4$) and iron phosphate ($FePO_4$), by various mechanisms, such as acidification, chelation, exchange reactions and the production of organic acids, which leads to the promotion of plant growth (Masdariah et al., 2019). Generally, PSBs

can synthesize low-molecular-weight organic acids, such as gluconic and citric acids that have hydroxyl and carboxyl groups to chelate the cations bound to the P, thereby converting insoluble Pi (Chawngthu et al., 2020). In addition, these species synthesize different phosphatases (phosphatase or phytase) to catalyze the hydrolysis of P and promote the mineralization of released nutrients (Wan et al., 2020). It has been estimated that under favourable conditions the application of PSBs can reduce the application of P fertilizer by 50% (Manzoor et al., 2017). In addition to P solubilization, PSBs can produce plant growth hormones, promote root expansion, increase root hairs and increase the area of soil P absorption by roots (S. K. Verma et al., 2018). Furthermore, PSB inoculation could increase the germination percentage of seeds by secreting IAA. Song et al. (2022) demonstrate that PSBs from the rhizosphere soil of intercropped soybean not only can secrete organic acids and phosphatase acid to dissolve insoluble P but also can produce IAA and siderophores that promote maize growth. In addition, bioavailable P positively affect the symbiosis of legumes and rhizobia making P solubilization one of the main mechanisms by which PSBs enhance N_2 fixation (Janati, Benmrid, Elhaissoufi, Zeroual, et al., 2021).

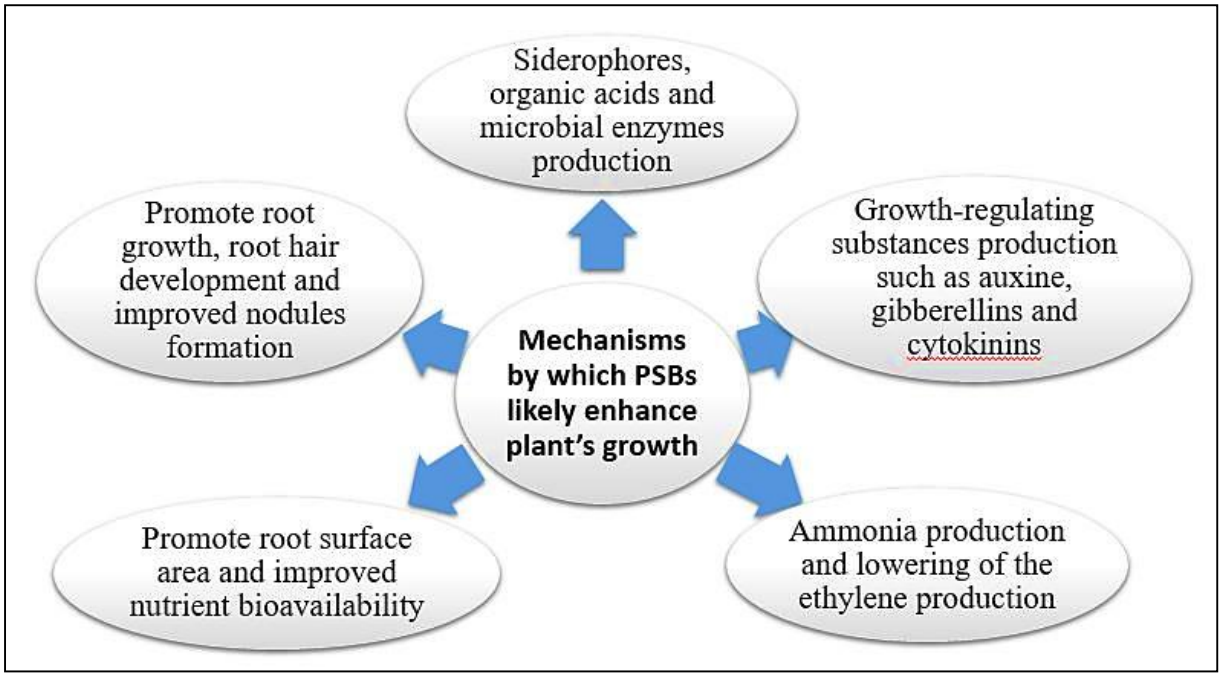


Figure 2. The possible mechanisms by which PSBs promote plant growth.

Several studies have reported that the application of PSB, to the soil, individually or in the consortia, increased plant growth by solubilizing insoluble P and subsequent increase of N_2 fixation (Timofeeva et al., 2022). For example, individually or in combination, *B. subtilis* AR5 and *B. thuringiensis* BR1 reduced the effect of salt stress and improved plant height, shoot dry weights, proline contents, enzyme activities, and mineral nutrient accumulation in shoot plants

including N (Mahgoub et al., 2021). Similarly, co-inoculation of wheat with a diazotrophic *Paenibacillus beijingensis* BJ-18 and P solubilizing *Paeni Bacillus sp*. B1 significantly increased plant growth and the content of P and N in the plant (roots and shoots) and the soil (Y. Li et al., 2020). Furthermore, the inoculation of chickpeas with N-fixing *Mesorhizobium ciceri*, P-solubilizing fungus *Penicillium* WF6, and/or PSB *Serratia* T1 resulted in increased availability and uptake of P and positively affected N_2 fixation (A. Zaidi & Khan, 2007). Legumes treated with biofertilizers containing consortia of three PSBs: *Pseudomonas sp*., *Burkholderia sp*., and *Enterobacter sp*. in combination with RP had increased P content both in the soil and plants, providing favourable conditions for nodulation, which in turn increased the efficiency of the N fixation (Bargaz et al., 2018). Applying P-solubilizing microorganisms as biofertilizers is one option to increase food production without posing a health risk while saving natural sources of P fertilizers and developing sustainable agriculture (Table 2). On the other hand, the co-inoculants should contain two or more ecologically distinct bacteria for coping with the negative effects of ecological diversity in the rhizosphere (Bargaz et al., 2017). In this way, beneficial bacteria can be expressed more continually, independent of ecological differences. The combination of diazotrophs with hydrolytic or producers of growth stimulators and phytohormones with P mobilizing bacteria can be used as an example of artificial microbial associations (A. Yadav & Yadav, 2022b).

Table 2. P-solubilizing bacteria additionally perform other functions.

PSBs strains	Other functions	References
Rhizobium, Bradyrhizobium, Mesorhizobium, Sinorhizobium, Azotobacter, Azospirillum, Agrobacterium, Bacillus, Burkholderia, Enterobacter, Erwinia, and *Pseudomonas*	Biological N fixation	(Forni et al., 2017; Janati, Benmrid, Elhaissoufi, Zeroual, et al., 2021)
Acidithiobacillus ferooxidans, Arthrobacter sp., Azotobacter sp., Bacillus sp., Klebsiella sp., Paenibacillus sp., Pseudomonas sp., and *Rhizobacterium sp.*	Mineral utilizing bacteria such as potassium and zinc	(Liu et al., 2018; Tripathi et al., 2022)
Pseudomonas, Bacillus, Serratia, Flavomonas, Curtobacterium, and *Trichoderma*	Antagonistic activities against plant pathogens	(Sahu, et al., 2022)
Pseudomonas spp., Bacillus spp., Mesorhizobium spp., and *Burkholderia spp*	Secretion of plant hormones and resistance to abiotic stressors	(Mahanty et al., 2017; Laranjeira et al., 2021; Alemneh et al., 2022)

To date, it was reported that the major taxonomic groups of PGPR belong to Proteobacteria and Firmicutes, in which, *Bacillus sp*. is the most known genus of Firmicutes (Ferreira et al., 2019). However, strains ascribed to the genera Rhizobium, Azospirillum, Burkholderia, Achromobacter, Pseudomonas, Acinetobacter, Serratia, Pantoea, Enterobacter, and Rahnella are the most representative of the phylum Proteobacteria (Table 2) (Batista et al., 2018). Although, various species of Bacillus and Pseudomonas have been isolated from the soil rhizosphere of different crops, which has shown the most popular inoculants with P-solubilizing attributes (Amy et al., 2022). In addition, it is reported that P solubilizers including Bacillus, Escherichia, Arthrobacter and Pseudomonas have been reported to nearly add about 30–35 kg P2O5/ha (Saha et al., 2021). Other bacteria identified include *Alcaligenes sp., Aerobacter aerogenes, Actinomadura oligospora, Agrobacterium sp., Bradyrhizobium sp., Brevibacterium sp., Citrobacter sp., Corynebacterium sp., Escherichia freundii, Erwinia sp., Flavobacterium sp., Micrococus sp., Mycobactreium sp., Rhodococcus sp*., and *Sarcina sp* (Mishra et al., 2014). Additionally, a large number of PSBs colonize plant roots with a different degree of intimacy, ranging from bacteria living in the rhizoplane to endophytic bacteria, and with different effects on plant growth and health. Among them, symbiotic or free-living bacteria in the soil can efficiently colonize the root and induce a beneficial impact on the host plant (Gamalero et al., 2020); *Flavobacterium sp., Nitrosomonas sp., Erwinia sp., Micrococcus sp., Escherichia intermedia, Enterobacter asburiae, Nitrobacter sp., Thiobacillus ferroxidans, T. thioxidans,* and *Xanthomonas sp* (González-López et al., 2005) . Furthermore, among the most studied and diverse bacteria that have been proven to be among the best P-solubilizers were found *Azospirillum brasilense, Bacillus circulans, B.cereus, B.fusiformis, B. pumilus, B. megaterium, B. mycoides, B. polymyxa, B. coagulans, B.chitinolyticus, B. subtilis, Burkholderia sp., Pseudomonas putida, P. striata, P. fluorescens, P. calcis, P. corrugate, Rhizobium meliloti,* and *Serratia phosphoticum* (Bhattacharyya & Jha, 2012). These microbes are predicted to be beneficial for the increase in P bioavailability through agricultural practices. More studies are required on co-inoculant PGPRs or a mixture of strains for ensuring their successful root colonization. Identifying PSBs and utilizing them for the release of recalcitrant P that is bound to rocks or minerals have implications for improving crop nutrient acquisition and crop productivity.

3.2. Quorum sensing and symbiosis influence on the PSBs occurrence and diversity

Microbial interaction is an important factor that affects the bioinoculant population in the soil. A lot of work has been done to study the diversity of bacteria in rhizospheric soil and their role

in plant improvement, but only a few pieces of literature are available for microorganism interactions especially quorum sensing (QS) capabilities of these bacteria. Rhizobacteria are found to form mutual interactions between plants and microbes and also to produce signal molecules and contain a QS system through which gene expression is regulated (Chamkhi et al., 2020). As the rhizosphere and plant roots are the potential source of QS molecules for microbes, root colonization is regulated by the network of the QS system, which depends on small molecules known as autoinducers produced and recognized by the network of rhizobacterial biofilm (Altaf et al., 2017). Based on the chemical structure and action mechanism of signal molecules, they belong to one of three classes: the first one is acyl homoserine lactone (AHL), commonly used by Gram-negative bacteria. The second class is identified as oligopeptides or their derivatives majorly used by Gram-positive bacteria, while the third signal molecule known as autoinducer-2 (AI-2) is used by both Gram-negative and Gram-positive bacteria (P. Kumar, 2023). The QS in a root-associated biofilm regulates and controls other traits of plant growth regulation and ultimately defines the mineral acquisition of plants from the active rhizosphere (Meryem et al., 2022). In addition to biofilm formation, QS regulation plays vital roles in many other processes, including the interaction of bacteria with higher organisms, regulation of virulence, conjugation, expression and regulation of genes related to the production of toxins, enzymes, antibiotics and other secondary metabolites (Ghosh et al., 2019). Sionov & Steinberg, (2022) mentioned that QS is sometimes linked to antibiotics resistance activity via efflux pump, biofilm and secretion system. Several ecological and interdependent key characters of bacteria, such as antibiotic, siderophore, or enzyme secretion, virulence factors of phytopathogens, as well as plant-microbe communications (which represent a major trait of PSBs) are coordinated through QS (Ditta et al., 2022). In addition, as noted by many authors, the QS allows bacteria to regulate their physiological activities by sensing the bacterial communities and the population density. The QS regulate the bacterial population and the behaviour of microbial cells in a particular environment (Rattray & Brown, 2023). According to Tamad et al. (2020) PSBs secrete AHLs, and they noticed that butanol-AHL (C_4AHL) was mainly found among all AHLs. Ghosh et al. (2019) reported that Burkholderia species use the QS system to indirectly regulate P-solubilization by inducing biofilm formation. Although the secretion of organic acids by Gram-negative bacteria was considered one of the key mechanisms for inorganic P-solubilization, involvement of biofilm formation, extracellular polymeric substances (EPS) production (Ghosh et al., 2019; Saeed et al., 2021). It was found that biofilm formation and EPS production by *B. tropica* P4 as well as *B. unamae* P9 were highly correlated with available P in the medium (Ghosh & Mandal,

2022). Furthermore, it is mentioned that the PSB's ability to release P was related to the PSB population, while the PSB population (Gram negative) was monitoring by the concentration of N-AHL (Shrestha et al., 2019). Developing an inoculant with QS-mediated biofilm formation may increase the association between the PGPR and root and thereby improving crop health (Balasundararajan & Dananjeyan, 2019). Biofilm-forming rhizobacteria can increase the availability of P, potassium, and zinc by solubilization, producing siderophores and indole-3-acetic acid, thereby benefiting crop growth.

4. Phosphate solubilizing bacteria survival

4.1. Diversity of P chemical forms and their impact on PSBs survival

For instance, research has been done on how soil microbial communities are affected by P inputs and levels. Studies on the effects of P on microbiomes typically concentrate on total or available P content data, while changes in the composition of the microbial community have only frequently been analyzed and significantly correlated with the wide range of soil P compounds brought about by P cycling (Ducousso-Détrez et al., 2022). In response to P fertilization, soil communities have adapted to various P levels. Changes in community structure, control over enzymatic abundances, and the gain or loss of metabolic capacities were key indicators of this adaptation. For instance, the most important edaphic factor in determining the differences in diazotrophic community structures in soil was discovered to be P availability. It has been shown that adding P to acidic soil makes diazotrophic abundances increase and changes the community composition significantly in both the bulk and rhizosphere soils (C. Wang et al., 2020). Numerous studies also concluded that P can affect how microbial alpha and beta diversity indices vary depending on the P level (Silva et al., 2017) or the P fertilization treatment (RP, or TSP-supplemented soils). According to Gumiere et al. (2019), P sources can account for 39.1% and 45.77%, respectively, of the variability in the bacterial and fungal community assemblages. When Gomes et al. (2018) looked for changes in bacterial taxa in the roots and rhizosphere of maize grown in oxisoils after fertilization with TSP, they discovered significant differences in the proportion of proteobacteria between P-rich and P-poor soil samples. The predominance of γ-proteobacteria in situations where P levels are high supported these significant differences. In low-P conditions (without TSP addition), they also noted a decline in the Proteobacteria / Acidobacteria ratio because of Acidobacteria benefiting from low-P content. Additional research at the lower family level showed that high-P soils (with TSP) were enriched in fast-growing, copiotrophic Enterobacteriaceae and Pseudomonadaceae, whereas low-P soils were enriched in slow-growing bacterial taxa, such as Burkholderiaceae

(Ducousso-Détrez et al., 2022). According to Gumiere et al. (2019), the number of bacterial-bacterial interactions increased in direct proportion to the amount of P_2O_5 present in fertilizers. The increase in P content may encourage the system to become more acidic, which would be advantageous to the bacterial community by increasing their interactions and luring back the soil fungi. Additionally, in both bulk and rhizosphere soils, soil P availability was found to be the main predictor of the structure of the soil diazotrophic community.

It is important to note that the availability of P in the soil influences the response to P fertilisation and affects the relative abundance of diazotrophs at the genus level. As a result, when using diazotrophic strains as plant inoculants, soil P levels should be taken into account (C. Wang et al., 2020). In this sense, a predominance of Oxalobacteraceae was found in the RP-fertilized soil compared to the control and TSP treatments when Silva et al. (2017) compared the effects of long-term fertilization practices with RP versus TSP on the microbiomes of maize in tropical oxisol soils. However, according to Margalef et al. (2017), applying RP to uncultivated soil at the same rate primarily stimulated Proteobacteria, Actinobacteria, and Firmicutes. However, in the uncultivated soil, increasing the RP rate resulted in a significant decrease in species richness, primarily affecting Proteobacteria and Firmicutes. Besides, Wang et al. (2020) found that P fertilization significantly affected the diazotrophic communities in the bulk and rhizosphere but not in the root samples. Bodenhausen et al. (2019) also reported that in low-P conditions, the root microbiota of Petunia was enriched in slow-growing bacterial taxa like Burkholderiaceae, whereas in high-P conditions were enriched in Enterobacteriaceae and Pseudomonadaceae.

4.2. Abiotic factors influencing PSBs survival

Microorganisms on the soil rhizosphere create compositionally complex communities, but several variables influence their diversity and abundance. The population density of PSBs may be significantly influenced by geographic, climatic, and environmental factors even though PSBs are widely distributed with a variety of populations and forms in various soils (Crowther et al., 2019). Many studies mentioned that the population density of PSBs in the environment may be significantly influenced by soil types, geographic, climatic, and environmental factors (Crowther et al., 2019; J. Li et al., 2021). According to Alori et al. (2017), factors like soil ecology, plant species, agronomic techniques, and soil physic-chemical characteristics like organic matter and pH can influence the prevalence of microbial P-solubilization. It is widely acknowledged that recognizing the impact of environmental factors is essential to raising the standard and dependability of PSB. Different soils have different PSB prevalence, abundance,

diversity, bioactivity, and survivability rates. This variation is a result of the various soil characteristics, including the physiochemical and nutritional conditions. For that, an introduced PSB's capacity to colonize roots and carry out its activity is influenced by readily available nutrients (Malgioglio et al., 2022). In this sense, Li et al. (2021) noted a positive correlation between soil NO_3-N and soil-dissolved organic carbon and the population density of soil PSMs across China. The same authors showed that the density of PSMs was positively correlated with longitude and mean annual precipitation but negatively correlated with latitude. Additionally, the availability of nutrients and the site's redox reactions are influenced by soil chemical factors such as soil pH, cation exchange capacity, and oxygen supply, which are indirectly linked to PSB's survival. Furthermore, carbon abundance decreased tolerance to a variety of environmental stresses, including high temperatures. It is noted that the temperature and water content of the soil also affects the microbial community and rhizobacterial chemotaxis toward roots (Bhat et al., 2023). Similarly, salinity prevents microorganisms from colonizing the root tips, accumulates sodium and pigments, and reduces antioxidants and potassium (Gamalero et al., 2020). Likewise, the physical characteristics of the soil play a significant role in water and airflow as well as being connected to oxygen diffusion properties, which is why soil texture and pore space are crucial for PSB survival.

In addition, the chance of microbial rhizosphere establishment depends on soil nutrients and root exudates. They contain compounds, which can either attract or repel soil microbes. The beneficial strains are given nutrients by plant root exudates, which also aid in their establishment in the rhizosphere (Mavrodi et al., 2021). Additionally, the root exudates modify the immediate environment and determine whether rhizosphere inhabitants will survive. They also alter the soil's chemistry and structure, which could once more pose a threat to PSB survival. Because root exudates and soil nutrient status decide the fate of the establishment of microbes in the rhizosphere, compatibility with host root exudates and other compounds released by rhizosphere microorganisms appears to be more important for the success of the PSB to establish a sufficient population on the host roots (Dutta & Podile, 2010).

4.3. Biotic factors influencing PSBs survival

The competitiveness of PSBs in natural environments depends on their capacity to survive and grow in soil. The PSB populations typically decrease quickly after being introduced to the rhizosphere. The question of how the immunized PSB will behave and how effective it will be in particular environments is what limits PSB use the most. The morphological and biochemical characteristics of the bacterial partner play an equal role in determining whether it will survive

on the roots of plants (F. Hussain et al., 2019). Therefore, one of the criteria of the PSBs to increase their comfort level in the roots may be changing the root morphology (Dutta & Podile, 2010). In addition, for the survival of PGPR, direct antagonistic effects of pathogen secretions are a formidable opponent. The pathogen may also directly affect PGPR by producing several toxins with broad-spectrum activity (Dutta & Podile, 2010). The competition between the native microflora and the PSB-inoculant harms the local microbial population. Likewise, slow-growing bioinoculants are at a disadvantage in a micro-ecosystem with lots of competition. They may not have enough time to multiply because they are being heavily grazed by the protozoal and nematode population (A. Yadav & Yadav, 2022b). The predation of bacteria by protozoa and nematodes in the soil is represented, and earthworm activity influences the metabolic processes of microorganisms by releasing nitrogen into the soil (Zheng et al., 2022). To avoid this, the presence of flagella, pili, and fimbriae is one adaptation of PSBs to survive, which increases bacterial movement and improves adherence to plant surfaces (Rani et al., 2023). Furthermore, inside the soil microbiota, where microbe-microbe interactions are significant selective forces shaping and probably stabilizing the complex microbial assemblages associated with plants, there are numerous biotic interactions taking place (Ducousso-Détrez et al., 2022). Many biotic factors can hinder the successful application of PSBs as commercial biofertilizers and biocontrol agents, but there are also plenty of factors that can support PSBs and their activities. The physiological state of the introduced microbes affects how effective they are as well. In a recent study, it was discovered that the initial inoculum density was a principal factor that determined whether inoculant strains of different bacteria survived (C. S. Young & Burns, 2021). Even though what happens in nature is extremely complex and challenging to control, understanding the guiding principles before using PSB strains in the field could greatly reduce some of the challenges. We advise paying close attention to: the physical and nutrient status of the soil for the chosen PSBs, a group of mixed and compatible PSBs engaged in various activities and the PSBs' combined effects with those of other advantageous microbes like mycorrhizae and Rhizobium.

5. Phosphate biofertilizers: current trends and prospects

5.1. PSB-biofertilizer

Rhizobacteria that encourage plant growth colonize plant roots and foster the growth of plants in the rhizosphere; as a result, they are used as biopesticides, biocontrol agents, phytostimulants, and biofertilizers. Microbial inoculants, also referred to as biofertilizers, are solutions that include live bacteria, fungi, or algae, either individually or collectively, to promote plant growth

and boost crop yield (Thomas & Singh, 2019). In the face of various environmental stresses and as a means of addressing the issue of feeding a growing global population, biofertilizers are anticipated to be a viable alternative system for sustainable agricultural cultivation. The use of beneficial microbes as a biofertilizer has become extremely important in the agricultural industry due to their potential contribution to food safety and sustainable crop production with a variety of applications (Itelima et al., 2018) as seed or soil inoculants. Through N_2 fixation, cellulolytic activity, or P solubilization, the beneficial microbes in biofertilizers enhance the chemical and biological properties of soil. These environmentally friendly techniques also have the advantages of longer shelf life without harming the ecosystem, maintaining a soil environment rich in all types of micro- and macronutrients through N-fixation, P and K solubilization or mineralization, releasing compounds that control plant growth, producing antibiotics, and decomposing organic matter in the soil (Itelima et al., 2018). For these reasons, cereals, vegetables, oilseeds, and pulses are the largest tested crops with PSB-biofertilizers (M. Khan et al., 2022). Although, PSB-biofertilizers can increase the P use efficiency (PUE) in agricultural soils when used in microbial formulations (Bargaz et al., 2017). However, the most popular PSB-biofertilizer application techniques are seed treatment, seedling dipping, and soil application. However, the most popular and straightforward application method in the past has been seed surface inoculation (M. Khan et al., 2022). Broadly, biofertilizers which are considered as PSBs and commercially available to the farmers include; Rhizobium, Azotobacter, *Azospirillum brasilense*, *Azotobacter chroococcum*, *Acetobacter diazotrophicus*, *Bacillus licheniformis*, *B. megaterium*, *B. mucilaginous*, *B. edaphicus*, *B. subtilis*, Actinomyces, *Herbaspirillum seropedicae*, *Rhizobium phaseoli*, and others are the most biofertilizers used to date (Seenivasagan & Babalola, 2021). It is important to remember that the efficiency of PSB biofertilizers can change depending on elements like the particular bacterial strain utilized, soil characteristics, and crop species. In light of the target crop's unique needs and the current soil conditions, it is advised to choose the proper PSB biofertilizer. Due to its ability to withstand heat and desiccation, the Bacillus genus has a longer shelf life in commercial formulations and is a preferred genus in the biofertilizer industry (A. Yadav & Yadav, 2022b). The advantages of PSB-biofertilizers must be understood, though, to incorporate them into contemporary agricultural practices. As a result, biofertilizer quality control will be required as the use of microbial-based biofertilizers rises in the future (Harahap et al., 2022). The creation of novel approaches for the uses of PSBs and their appropriate implementation in agriculture are essential to the success of biofertilizer science. In addition, one of the few reasons why

ecologists and agriculturalists are still unaware of many useful PSBs is a lack of knowledge about improved fertilizer application protocols in the field.

5.2. Mechanism of action of PSB-biofertilizers: phyto-benefice

The use of PSB-biofertilizers in plant inoculation appears to meet modern agriculture goals, it boosts plant biomass when used singly or in combination with other fertilizers, enhances the nutritional value of seeds and fruits of food crops, and increases plant yields by secreting various phyto-beneficial compounds (Chungopast et al., 2021). The availability and uptake of P by plants are significantly improved by these biofertilizers. The production of organic acids, P solubilization, and root growth promotion are just a few of the processes that go into how PSB-biofertilizers work (M. Khan et al., 2022). By bringing down the pH of the soil, these bacteria release organic acids that solubilize P and become available for plant uptake, promoting their growth and development. As was already mentioned, these acids help to solubilize P, in addition to enhancing soil structure and fertility, they also increase the availability of other nutrients in the soil by chelating them and making them more accessible to plants (Kalayu, 2019). Additionally, by producing phytohormones like indole acetic acid and gibberellin, PSB-biofertilizers act as growth regulators and enhance root growth and morphogenesis to explore a larger volume of soil and absorb nutrients more effectively (Figure 3) (Kang et al., 2019). In addition, such plant growth promotion traits exhibited by valuable PSB-biofertilizers include; asymbiotic or symbiotic N_2 fixation (Janati et al., 2021); production of siderophores and cyanogenic compounds (Mendoza-Arroyo et al., 2020; Boubekri et al., 2021); management of plant pathogens through antibiotics or hydrolytic enzymes (Hamane et al., 2020; Mitra et al., 2020). Similarly, microbial excretion of HCN has been identified as an important antifungal feature for managing root-infecting fungi and is regarded as an environmentally sustainable strategy for biological weed control (Chaiharn et al., 2020). Furthermore, many PSB-biofertilizers produce the enzyme ACC deaminase, which reduces ethylene levels in plants (G. Zhao et al., 2021). It was confirmed that ethylene could cause senescence, chlorosis, and abscission in plants, exacerbating the pathogens' lethal impact (Etesami & Glick, 2020). Thus, the enzyme ACC deaminase converts ACC, a precursor of ethylene synthesis, into ketobutyrate and ammonia, lowering the precursor levels and, as a result, ethylene production (Orozco-Mosqueda et al., 2020). Reduced ethylene levels allow plants to grow normally under stress and thus improve their growth. These biofertilizers have been studied and used extensively in agricultural research. A study by Cavite et al. (2021) discovered that isolates capable of producing siderophores produced higher agronomic yields than 100% chemical fertilizers when

using biofertilizers in rice cultivation. A biofertilizer treatment and a 50% chemical fertilizer dose resulted in a weight of 3.29 g for 100 grains of dry grain, whereas a 100% chemical fertilizer treatment resulted in only 2.70 g. According to Harahap et al. (2022), PSB-biofertilizers have the potential to stimulate plant growth through coleoptile elongation and/or root length in plants, with a significant effect on the height of wheat and mustard plants but no effect on the height of rice plants.

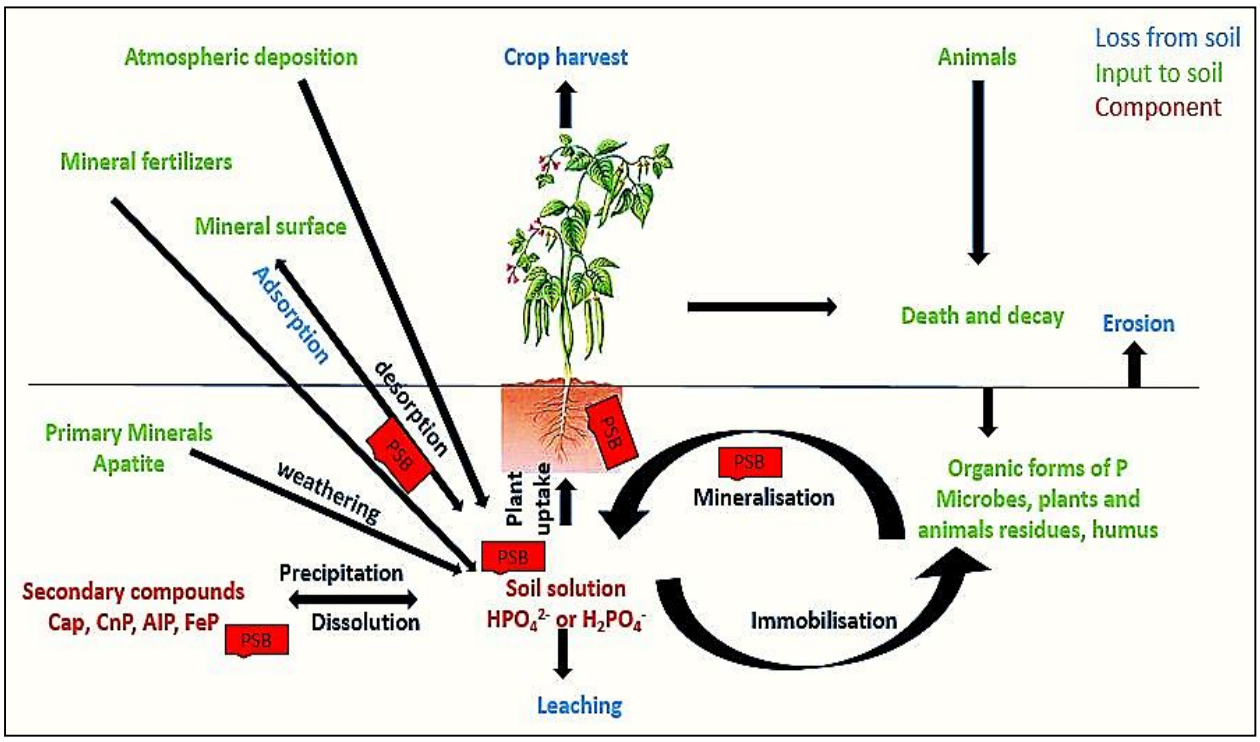


Figure 3. The mechanism of action of PSB-biofertilizers as phyto-benefices.

Additionally, they propose that siderophore and bacteria that increase plant growth could be developed as potential biofertilizers to boost upland rice yield, growth, and climate-resilient. A study conducted by Bilal et al. (2021) examined how PSB-biofertilizers affected crop yield. The yield of various crops, including wheat, maize, and rice, that were given PSB-biofertilizer treatments and crops grown without biofertilizers were compared. In comparison to the control group, the PSB-treated plants' crop yield increased significantly, according to the findings. In particular, Harika et al. (2023) looked into the use of PSB-biofertilizers on black gram. The researchers investigated the effect of PSB-biofertilizers on plant development, nodulation, and nitrogen fixation in legumes. The findings showed that PSB-biofertilizers improved nitrogen fixation and nodulation, which resulted in increased growth and yield in legume crops. According to Masrahi et al. (2023), the use of mycorrhizal fungi or PSB plus superphosphate as a bio-fertilizer has a significant chance of increasing grain yield, barley production, and

mineral content in salinity-prone environments. This suggests that bio-fertilizers, especially in saline soils, are thought to be a good tool for promoting barley growth, nutrient uptake, and yield. Additionally, a study by Fouda, (2020) examined the potential of PSB-biofertilizers in organic farming systems and looked into how they worked together to affect crop productivity and nutrient availability. The findings showed that biofertilizer techniques combined with chicken manure as organic manure under 75% of P fertilization, gave the highest total and marketable yield of the garlic crop. On the other hand, (Shinde & Khade, 2021) found that using a combined treatment of biofertilizers Azotobacter and PSB had an impact on the maize seed's DPPH activity in both the fresh and dry kernel stages. These studies are merely a few of the many that have been done on PSB-biofertilizers, from which we can conclude and emphasize the positive effects of these biofertilizers on crop growth, yield, nutrient uptake, and soil fertility.

5.3. Utilization of microbial consortia biofertilizer

The microbial consortia (MC), which aimed to establish the boundaries between two groups of microbial products, which were undefined, based on scientifically supported criteria, have become more popular in recent years than the microbial-based on single strains. Broad-spectrum biofertilizers include Blue-Green Algae (BGA), Rhizobium, and Azolla are crop-specific bio inoculants, such as *Azospirillum*, *Azotobacter*, phosphorus solubilizing bacteria (PSB), vesicular-arbuscular mycorrhiza (VAM), and Anabaena, as nitrogen-fixing cyanobacteria (Seenivasagan & Babalola, 2021). The use of these biofertilizers on MC is a modern agricultural tool, crucial for integrated nutrient management, a decrease in the use of harmful chemicals, cost-effectiveness, and as a source of renewable energy for plants, according to Ramasamy et al. (2020). A recently proposed strategy for increased activity and improved viability of PGPMs, the microbial consortium for the PGP, has been made (Kour et al., 2020). For that, biofertilizers from the same or different families of microorganisms can be used singly or in consortia. According to Deveau et al. (2018), the behavioural characteristics of one or both of the interacting partners intrinsically modulate bacteria-fungi interaction (BFI). The development of bacterial-fungi interdependency typically results from intimate biophysical and metabolic associations that occur during their coexistence (Mekonnen et al., 2023). A study done by Saxena et al. (2015) evaluates the effects of *Bacillus sp.* RM-2 and *Aspergillus niger* S-36 co-inoculation with TCP on the growth and yield of chickpea plants were compared to those of their single or individual inoculation.

Table 3. PGP effect of PSB on plant crop growth and nutrient uptake.

Crop	Bacterial species	PSBs potential	Growth	Effects on plants	References
Zea mays	*Klebsiella sp*. Br1 + *K. pneumoniae* Fr1, + *B. pumilus* S1r1	N_2 fixation and Auxin production	Greenhouse	Ability to BNF and increased the total N content.	(Kuan et al., 2016)
Solanum lycopersicum	*Acinetobacter* + *Ochrobactrum*	IAA and siderophores Producer	Greenhouse	Significantly promoted the shoot dry weight up to 32.6%.	(J. Zhang et al., 2017)
Cicer arietinum	*Bacillus sp*. AZ17 + *Pseudomonas* sp. strain AZ5	Zn solubilization, IAA, and organic acids producer	Field experiments	Increase grain yield, straw weight, nodules number, nodules dry weight, and Zn and P uptake.	(Zaheer et al., 2019)
Gossypium hirsutum	*A. vinelandii* + *B. niabensis*	Phyto-hormones secretion	Controlled condition	Effectively increase shoot height and root height.	(Patel & Panchal, 2020)
Triticum aestivum	*Bacillus sp* + *Agrobacterium tumefaciens* + *Klebsiella pneumoniae* + *Azotobacter chroococcum* + *Pseudomonas* sp	IAA producer, nitrogen fixation, inorganic/organic P and potassium-releasing ability	Greenhouse	Increased shoot height and plant dry and maximized the dry weight and height.	(J. Wang et al., 2020)
Lactuca sativa	*Rhizobium radiobacter* LB2	Salt tolerance, Zn solubilization, N_2 fixation, IAA, exopolysaccharides and siderophores production	Field experiments	Increment in all the plant growth parameters with improved nutritional content even in saline soil.	(M. Verma et al., 2020)
Oryza sativa	*Enterobacter ludwigii* GAK2	organic acids, IAA and gibberellic acid produced, and Si solubilization	Growth chamber	Promoted seed, increased root and shoot length, fresh biomass and chlorophyll content.	(Lee et al., 2019)
Saccharum officinarum	*Kosakonia radicincitans* and *Bacillus subtilis*	ACC deaminase, IAA and siderophores producer	Field experiment	Increased yield, CCS%, and soil P amount, improvement in juice sucrose and purity.	(Aye et al., 2021)
Acacia cyanophylla	*Agrobacterium sp*. NA11001, *Phyllobacterium sp*. C65, *Bacillus sp*. CS14, and *Rhizobium sp*. V3E1	IAA producer and inorganic P solubilizer	Greenhouse	Great increase of shoots and roots' dry weights, and shoot length.	(Lebrazi et al., 2020)
Mentha arvensis	*Bacillus* sp. STJP	IAA and siderophores producer	Greenhouse	Increase in the plant growth parameters, oil yield and P uptake	(Prakash & Arora, 2019)

The co-inoculation of PSB and fungal strains was found to be superior to their individual or single inoculations, as evidenced by a significantly higher content of chlorophyll in the leaves, longer shoots and roots, and heavier (fresh and dry) shoots and roots. Additionally, it was reported by Abdullahi et al. (2016) that seed treatment with a mixture of *P. fluorescens* and *T. viride*, as well as between *B. thuringiensis* and *T. viride*, can become a part of the integrated management of early blight disease of potato and plant growth. Since the lack of activities in one additional microbe can be found through the action of the other, using a mixture of two or more compatible microorganisms of different species (or strains) can facilitate positive additive or synergistic results (Nadarajah, 2019). Therefore, the addition of microbial consortia can restructure and stimulate mechanisms that support plant growth both in ideal circumstances and in response to various biotic and abiotic stresses (Woo & Pepe, 2018). In some circumstances, a combination of various strains from the same species can display improved behaviours and be referred to as a consortium (Table 3). In addition, it is reported that the QS is a key factor in bacterial compatibility in consortium formulations and is an important molecular signal for bacterial consortium communication (Mekonnen et al., 2023). Kapadia et al. (2022) examine the effects of MC made up of *Bacillus sp*., *Delftia sp*., *Enterobacter sp*., and *Achromobacter sp*. on tomato (*Solanum Lycopersicum L.)* growth and mineral uptake under salt stress and in typical soil conditions. In comparison to the control (without MC), bacterized seedlings sown under saline soil significantly increased leaf (105.17%), shoot (105.62%), root (109.06%), and chlorophyll content (61.49%) dry weight, leaf number (75.68%), shoot length (92.95%), root length (146.14%), and secondary roots (91.23%). According to Kapadia et al. (2022), microbial consortia may be the most effective, sustainable, and cost-effective method for managing soil salinity and enhancing plant growth under salt stress. Similarly, Barra et al. (2016) tested the MC using halotolerant bacteria from Enterobacter, Serratia, Microbacterium, Pseudomonas, and Achromobacter genera that produce IAA and ACCD. The effect of NaCl stress on the emergence, growth, and biomass of wheat seedlings exposed to salt stress in a growth chamber and greenhouse conditions was significantly reduced by seed inoculation. Additionally, co-inoculation of three PSBs *Enterobacter*, *M. arborescens*, and *S. marcescens* performed best in promoting wheat's growth, yield, and uptake of nutrients (N, P, Cu, Zn, Mn, and Fe). According to Kumar et al. (2017), co-inoculation of *Enterobacter* with *S. marcescens* and *M. arborescens* may be used to create a potent PGP consortium formulation for the environmentally friendly and sustainable production of wheat. Additionally, Yadav & Yadav, (2022) investigate the capacity of cumin plants treated with talc and glycerol-based PSB consortia to promote growth. The treatment with the highest per-plant cumin seed yield of 0.42 g with talc-based PSB

inoculations contained a combination of *K. pneumoniae* MF351845, *Erwinia sp*. MF351846, and *P. nitritireducens* MF351919. Overall, talc-based PSB combinations improved all the cumin plant's height, dry weight, 100 seed weight, and yield. Likewise, Admassiea et al. (2017) found that the dual culture technique on *Colletotrichum* isolates from pepper showed isolates of *Bacillus* and *Trichoderma* in combination noted the greatest reduction in pepper anthracnose disease compared to pathogen-inoculated plants. For these reasons, interest in creating synthetic consortia for use in industrial biotechnology is sparked by the potent characteristics of natural consortia.

6. Biological and nano-fertilizers: Fate and behaviour in Agroecosystems

6.1. Formulated commercial biofertilizers: preparation and field applications

Given the numerous environmental issues, it is now necessary to improve plant health using sustainable techniques, and biofertilizers are one method of overcoming these problems. Naturally, bio-fertilizers include microbes, which are an important component of sustainable agriculture because they keep plants healthy by combating pathogens and promoting growth by supplying various nutrients. The biofertilizer formulations proposed are designed to be sustainable while also improving productivity and soil fertility (Elhaissoufi et al., 2021). Successful biofertilizer formulations must have certain desirable characteristics, such as being non-toxic, biodegradable, and environmentally friendly. They also need to be able to incorporate nutrients, adjust pH, be made of inexpensive raw materials that are readily available in sufficient quantities, have a long shelf life, and maintain high levels of viable metabolites under unfavourable conditions (Kour et al., 2020). Inoculating seeds with dry or liquid biofertilizers are just two of the many ways that formulated biofertilizers can be added to the soil. There are different types of commercial biofertilizers including using carrier materials such as peat formulations, granules, wheat bran, talc, and vermiculite, and liquid biofertilizers (Saif et al., 2021). Liquid formulations have gained attractiveness as they are easy to handle and can easily be applied to seeds or soil (Gopi et al., 2020). Furthermore, liquid biofertilizers have numerous advantages, including high export potential, purity, and ease of identification, application, and maintenance (Amenaghawon et al., 2021). As well as, a shelf life of up to two years, allowing the manufacturer to add an adequate amount of nutrients, cell protection agents, inducers that can contribute to cell formation, and spores or cysts (Kour et al., 2020).

According to research, formulations once inoculated into the host plant, increase their numbers and activity largely (Kour et al., 2020). However, microbial formulations, as biofertilizers, are influenced by soil characteristics, inoculant properties, and the environment (Sahu et al., 2022). Although, the effects of bio-fertilizers on soils include microbial community diversity, soil

nutritional status and fertility, and crop productivity. Thus, the soil microbes have the magic key to create a complex network for microbial interactions with agroecosystems or plants and soil components (Sahu et al., 2022). Broadly, biofertilizers that are commercially available to the farmers have been categorized as follows: (i) N_2-fixing biofertilizers (Rhizobium, Bradyrhizobium, Azospirillum, and Azotobacter), (ii) P-solubilizing biofertilizers (Bacillus, Pseudomonas, and Aspergillus), (iii) P-mobilizing biofertilizer (mycorrhiza), and (iv) PGP biofertilizers (M. Khan et al., 2022). Among PGPR, *Azospirillum* has been assessed in different crops including barley, maize, sorghum, and wheat, which make a range of companies develop new products based on *Azospirillum* and other benefits. Many PSBs are proven to be effective biofertilizers or biocontrolling agents especially *Bacillus megaterium*, *Bacillus circulans*, *Bacillus subtilis*, and *Pseudomonas striata* are effective biofertilizers (Satyaprakash et al., 2017). Main manufacturers of biofertilizers based on P-solubilizing microbes include:

- Agro Bio-Tech Research Centre Ltd (India), powder or liquid formulation using *Bacillus megaterium var. phosphaticum.*
- AgriLife (India), powder (spore) formulation using *Bacillus megaterium.*
- Varsha Bioscience and Technology (India), powder formulation using *Bacillus megaterium*, increase crop yield by 15%–25%.
- T. Stanes Company (India), liquid formulation *Bacillus megaterium var. phosphaticum*, save up to 50% over the cost of P chemical fertilizer.
- Novozymes Biologicals (Canada), Powder or granular formulation using *Penicillium bilaii*, solubilize 8.25 $kg.ha^{-1}$.

Despite the widespread adoption of several microbial inoculants for numerous crops, the positive effects of these inoculants are still not consistently applied. Extreme events, salinity, drought, and other environmental stresses are among the major threats that have an impact on the survival and establishment of these organisms in the rhizosphere. Because of the potential for multiple interactions between biofertilizers and soils that could affect their effectiveness, more research into these formulations is necessary.

6.2. Nanotechnology in nano-fertilizers: Biosynthesis and applications

One of the most crucial tools in contemporary agricultural technology is nano biofertilizers. Its cutting-edge agricultural inputs are designed to release nutrients into the soil gradually and in a controlled way to support crop development while minimizing environmental impact. Microbiological cultures, bioinoculants, bacterial inoculants, and bacterial fertilizers are other names for nano biofertilizers (Sahu et al., 2022). They could be characterized as nanomaterials

or nanoparticles that enable the delivery of one or more necessary or advantageous nutrients to plants at the nano-scale dimension to promote plant growth and increase output (B. Sharma et al., 2023). These biomaterials have unique properties when scaled down to the nanometer level, allowing for novel applications (Alvarado et al., 2019). Such supplies are superior and essential in many sustainable fields due to their special size-dependent properties. The development of new nanodevices (biosensors, enzyme encapsulation), and nanomaterials (nanotubes, nanowires, fullerene derivatives, and quantum dots) with the emergence of nanotechnology signal the likelihood of narrative applications in the fields of agriculture and life sciences (Gouda et al., 2018). Nanoparticles can be created biologically, which is less expensive and better for the environment (Figure 4). Synthesize nano-nutrients based microorganisms started with cultured microbes on the chosen nutrient sources and given the right growth conditions. Following the completion of the growth process, the biomass is separated, and the filtrate can be used to separate particular extracellular proteins, which are then synthesized into nanoparticles (Sahu et al., 2022). Fungal proteins that are soluble in water encase biologically created tiny functional nanoparticles, making them economically significant, comparatively stable, and environmentally safe (Sahu et al., 2022). Additionally, organisms that use biosynthesis are in line with the green chemistry principles that stipulate that bio-organisms should be environmentally friendly, with reducing and capping agents (B. Sharma et al., 2023). In contrast to conventional fertilizers, it appears that nano fertilizers may be able to release nutrient compounds, within a controlled manner when exposed to changes in environmental factors like soil acidity, moisture, and temperature (Sahu et al., 2022). Furthermore, nanoparticle moieties enable efficient biofertilizer delivery to crops and protection against external physical harm by crosslinking advantageous microbes (B. Sharma et al., 2023). Ojuederie et al. (2019) reported the use of Titania (TiO_2) nanoparticles as plant growth enhancers in agricultural practices gaining more attention due to their adhesive effect on bacteria cells, which binds them to the roots of plants. In addition, organic polymers encourage the accumulation of biologically necessary components and soil moisture retention after soil particle aggregation. According to Gouda et al. (2018), the context of nano bio-fertilizers in the present offers a great opportunity to develop environmentally friendly compounds that can easily replace chemical pesticides, insecticides, fungicides, and nematicides, which can be nano-microencapsulated, or combined to create formulations that offer effective pest control without leaving residue in the soil. Agriculture development nanoparticles in plant pathology target particular agricultural issues in the interactions between plants and pathogens and offer novel crop protection strategies. This includes the early detection and management of biotic

stresses, improving input use effectiveness, and extending the shelf life of perishables (fruits, flowers, and vegetables). To protect cells from a hostile environment, extracellular polymeric substances and nano bio-fertilizers are involved in cell aggregation, cell adhesion, and biofilm formation (B. Sharma et al., 2023). For example, PSBs (*Pseudomonas fluorescens*, *Bacillus subtilis*, *Paenibacillus elgii*, and *Pseudomonas putida*) treated with gold, aluminium, and silver-coated nanoparticles have been reported to inhibit the growth of harmful fungal parasites within the rhizosphere, acting as potential nano-biofertilizers (Gouda et al., 2018). For this purpose, it is possible to control the fertilizer's release into the target cell while preventing unintended loss by microencapsulating the nano bio-fertilizers. Likewise, pre-treatment with ZnO nanoparticles has also been shown to increase the rate of seed germination in a variety of monocots and dicots (Szőllősi et al., 2020). However, microbial community diversity, soil nutritional status and fertility, and crop productivity are all affected by bio- and nano-fertilizers. As a result, the efficacy of biologically mediated nano fertilizers and biofertilizers is primarily determined by the surrounding agro-environment. Although many microbial inoculants have been widely adapted for many crops.

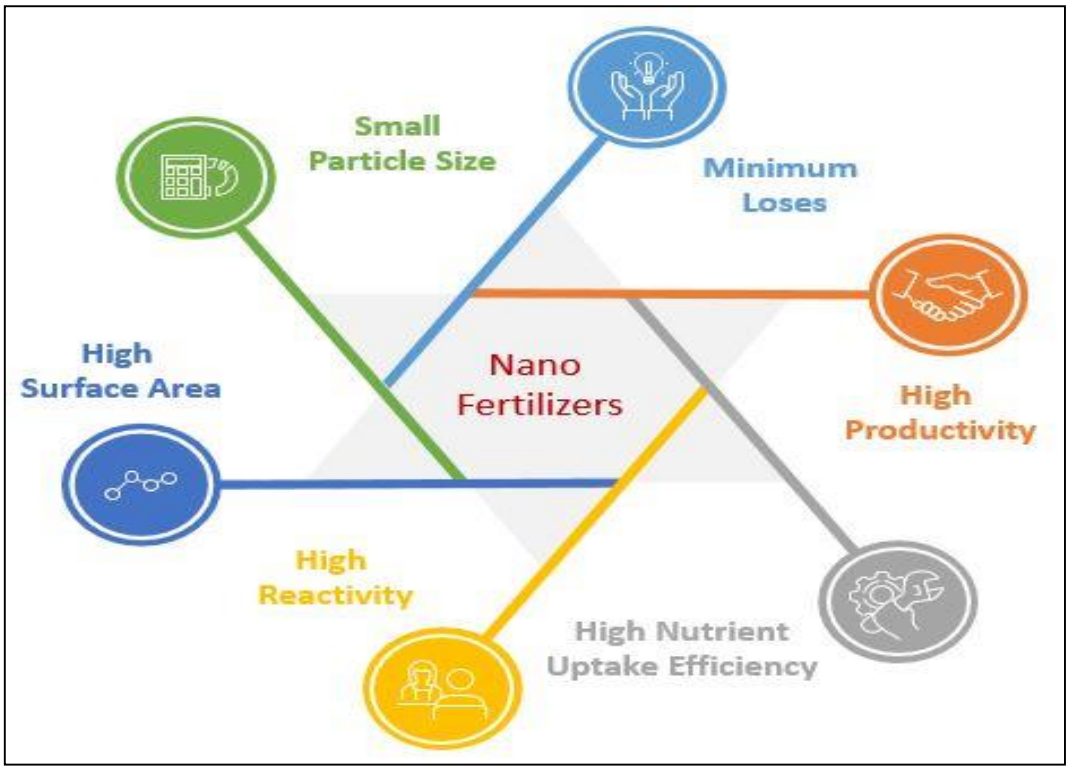


Figure 4. Certain properties and superior quality of nano-fertilizers for microbial inoculants.

Furthermore, the functional balance between plant productivity and soil processes (nutrient recycling, soil microbial activity, soil organic decomposition, etc.) completely controls the sustainability of this agroecosystem. As a result, nano biofertilizer applications differ and

depend on the crop type involved. Generally, applying nano fertilizer methods typically involves spraying or coating crops with micronutrient formulations for improved foliar uptake, the soaking method, which has positive results and reduces the amount of fertilizer needed, and soil application, which significantly boosts yield (Kumari & Singh, 2020; B. Sharma et al., 2023). The cultivation technique and fertilizer characteristics both affect the most effective method. For these reasons, numerous formulations have been developed to improve crop production, including (nano-Zn + biofertilizer) that reported to improve the physiochemical characteristics of sugar beet plants (Jakienė et al., 2015) and (nanopharmax + humic acid) that may improve the nutritional component of cumin (Safaei et al., 2014). The application of Nano-biofertilizer with a particular bio-inoculant, such as *Azetobacter sp*, *Pseudomonas putida*, or *Pantoea agglomerans*, has been shown to increase the concentration of nutrients and elevate the level of pigment content on different plants, which also enhances other parameters like photosynthetic productivity, leaf area, and carbohydrate content (Mir et al., 2015). Likewise, the control fungal-nematode disease in Rabi crops and to give crops resistance against abiotic stressed conditions, nano clay-coated biological agents containing *Trichoderma sp*. and *Pseudomonas sp*. can be used (Mukhopadhyay & De, 2014). Similarly, the effectiveness of nano fertilizer alone versus nano fertilizer + biofertilizer (*Ascophyllum nodosum*) on grapevine plants was tested by Sabir et al. (2014). The results showed that nano-biofertilizer in a combined state significantly improved vine growth, yield, berry quality attributes, and leaf nutrient level in grapevines grown in alkaline soil conditions compared to nanofertilizer alone. On the other hand, Mardalipour et al. (2014) investigated the spring wheat variety and found that the foliar application of Biozar as a nano biofertilizer significantly increased crop growth, seed yield, and yield components. Additionally, it effectively increases the ability of wheat plants to absorb nutrients. On their part, Thirugnanasambandan (2018) used gold nanoparticles and rhizobacteria to assess their impact on a variety of horticultural *Fabaceae* crops, and the in vitro study results are very promising. According to other reports, nano- and micro-encapsulation of insecticides, fungicides, or nematicides-based PGPR help create a formulation that offers effective pest control while preventing residue in the soil. Currently, developed nations like the United States, China, Germany, France, Japan, Switzerland, and South Korea use nanotechnology and products for agricultural growth. To meet the demands of the expanding population, large-scale implementation of such products is still limited to a few biotechnological products in India, such as Golden rice, BT cotton, BT Brinjal, Cucumber, seedless bananas, tomatoes (Gouda et al., 2018).

One of the most pressing issues in environmental science is the fate and behaviour of biological fertilizers (nano and biofertilizers) in various agroecosystems. Nano fertilizers were reported to increase and maintain soil fertility; reduce environmental pollution by using fewer chemical fertilizers; improve plants' ability to absorb water and nutrients from the soil; lower the need for irrigation and fertilization doses for crops; and improve crop yields both in the field and in greenhouses (Kumari & Singh, 2020; Shanware & Taiwade, 2022). Because they have an impact on agroecosystems (water, plants, microorganisms, people, and animals), numerous studies point to the benefits of using nano-fertilisers, but the harmful effects on the soil remain a concern for the future and need to be taken into account. By enhancing soil fertility, plant tolerance, crop productivity, and maintaining a balanced nutrient cycling, the use of contemporary tools and techniques for PSB enhancement can play a crucial role in sustainable agriculture (Gouda et al., 2018). Additional research on the production of microbial communities, the selection of appropriate rhizosphere microbes, chemical engineering and material science applications, as well as multidisciplinary research combining ecological and functional biological approaches, can lead to novel formulations and opportunities with enormous potential. Because of the production of nanoparticles and microbial inoculants, it is crucial to develop safe, effective, dependable, clean, non-toxic, and environmentally friendly processes.

6.3. Advantages of bio- and nano-fertilizers in the economic improvement

Chemical fertilizer use was reported to reduce N_2 fixation in the soil, increase pathogen resistance to pests, and allow for pesticide bioaccumulation and the loss of suitable habitat for birds, which eventually led to economic loss (Jain et al., 2021). According to Avila-Quezada et al. (2022) approximately 40-70% of nitrogen, 80-90% of phosphorus, and 50-90% of potassium fertilizers are lost in soil and thus may not reach the target plant portions, resulting in economic failure. Inadequate use of artificial/synthetic fertilizers and pesticides is thus extremely damaging to the plant ecosystem, which is why employing various types of nanoparticles as an ecological means of protecting our ecosystem/agroclimatic zones is necessary (Jakhar et al., 2022). Since plants will be able to absorb the majority of the nutrients without leaching, the use of slow-release nano-fertilizers may therefore be a useful substitute for soluble mineral fertilizers for crop development (Guo et al., 2018). It is well known that nanotechnology has already found use in several fields, including biomedicine, targeted drug delivery, cancer therapy, the cosmetics industry, electronics, biosensors, wastewater treatment, and the delivery of fertilizer and pesticides to specific targets. According to estimates from Sahu et al. (2022),

worldwide investments in the field of nanotechnology increased to about $10 billion and $1 trillion, respectively, in 2005 and 2015. However, the Indian government and other organizations are developing revitalization strategies for the economic growth of sustainable agriculture, such as promoting the nascent biofertilizer market at the level of both the user-farmer and the producer-investor. In addition to affecting the economic growth of sustainable agriculture in India, the use of biofertilizers will also benefit the nation's ecosystem sustainability and overall well-being. In line with Jakienė et al. (2015), the most cost-effective treatment increased incomes from 20 to 90 euros per hectare ($L.ha^{-1}$) with a single application of bioorganic fertilizer. It was also claimed that the novel application of chitosan-based Nano fertilizers would boost soil heterogeneity by lowering the rate of soil additive loss, cutting back on the use of chemical fertilizers, and minimizing soil pollution (Shahrajabian et al., 2021). According to Feregrino-Perez et al. (2018), using unconventional and economically sound chitosan-based technology (nano-fertilizers) will be essential to scaling up agriculture in the future and addressing the world food crisis. To achieve precision agriculture with ecological and financial viability, recent developments in the form of chitosan may be investigated (Jakhar et al., 2022). Similar to this, precise and targeted nitrogen application via foliar application of nano nitrogen reduces urea losses, improves nutrient uptake efficiency, and addresses environmental problems related to soil, air, and water pollution (Y. Kumar et al., 2021), while requiring less nitrogen to be applied per unit area, improving farm economics.

7. Conclusion and perspective

Different studies in greenhouses and fields have evaluated the stimulation of plant growth and crop yield by helpful plant growth-promoting microbes to either decrease the use of agrochemicals or pollution caused by them. According to an estimate, however, up to 99% or more of microbial life is still incapable of being grown in a lab, making it impossible to study and comprehend it in the manner that microbial ecologists have come to expect over the past century. Therefore, to promote economic development for sustainable agriculture, research must focus more on locating and choosing the best crops, soil, and PSB strains that can fix nitrogen, bind phosphorus, and absorb zinc under a variety of agroclimatic conditions. Additionally, biofertilizer cultures should be exchanged between nations with similar climatic conditions, evaluated for performance to find a better strain for a particular crop, and monitored for activity during storage to prevent natural mutants. The same goes for creating appropriate substitute formulations, such as liquid inoculants or granular formulations for all bioinoculants, as well as standardizing the media and inoculation method for the new formulations. Therefore,

it is imperative to create safe, efficient, dependable, clean, non-toxic, and environmentally friendly processes for the production of nanoparticles and microbial inoculants. Future research must also concentrate on comprehending how bacteria-derived bio- and nano-fertilizers interact with nanoparticles, which are also beneficial micronutrients for microbial life and plants. The similarities and differences between bio- and nano-fertilizers, as well as advancements in plant and soil sciences will be covered in the current review. To create better consortia with a longer shelf life that could also protect biofertilizers as nanoparticles or bioinoculants after field application, more research is needed to understand PSBs survivability, population dynamics, and interactions in rhizosphere soils.

Chapitre II

Bactéries solubilisant le phosphate issues de sols aux conditions environnementales variées : Occurrence et fonction

Bactéries solubilisant le phosphate issues de sols aux conditions environnementales variées : Occurrence et fonction

Synthèse

Le phosphore (P) est le deuxième élément nutritif le plus essentiel, après l'azote, que les plantes ont besoin pour leur croissance et leur développement. La faible disponibilité du P dans le sol est principalement due aux complexes qu'il forme avec d'autres éléments, devenant ainsi non disponible pour la plante sans l'intervention des microorganismes. La solubilité du phosphore est assurée par la sécrétion d'acides organiques par certains microorganismes qui assurent la chélation des ions liés au phosphore.

L'intérêt pour des solutions innovantes pour remédier à ce problème pédologique et écologique s'est considérablement accru au cours des trois dernières décennies pour deux raisons principales : premièrement, l'épuisement croissant des roches P extractibles et deuxièmement, on estime que 5,7 milliards d'hectares de terres arables dans le monde ont une quantité insuffisante d'orthophosphates libres pour une production végétale optimale. Les micro-organismes bénéfiques, tels que les bactéries solubilisant le P (PSB), un groupe de la communauté des rhizobactéries favorisant la croissance des plantes (PGPR), est considéré comme un moyen avantageux pour fournir du P aux plantes en plus des phytohormones et des minéraux. C'est pourquoi l'attention s'est portée sur la découverte des espèces de PSB, qui peuvent réduire l'utilisation d'engrais chimiques à base de P grâce à la production d'acides organiques qui aident à convertir les formes divalentes et trivalentes non-assimilables de P en formes monovalentes assimilables tout en protégeant les plantes du stress abiotique dans une gamme de conditions bioclimatiques. Ces communautés microbiennes contribuent aux services éco-systémiques essentiels du sol, notamment la décomposition de la matière organique, le cycle des nutriments, la lutte contre les maladies et la nutrition des plantes.

Le climat méditerranéen du Maroc, en particulier les zones de basse altitude, est semi-aride avec des sols pauvres en nutriments dans lesquels les petits agriculteurs à faibles revenus dominent sans avoir accès à des engrais inorganiques coûteux. Les effets combinés de la diminution des précipitations et de l'augmentation des températures font de la région méditerranéenne un point chaud du changement climatique et l'un des écosystèmes les plus vulnérables. De plus, les communautés microbiennes du sol ne sont pas distribuées au hasard, mais présentent une agrégation spatiale à la suite d'un stress à long terme par le biais d'une évolution adaptative, qui conduit à la sélection de micro-organismes du sol présentant des caractéristiques génétiques et métaboliques distinctes. La présence, l'abondance, la diversité et

la bio-activité des PSB varient d'un sol à un autre. Cette variation est attribuée aux différentes propriétés du sol, y compris les conditions nutritionnelles et les propriétés physico-chimiques. De même, les variables climatiques (précipitations annuelles (Pa) et température moyennes (Tm)) régulent diversement la population des PSB et les réserves de P (inorganique et organique) du sol en combinaison avec les variables édaphiques principalement déterminées par les facteurs de formation du sol. À l'échelle continentale et mondiale, la densité de population des PSB est corrélée au P total plutôt qu'au pH et cette corrélation est influencée par les conditions écologiques et les types de sol. Cependant, il n'existe pas une large gamme de PSB adaptées aux différentes situations agro-écologiques. Étant donné notre compréhension limitée des variables pédologiques et climatiques qui influencent le développement des PSB, nous avons émis l'hypothèse que les conditions environnementales pouvaient être corrélées avec les types de sol, la performance de solubilisation du P par ces PSB et, inversement, leur potentiel à promouvoir la croissance des plantes.

Compte tenu de ce qui précède, la présente étude s'est concentrée sur différents effets climatiques et conditions pédologiques sur l'abondance, la diversité et le potentiel des PSB dans la rhizosphère des légumineuses dans quatre stations bioclimatiques dissemblables de la région Fès - Meknès, au Maroc. Pour évaluer l'impact possible de ces facteurs sur la capacité de solubilisation du P par les PSB et sur les caractères de promotion de la croissance des plantes (PGP), nous avons analysé le sol et le climat de chaque station étudié. De même, nous avons testé l'efficacité de solubilisation du P, la production des auxines et des siderophores, la solubilisation de zinc et l'activité de l'ACC désaminase des isolats. Ensuite, nous avons évalué l'impact et la corrélation entre les conditions environnementales de chaque site étudié et l'abondance, la diversité et les compétences des souches de PSB.

Au total, 51 PSB ont été étudiées dans ce travail. La moyenne de solubilisation du P rocheux (RP) et du P tricalcique (TCP) de toutes les souches isolées dans chacune des quatre régions varie de 18,69 $mg.L^{-1}$ à 40,43 $mg.L^{-1}$ et de 71,71 $mg.L^{-1}$ à 94,54 $mg.L^{-1}$, respectivement. Les caractéristiques PGP des souches isolées sont positivement corrélées avec l'abondance des PSB et les caractéristiques de l'échantillon (sol et climat). La densité de la population de PSB était significativement différente entre les quatre sites en raison des conditions environnementales variées : température (T), humidité (H) et précipitations (P) des sites étudiés. Le site d'Ait Saleh, le plus chargé en PSB, montre 70% et 69% de la production d'auxines et de siderophores, respectivement. Les résultats ont montré que la diversité des communautés bactériennes, la densité et le potentiel des PSB sont étroitement liées à diverses conditions édapho-climatiques

telles que la température, les précipitations, le statut nutritif du sol et la texture du sol. Les caractéristiques morphologiques et biochimiques des souches nous ont permis d'identifier environ neuf genres bactériens différents, dont *Bacillus*, *Pseudomonas* et *Rhizobium*. Sur la base des résultats mentionnés, le climat semble exercer une forte pression sélective sur la structure des communautés de PSB et sur leur compétence à solubiliser le P.

Elargir le champ de travail et étudier différentes régions qui sont plus susceptibles de contenir des PSB efficaces avec des traits et des mécanismes écologiques, peuvent améliorer la production agricole et d'orienter la recherche vers des voies appropriées. Ce travail de recherche apporte une contribution significative au domaine de l'agriculture durable au Maroc en menant une enquête approfondie sur la performance des PSB associée aux conditions environnementales de leur source de sol. Cette étude fournit des informations précieuses sur la densité et le potentiel des PSB dans différents habitats, ceci pourrait être utilisé pour améliorer un système plante-PSB efficace et augmenter la production agricole en tenant compte de leurs caractéristiques écologiques spécifiques. En général, les résultats de l'étude pourraient fournir une approche efficace pour l'amélioration des inoculant microbiens au but agronomique afin d'augmenter la mobilisation du P du sol, que ce soit en production intensive ou dans des applications agro-écologiques.

Phosphate solubilizing bacteria from soils with varying environmental conditions: Occurrence and function

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Abstract

Phosphate solubilizing bacteria (PSB) is an advantageous way to supply phosphate (P) to plants. The Mediterranean climate of Morocco, especially the low-lying areas, is semi-arid with nutrient-depleted soils in which small-scale, low-income farmers dominate without access to expensive inorganic fertilizers. However, there is not a wide range of PSBs suitable for various agroecological situations. Furthermore, our understanding of the soil and climatic variables that influence their development is limited. This study aims to examine the impacts of specific environmental factors, such as climate and soil, on the abundance, potential, and diversity of PSBs in four agricultural regions of Morocco. To assess the possible impact of these factors on the P solubilization capacity of PSBs and plant growth-promoting (PGP) traits, we analyzed the soil and climate of each sample studied. Similarly, we tested the P solubilization efficiency of the isolates. The bacteria were isolated in a National Botanical Research Institute's phosphate (NBRIP) agar medium. A total of 51 PSBs were studied in this work. The P-solubilization average of Rock P (RP) and Tricalcium P (TCP) of all strains that were isolated from each of the four regions ranged from 18.69 $mg.L^{-1}$ to 40.43 $mg.L^{-1}$ and from 71.71 $mg.L^{-1}$ to 94.54 $mg.L^{-1}$, respectively. The PGP traits of the isolated strains are positively correlated with the PSBs abundance and the sample characteristics (soil and climate). The morphological and biochemical characteristics of the strain allowed us to identify around nine different bacterial genera, including *Bacillus, Pseudomonas, and Rhizobium*. The findings showed that bacterial communities, density, and potency are closely correlated to various edapho-climatic conditions such as temperature, precipitation, soil nutrient status, and soil texture. These findings could be used to improve an effective plant-PSBs system and increase agricultural output by taking into account their specific ecological traits and plant growth mechanisms.

Keywords: Environmental factors, Diversity, Phosphate Solubilising Bacteria, PGP traits, RP Solubilization, Sustainability.

1. Introduction

Phosphorus (P) is the second most essential nutrient after nitrogen, required by plants for growth and development. The low availability of P in the soil is mainly due to the complexes it forms with other elements when applied, thus becoming unavailable to the plant without the help of microbes (Koczorski et al., 2021). In acidic soil, P forms complexes with Al and Fe oxides, while in alkaline soil, it forms complexes with calcium (Aliyat et al., 2020). Phosphorus solubility is mediated through the secretion of organic acids that lower pH, the chelation reaction of phosphorus-bound ions, and competition with phosphorus for adsorption sites in the soil. Beneficial microbes, such as P-solubilising bacteria (PSB), a group of the Plant Growth Promoting Rhizobacteria (PGPR) community, can promote growth through plant-essential metabolites production, including phytohormones and minerals. PSBs interest has grown significantly over the last three decades for two main reasons; first, there is a growing depletion of extractable P rocks (Cordell et al., 2009), and second, an estimated 5.7 billion hectares of arable land around the world have insufficient free orthophosphate for optimal crop production (Hinsinger, 2001). For this reason, attention has recently been focused on the discovery of PSBs, e.g. the genera *Bacillus, Pseudomonas, Rhizobium, Enterobacter,* and *Burkholderia* which can reduce the use of chemical P fertilizers (Tagele et al., 2018; A. Sharma et al., 2019; Rizvi et al., 2021) by the production of organic acids which lowers the pH and helps convert non-assimilable divalent and trivalent forms of P into assimilable monovalent forms (Kalayu, 2019), while protecting plants from abiotic stresses under a wide range of climatic conditions. These rhizobacteria promote plant growth through direct and indirect plant growth promotion (PGP) mechanisms. Among these, auxin production, siderophores production, zinc solubilization, and P solubilization are desirable traits that are commonly suggested as mechanisms to promote plant growth by effective PGPs.

Soils contain diverse communities of microorganisms that interact with plants and respond to their specific physical and chemical characteristics. These microbial communities contribute to essential soil ecosystem services, including organic matter decomposition, nutrient cycling, disease control, and plant nutrition. Climate models show that the Mediterranean region is a hot spot for climate change. The combined effects of decreasing precipitation and increasing temperatures make the Mediterranean region one of the most vulnerable ecosystems. Soil microbial communities are not randomly distributed but show spatial aggregation (Zhou et al., 2020). In particular, microenvironments can develop following long-term stress through adaptive evolution (F. Zhao et al., 2016), which leads to soil microorganisms selection with distinct genetic

and metabolic characteristics (Oh et al., 2012). This adaptive evolution generates biodiversity in soil (Gómez & Buckling, 2013).

The occurrence, abundance, diversity, and bioactivity of PSB vary in different soils. The variation is attributed to the different soil properties including the nutritional conditions and physiochemical properties. However, PSBs are influenced by biotic or abiotic factors such as interactions with other microorganisms, agronomic activities, ecological conditions, and soil types. Research has also shown that PSBs are largely dependent on some produced metabolites and their release rate (Alori et al., 2017). Deiss et al. (2018) determined that climatic variables (average annual precipitation and temperature) diversely regulate the soil inorganic and organic P pools in combination with edaphic variables mainly determined by parent material and soil formation factors. In addition, well-aerated soil is more conducive to rapid phosphorus solubilization than saturated wet soil. Lime and compost, used as soil amendments, also have positive effects on phosphate solubilization (Alori et al., 2017). Amadou et al. (2020).observed that soil amendment with biochar and manure, as well as the addition of inorganic mineral fertilizers, changed the soil properties, especially the NH^{4+} content, and this had an impact on the structure of the bacterial community above and below the soil (rhizosphere and root). Among the different abiotic stresses, low temperature is a major limitation to the growth, reproductive stage, and plant grain yield (Rizvi et al., 2021). In addition, interactions between plants and microorganisms are influenced by temperature changes. Li et al. (2021) show that on a continental and global scale, the population density of PSB is correlated with total P rather than pH and is influenced by environmental factors. It is, therefore, remarkable that positive relationships exist between soil PSB population density and available P, nitrate-nitrogen, and dissolved organic carbon in the soil, reflecting functional couplings between PSBs and microbes that drive the biogeochemical cycles of nitrogen and carbon (J. Li et al., 2021).

Since PSBs can play a key role in enhancing the host plant's ability to withstand stressful conditions. We hypothesized that the environmental conditions observed at the sampling sites might be correlated with the soil types, the expression levels of the different P-solubilising traits of the isolated bacteria, and, conversely, their potential to promote plant growth. Therefore, we addressed the question: are the performance and plant growth promotion mechanisms of rhizobacteria associated with the environmental conditions of their soil source? Considering the above facts, the present study focused on the several effects of climate and soil conditions on the abundance, potential, and diversity of PSBs in the rhizosphere of legumes in four dissimilar bioclimatic stations of Fez - Meknes regions, Morocco. In addition, to assess the potential impact of these factors on the capacity of PSBs, we tested the P solubilization efficiency of isolates,

evaluate their plant's growth trait potential, and assessed the impact of environmental conditions at each study site on the PSB strains. A better understanding of microbial consortium patterns is essential and becomes a prerequisite for the sustainable use of these biofertilizers in agriculture. The study results could provide an effective approach for the agronomic improvement of microbial inoculants to enhance soil P mobilization for plant growth either in intensive production or in agroecological applications in Morocco.

2. Materials and methods

2.1. Soil samples and isolation of phosphate solubilizing bacteria

Soil samples were collected from cultivated and spontaneous plants from different rhizosphere sites of four dissimilar bioclimatic stations distributed in the sub-mountainous and central areas of the Fez - Meknes regions from December to January 2020 **(**Fig 1). As this is a simple soil sample that does not harm the environment, the regional authorities do not require us to obtain a permit or any legal obligation. Five samples are taken at each site, and the values at each site represent the average of the five samples. The selected fields from which samples are taken were under cultivation, and rhizosphere sampling was carried out after crop harvest at the end of wheat, barley, beans, peas, and beans planting season. From each sample, a part was consecrated to PSBs isolation.

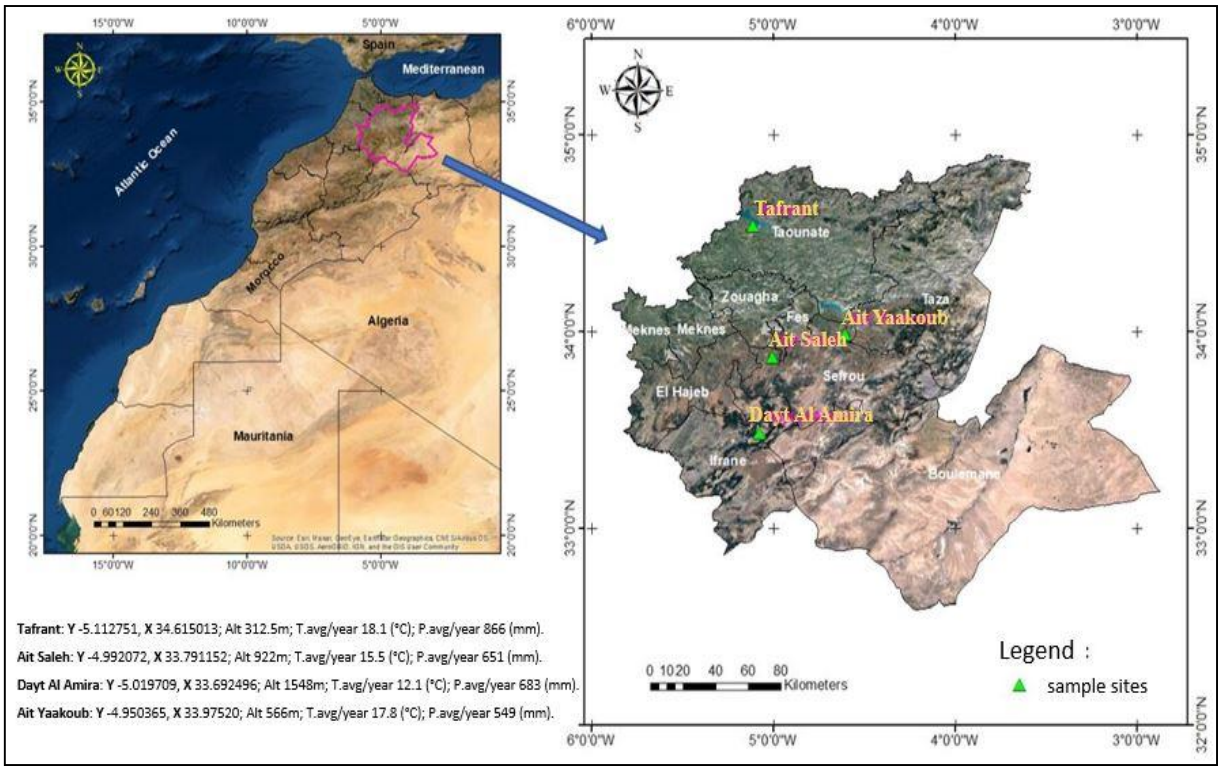


Figure 1. Soil sampling sites located at Fez - Meknes region (Morocco).

Phosphate solubilizing bacteria were isolated on National Botanical Research in P medium (NBRIP) agar plates (Nautiyal, 1999; Janati et al., 2022)**.** To determine the efficiency of P solubilization, the PSB isolates were grown in an NBRIP agar medium supplemented with 5 $g.L^{-1}$ TCP. After nine days of incubation at 28 ± 2 °C, the colonies' diameter and the visible halos were measured to calculate the P solubilization index (PSI) by the following formula (Equation 1)**:**

$$PSI = \frac{CD + HD}{CD} \qquad (1)$$

CD: Colony Diameter. HD: Halo zone Diameter.

2.2. Sites description

Samples collected from the crop field correspond to four sites named Ayt Saleh, Ayt Yaakoub, Tafrant, and Dayt Amira. Sites samples description and bacteria analyses are described in Table 1. Soil physiochemical characterization such as organic matter, pH, conductivity, soil texture, available nitrogen, phosphorus, and potassium in the soil, soil micronutrient status, total mineral content, etc. was analysed using standard methods at the pedology department at the National School of Agriculture, Meknes, Morocco. Soils in these regions are classified as alkaline with medium P content. Soil samples from all stations have major variations in clay, silt as well as in sand content. The value of silt content is higher than that of clay and sand, except for the Tafrant site where the clay represents the major part. For heavy metal concentration analysis, representative soil samples were air-dried and sieved (<2 mm), and concentrations of Zn, Pb, Co, Cd, Ni and Cr were determined, after extraction with mineralization, by Inductively Coupled Plasma (ICP).

Table 1. Sites samples description.

Stations/Parameters	**Tafrant**	**Ait Yaakoub**	**Dayt Al Amira**	**Ait Saleh**
Coordinates	**Y** -5.112751, **X** 34.615013	**Y** -4.950365, **X** 33.975209	**Y** -5.019709, **X** 33.692496	**Y** -4.992072, **X** 33.791152
Altitude (m)	312.5	566	1548	922
T. avg/year (°C)	18.1	17.8	8.3	15.5
P. avg/year (mm)	866	549	683	651
pH	8.26	8.32	8.07	8.27
Sand (%)	15.3	21.6	8	31.9
Silt (%)	33.5	46.8	55.0	35.6
Clay (%)	51.2	31.5	37.1	32.6
Texture Triangle USDA	Clay	Clay Loam	Silty Clay Loam	Clay Loam

2.3. Climatic study by Emberger bioclimatic Quotient

The current study has focused on the Emberger rainfall quotient to determine the climate type that governed each studied area. It is the ratio between precipitation amount and the average of thermal

extremes (warmest - coldest months), all corrected by the extreme thermal amplitude (M - m). Emberger noted that thermal amplitude is a significant factor in plant species distribution, which leads to microbial species diversity (Emberger, 1942; Costa et al., 2022). This approach places meteorological stations at the appropriate bioclimatic stage providing a better understanding of the Mediterranean region's aridity and its effect on PSBs abundance in studied sites. The following formulas were used to calculate the Emberger quotient (Table 2). To investigate the effects of climatic and edaphic parameters on PSBs population density in the environmental samples, we analyzed the relationships between these parameters and PSBs population density using univariate linear regressions. In addition, the rainfall and temperature of each site were studied using an umbrothermal diagram to give more details on how PSBs abundance and diversity can be affected.

Table 2. Emberger bioclimatic quotient formulas.

formulas	References	Coefficients
$Q_2 = 200*P/(M^2-m^2)$	(Emberger, 1942)	P: Annual rainfall in mm/m^2/year.
$Q_2= 3.43*P/(M-m)$	(Stewart, 1968)	M: Maximum temperature of the hottest month in °K. m: Minimum temperature of the coldest month in °K.
$Q_2= 2000*P/(M+m+546.4)*(M-m)$	(Mokhtari, 2013)	M: Maximum temperature of the hottest month in °C. m: Minimum temperature of the coldest month in °C.

2.4. Screening of bacterial plant growth promoting traits

2.4.1. Quantitative estimation of phosphate solubilization

To measure the solubilization capacity of isolates a liquid NBRIP medium supplemented with 5 $g.L^{-1}$ RP alone and 5 $g.L^{-1}$ TCP alone was used. The amount of assimilable P content was carried out by the ascorbic acid colourimetric method (Murphy & Riley, 1962) after seven days of incubation and with few modifications. A standard range of KH_2PO_4 (concentration from 0 to 1 $mg.L^{-1}$) was used to calculate P content. To identify PSB genera, we extended the morphological and biochemical tests conducted by Janati et al. 52022). In this paper, we examine the preliminary screening of PSB isolates and investigate the species affiliation of each potential isolate for phenotypic characterization of phosphate solubilizing bacteria based on growth in a different medium, according to Bergez's textbook (Systematic Bacteriology, 2nd ed., vol.5, eds., 2012).

2.4.2. Analysis of auxin production

Auxin production of PSBs was determined according to the method of (Naik & Sakthivel, 2006) with some modifications. The test strains were cultured into L-tryptophan-containing LB liquid

medium (addition of 1 L LB + 0.1 g L-tryptophan) and incubated on a shaker at 180 rpm at 28 ± 2°C for 4 days. The broth was centrifuged at 6800 × g for 10 min. The supernatant was isolated and 0.1 mL was mixed with 0.1 mL Salkowski's reagent (4.5 g $FeCl_3$ and 587.4 mL of 98% H_2SO_4, solution) and kept in the dark at room temperature for 30 min. The development of a pink-red colouration indicates the production of auxins. The absorbance of each sample was measured with a spectrophotometer at 530 nm, and auxin concentrations were determined based on a standard range prepared with standard solutions ranging from 0 to 50 $\mu g.mL^{-1}$ auxins.

2.4.3. Siderophores production activity assays

The culture on agar medium containing chromium azurol S (CAS) allows the detection of the secretion of siderophores by the microorganisms. The principle is that the culture medium initially has a blue colour due to the Iron/CAS/HDTMA complex, which turns orange-red following the displacement of iron by the siderophores produced by the microorganism. All the test strains were inoculated into TSA liquid medium and were shaken at a 180 rpm incubator for 2 days at 28 ± 2°C. The fermentation broth was centrifuged at 6800 × g for 10 min. A solution consisting of 60.5 mg CAS in 50 mL of deionized water was amended with 10 mL $FeCl_3$.6H2O solution. Then, 72.9 mg HDTMA (hexadecyltrimethylammonium bromide) dissolved in 40 Ml of deionized water was added to CAS to a final volume of 100 mL (Shin et al., 2001; C. Song et al., 2022). Four millilitres from the prepared CAS solution were mixed thoroughly with 4 mL of the culture supernatant, followed by incubation for 1 h. The absorbance of each sample was measured with a spectrophotometer at 630 nm. Siderophores production by bacterial strains was evaluated as the percentage of siderophores units (Equation 2).

$$SUs = [(Ar - As)/Ar] \times 100 \qquad (2)$$

Ar = absorbance of reference (CAS reagent) and As = absorbance of the sample.

2.4.4. Qualitative test for Zinc solubilising bacteria

The zinc-solubilizing ability of these strains was evaluated in mineral salt medium (MSM: NaCl 1g, $CaCl_2$ 0.1 g, $MgSO_4$ 0.5 g, KH_2PO_4 1 g, K_2HPO_4 1 g, yeast extract 4 g, agar 15 g in 1 L, and pH was maintained at 7.2). Different sources of insoluble zinc salts like zinc oxide (1.244 $g.L^{-1}$ = 15.23 mM), zinc phosphate (1.9882 $g.L^{-1}$ = 5.0 mM), zinc carbonate (1.728 $g.L^{-1}$ 5.2 mM), and zinc sulfide (1.124 $g.L^{-1}$ 11.54 mM) at a final concentration of 0.1% were added in the medium individually and autoclaved at 121°C for 30 minutes (R. Yasmin et al., 2021). A loop was full of bacterial overnight growth in nutrient broth was spread on an MS medium. Plates were incubated at 30°C for 7 days. Strains showing a clear zone around the colony are considered zinc-solubilizing

strains (Khanghahi et al., 2018). The Zinc solubilizing index (ZSI) was calculated as the following formula (Equation 3).

$$ZSI = (halo + colony)/colony\ diameters \quad (3)$$

2.4.5. ACC deaminase activity

The isolates were checked for their ability to utilize ACC as an N source by assessing their growth in DF minimal salts medium (Dworkin & Foster, 1958) supplemented with 3 mM ACC. The ACC deaminase activity of the isolates was measured using a spectrophotometer that measured the absorbance at 540 nm, the α-ketobutyrate produced from the enzymatic cleavage of ACC through ACCD as discussed previously by Honma & Shimomura, (1978). The α-ketobutyrate produced by this reaction is estimated by comparing the absorbance at 540 nm of a sample to a standard curve of α-ketobutyrate ranging between 0.1 and 1.0 mM (Chinnadurai et al., 2009).

2.5. Identification of phosphate-solubilizing bacteria

Following Bergey's Manual (Systematic Bacteriology, 2nd ed., vol. 5, eds., 2012), the preliminary screening for PSB isolates was performed to investigate species affiliation of each potential isolate based on growth in a different medium; King A and King B medium to identify pseudomonas (Goto & Enomoto, 1970), Yeast extract mannitol agar medium (YEMA) to select rhizobium (Paudyal & Gupta, 2017). Mannitol Salt Agar medium (MSA) to identify staphylococcus (Lally et al., 1985), Eosin methylene blue medium (EMB) to identify E. Coli (Leininger, 1993), De Man, Rogosa and Sharpe medium (MRS) to identify lactic bacteria (Nikita & Hemangi, 2012). Motility test, Gram staining, catalase, gelatinase, and urease activities, starch glucose, lactose as a source of carbon, citrate utilization and H_2S production were performed.

2.6. Statistical analyses

The region map was prepared using the SIG software. Data were analyzed using SPSS software, and results were expressed as the means ± standard deviation of three replicates. Data were examined by ANOVA I analysis, and mean comparison was performed by Duncan's multiple range test at $p \leq 0.05$. Principal Component Analysis (PCA) was performed to give a summarising view of obtained results, how soil and environmental characteristics influenced isolates biological attributes, and to determine which inter-related parameters most influenced isolates P solubilizing potential of the genera diversity indices were calculated using PAST free software version 3.

3. Results

3.1. Soil samples analysis

One hundred sixty-four PSBs strains were obtained from the rhizosphere of legumes, cereals, and spontaneous plants based on morphologically different colonies and remarkable halo solubilization formed in the NBRIP-TCP medium. Fifty-one strains showed different capacities for P-solubilization and soluble P concentration in NBRIP-TCP and NBRIP-RP mediums and were named as PSBs. Ait Saleh region showed the highest PSBs load of $3.16x10^4$ (Cells.Kg^{-1} of soil). The value of air-filled porosity present in the studied soils indicates adequate pore distribution and continuity; Dayt Al Amira and Ait Saleh present the highest value of 15%. The result confirms that Ait Saleh has the maximum organic matter (5.64%) and permeability (29 mm.hr^{-1}). On the other hand, the Dayt Al Amira site showed the highest amount of P_2O_5, K_2O, and MgO (98.7 mg.Kg^{-1}, 990.7 mg.Kg^{-1}, and 1213.3 mg.Kg^{-1} respectively), however, Tafrant site has the highest value of CaO with 9450 mg.Kg^{-1}. There was a positive correlation between total N and mineral N in the studied regions, Ait Yaakoub and Dayt Al Amira have the highest mineral N value (8.9 and 8.2 mg.Kg^{-1}), respectively, against 0.3 % of total N, followed by Ait Saleh and Tafrant. In terms of soil pH, it was close to neutral or slightly basic (pH = 8.23 on average), and the soil was moderately calcareous. Cation exchange capacity ranged between 31.6 and 41 cmol.kg^{-1}. Maximum electrical conductivity, reflecting soil salinity, was recorded in the Ait Yaakoub sample at 300.33 (ds.m^{-1}).

Table 3. PSBs load and Physico-chemical analysis of the soil studied sites.

Sites/Parameters	Tafrant	Ait Yaakoub	Dayt Al Amira	Ait Saleh
PSBs load (Cells.Kg^{-1})	59.5	198	1040	31600
Bulk Density (%)	1446	1459	1464	1462
Air Filled Porosity (%)	13	14	15	15
Specific Gravity (kg.m^{-3})	2.81	2.74	2.65	2.67
Permeability (mm.hr^{-1})	26	28	28	29
P_2O_5 (mg.Kg^{-1})	10	46.7	98.7	23.3
K_2O (mg.Kg^{-1})	432	846.3	990.7	373
CaO (mg.Kg^{-1})	9450	8550	5250	6375
MgO (mg.Kg^{-1})	410.7	854	1213.3	917.3
P (mg.Kg^{-1})	0.04	0.02	0.04	0.03
K (mg.Kg^{-1})	0.21	0.21	0.46	0.26
Fe (mg.Kg^{-1})	1.29	0.62	1.24	0.94
Al (mg.Kg^{-1})	1.02	0.79	1.19	0.95
Ca (mg.Kg^{-1})	2.18	0.54	2.14	1.79
Mg (mg.Kg^{-1})	0.39	0.17	1.41	0.89
Na (mg.Kg^{-1})	0.44	0.68	0.84	0.97

Ag, As, Be, Bi, Co, Nb, Ni, Pd, Pt, Se, Zr < 0.001. B, Ba, Cd, Cr, Cu, Li, Mn,Mo, Pb, Sb, Si, Sn, Sr, Ti, V, Zn < 0.01

The average percentage of nutrient parameters in the soil showed important mean values. Agricultural legume soils analyzed for physicochemical characteristics revealed that Dayt Al Amira has the highest mineral content region with remarkable values of Al, Ca, Mg, and P (Table 3). We noted that this region has a silty clay loam texture with just 8% of sand. Moreover, the Dayt Al Amira region has minimal values of total limestone (8.3 %) and active limestone (2.5 %). In addition, from the mineralogical compositions of different sites analyzed with ICP, we notice that Al, Ca, Fe, K, Mg, Na, and P are the most abundant minerals in various sites' distribution.

3.2. Climatic study using Emberger bioclimatic quotient

The regions studied are characterized by different thermal and water profiles. Rainfall-thermal quotients (Q_2) for the different regions using three methods show that all formulas give approximately the same results. The value of Q_2 using Stewart's formula differs only slightly from the Q_2 developed by Emberger and Mokhtari (Table 4).

Table 4. Rainfall-thermal quotients (Q_2) of the four studied sites.

Regions/Q_2	Tafrant	Ait Yaakoub	Dayt Al Amira	Ait Saleh
Q_2 Emberger	172.52	101.24	127.71	126.35
Q_2 Stewart	172.70	101.24	125.28	125.45
Q_2 Mokhtari	172.49	101.22	127.68	126.33
Average	172.57	101.23	126.89	126.04

Emberger subdivided the climatic zone into characteristic zones combining the rainfall-thermal quotients Q_2 obtained and the minimum temperatures of the coldest month (m) (limiting factor). The Q_2 values plotted on the Emberger diagram illustrate the shift in Fig 2. We noted that Dayt Al Amira and Ait Saleh sites have close values of Q_2 (126 approximately). However, the Tafrant site has the most significant value and the Ait Yaakoub site has the minimum value. These results give an idea of the PSBs distribution in these regions given that the Ait Saleh and Dayt Al Amira sites have the most important PSBs load respectively. The climatogram analysis reveals that the Tafrant site is the only site subjected to the humid bioclimatic stage in warm winter with Q_2 being approximately 172.57. On the other hand, the Ait Yaakoub site is placed at the limit of sub-humid and semi-arid climate with warm winter and Q_2 close to 101.23, whereas the two other sites Dayt Al Amira and Ait Saleh are in sub-humid climate in soft winter, with Q_2 close to 126.89 and 126.04, respectively. Furthermore, the umbrothermal diagram shows that the Tafrant site has the most precipitation but is countered by high temperatures, such as the Ait Yaakoub site, which exceeds 17.8 degrees Celsius on an annual basis. This is not the case for the Ait Saleh and Dayt Al Amira sites, which have significant rainfall and moderate to low temperatures respectively.

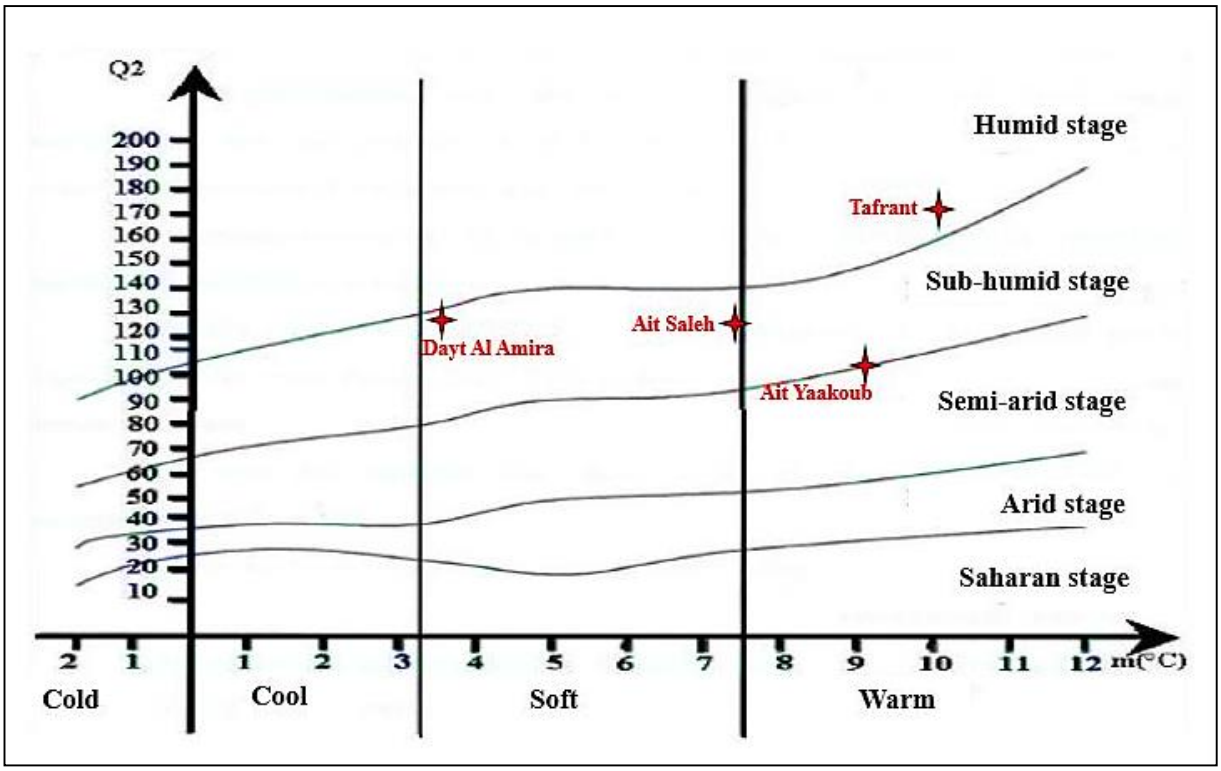


Figure 2. Emberger climatogram of soil studied sites.

The lowest average rainfall in all regions makes July the driest month of the year, with only 2 mm in Tafrant, 4 mm in Ait Yaakoub, 13 mm in Ait Saleh, and 25 mm in Dayt Al Amira. While an average of 136 mm makes December the month with the highest rainfall in Tafrant. Although, Ait Yaakoub record's rainfall is recorded in November with an average of 70 mm. However, in Ait Saleh and Dayt Al Amira, April has the highest rainfall of the year with an average of 79 mm and 80 mm, respectively (Fig 3). This figure is depicting a negative correlation among temperature and PSB load. We note that the high microbial load at the Ait Saleh site may also be influenced by other parameters studied. The strong negative correlation between rainfall and microbial load is clearly observed at the Tafrant site. On the other hand, moderate rainfall favours microbial load at the Ait Saleh and Dayt Al Amira sites.

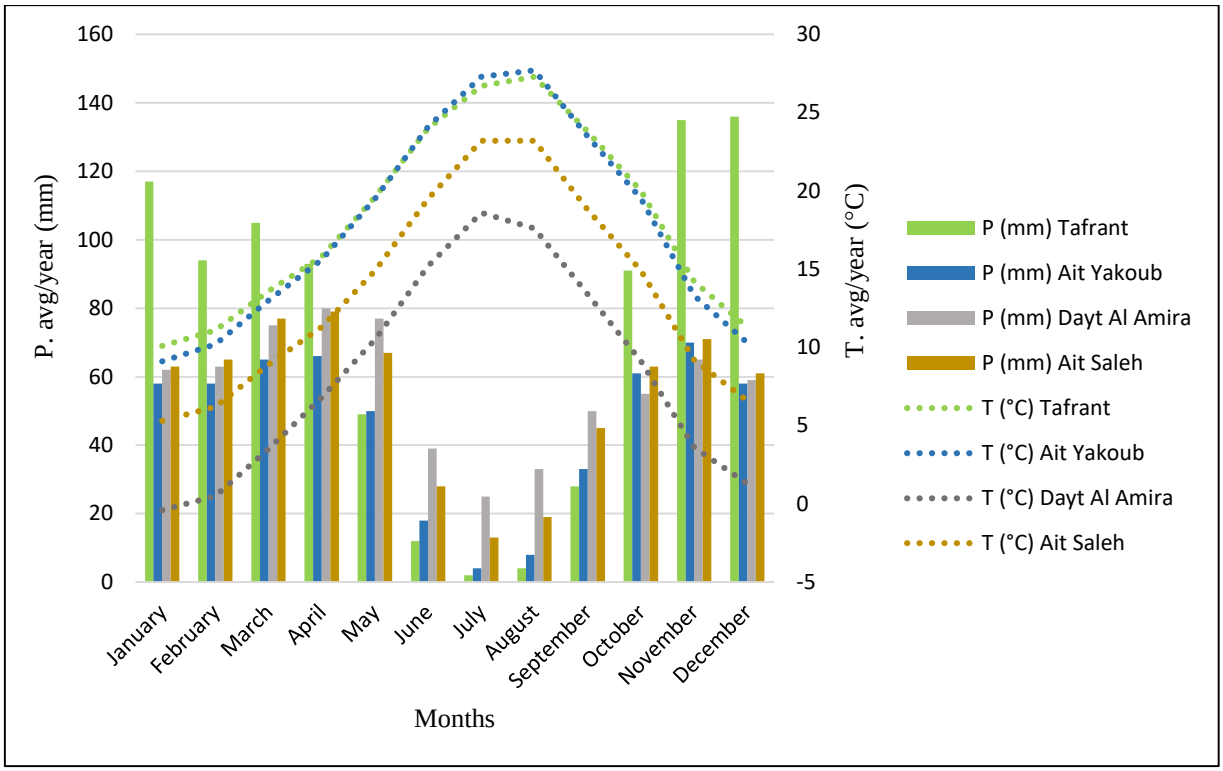


Figure 3. Umbrothermal diagram of the studied sites.

3.3. Screening of bacterial plant growth promoting traits

3.3.1. Phosphate solubilizing assessment

Results revealed that all isolated strains from studied regions were able to produce halo-forming colonies on the NBRIP-TCP amended solid medium. Although, there was a significant difference in all tested strains. The solubilization halo zone ranged from 1.1 cm to 4.2 cm, and the appearance of the halo zone in all strains was noticed after the 4th day of incubation. Isolate WJEF8 shows a maximum solubilization index with PSI= 3.84 (Janati et al., 2022) (Table 5). Furthermore, we noted that an increase in incubation time leads to an improvement in the halo zone size of each isolate, with a correlation between incubation time and halo zone size. The isolated strains were further screened to confirm their inorganic phosphate solubilizing efficiency on TCP- and RP-NBRIP broth medium. The results of 9 days of solubilization experiments and pH variation are presented in (Fig 4). The obtained results show that when TCP was used in NBRIP broth as the only P source, the concentration of soluble phosphorus released by the isolates in each site varied between 71.71 mg.L^{-1} and 94.54 mg.L^{-1} accompanied by significant variations between the different isolates ($p \leq 0.05$). Maximum TCP solubilization was observed for the WJEF61 strain.

Table 5. The average PSI in NBRIP-TCP solid medium of all studied strains of each sampling site.

Sites	Strains	PSI
Tafrant	WJEF2	2.29
	WJEF3	2.47
	WJEF4	2.31
	WJEF4'	2.56
	WJEF5	2.50
	WJEF8	3.84
Ait Yaakoub	WJEF9	1.90
	WJEF10	2.15
	WJEF11	2.54
	WJEF12	2.38
	WJEF13	2.07
	WJEF15	2.46
	WJEF16	2.22
	WJEF17	2.29
	WJEF18	2.36
Dayt Al Amira	WJEF21	2.09
	WJEF22	2.75
	WJEF24	2.85
	WJEF25	2.90
	WJEF26	2.67
	WJEF28	2.67
	WJEF29	1.40
	WJEF30	2.57
	WJEF31	2.15
	WJEF32	2.33
	WJEF33	2.50
	WJEF34	2.33
	WJEF36	2.31
	WJEF37	2.39
Ait Saleh	WJEF38	2.30
	WJEF39	2.50
	WJEF40	2.78
	WJEF41	2.27
	WJEF43	2.20
	WJEF44	2.09
	WJEF45	2.30
	WJEF46	2.27
	WJEF49	2.50
	WJEF50	2.33
	WJEF51	2.33
	WJEF52	2.40
	WJEF53	2.33
	WJEF54	2.22
	WJEF55	2.11
	WJEF56	2.33
	WJEF57	2.30
	WJEF59	2.30
	WJEF61	2.14
	WJEF62	2.64
	WJEF63	2.33
	WJEF64	2.17

On the other hand, the concentration of soluble P in the RP-NBRIP medium in each site varied between 18.69 $mg.L^{-1}$ and 40.43 $mg.L^{-1}$ accompanied by significant variations between the different isolates ($p \leq 0.05$). The maximum solubilization of RP was observed for the WJEF51 strain. These isolates were found to solubilize TCP more than RP and the most occurred ones were in the Ait Saleh site. The results of the experiments indicated that P solubilization varied according to the microbial isolates and the type of insoluble phosphate (TCP or RP). The solubilization of TCP and RP in the NBRIP liquid medium by the tested strains was accompanied by a significant decrease in pH. The pH of the medium inoculated with the selected strains decreased significantly from 4.88 to 4.47 (TCP) and 4.67 to 4.50 (RP), respectively, after nine days of incubation. Based on the one-way analysis of variance (ANOVA), we noted a significant difference between the solubilization of RP and TCP within the four sites. It is interesting to mention that the Ait Saleh site has the highest solubilization potential, taking into account that this location has the maximum PSBs load.

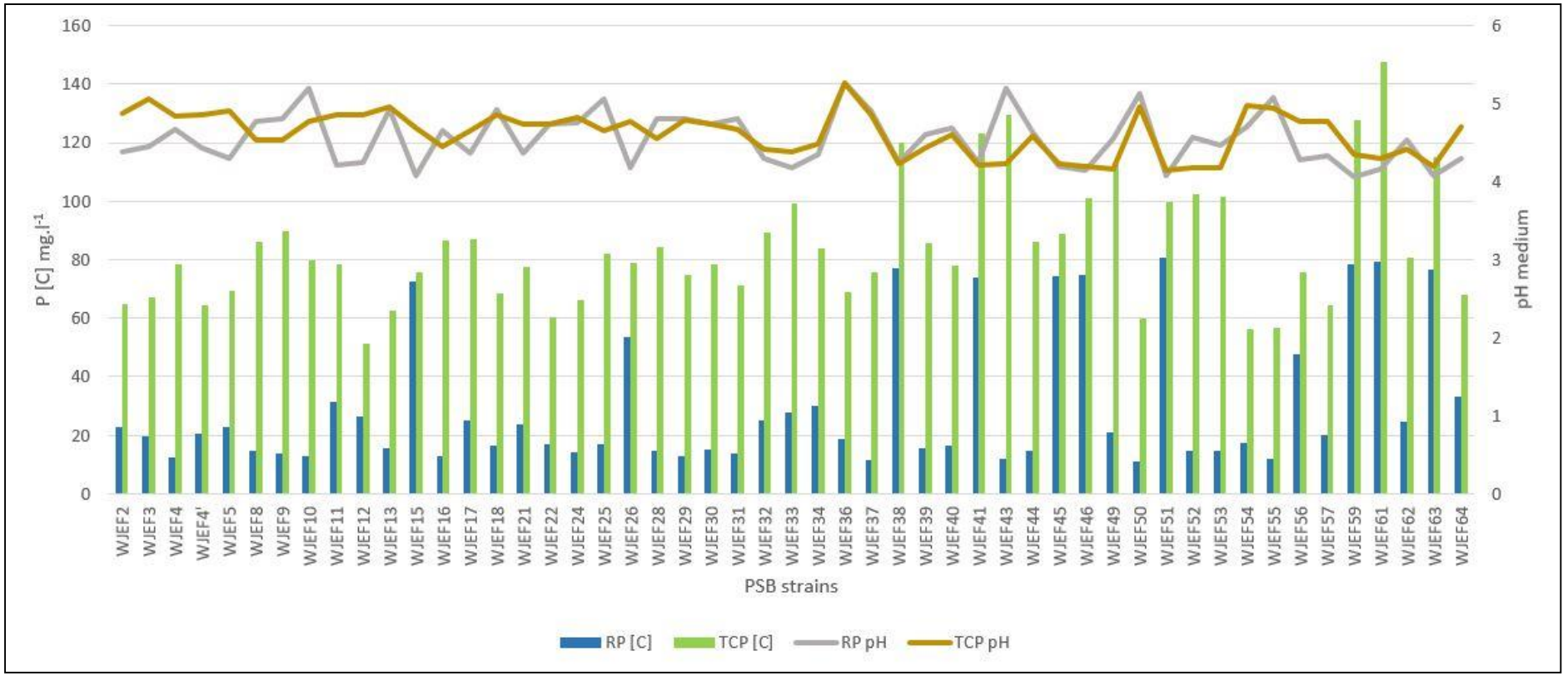


Figure 4. The average concentration of RP and TCP solubilization accompanied with pH variation in NBRIP liquid medium of all studied strains after nine days of inoculation.

3.3.2. Analysis of auxins production

The data obtained on auxins production showed that from 51 isolates approximately 33.34 % can produce auxins (Fig 5). It was observed that the isolates produce maximum auxins in the medium, by giving intense pink colour with the highest absorbance expressed as an optical density (OD) value. These isolates showed varying rates of production from 101.87 µg.mL^{-1} to 8.426 µg.mL^{-1}**.** We emphasize that the Ait Saleh site is the source of 70.59% of the bacteria that produce auxins, in which WJEF59 has the highest auxins concentration of 101.87 µg.mL^{-1}**.**

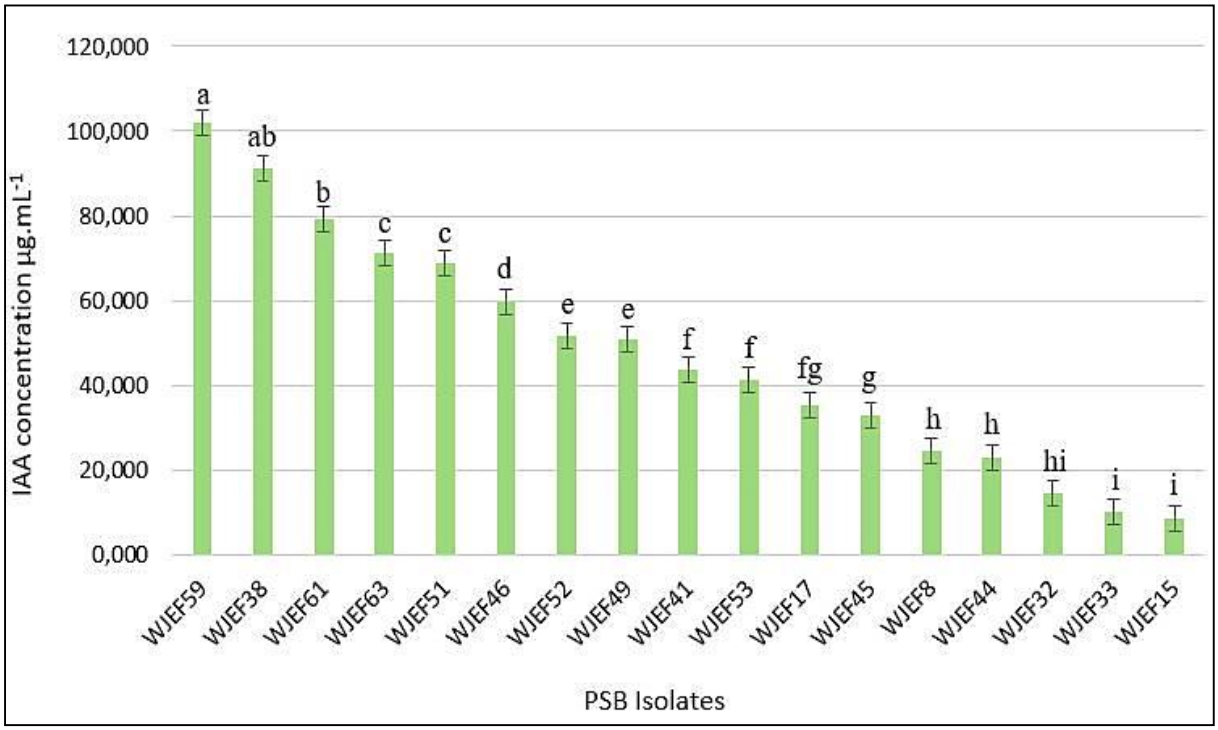


Figure 5. Auxins concentration levels produced by bacterial isolates. The different letters indicate a significant difference ($p<0.05$), Mean values ($n = 3$).

3.3.3. Analysis of siderophores production activity

Based on the data collected on siderophores production, out of 51 isolates, approximately 25.49 % can produce siderophores (Fig 6). These isolates produced between 12.06 and 72.26 Units. Like auxins production, the Ait Saleh site also contains the bacterial strains that produce siderophores accounting for 69.23% of the total bacteria isolated, where WJEF63 has the highest value of 73,26 Units.

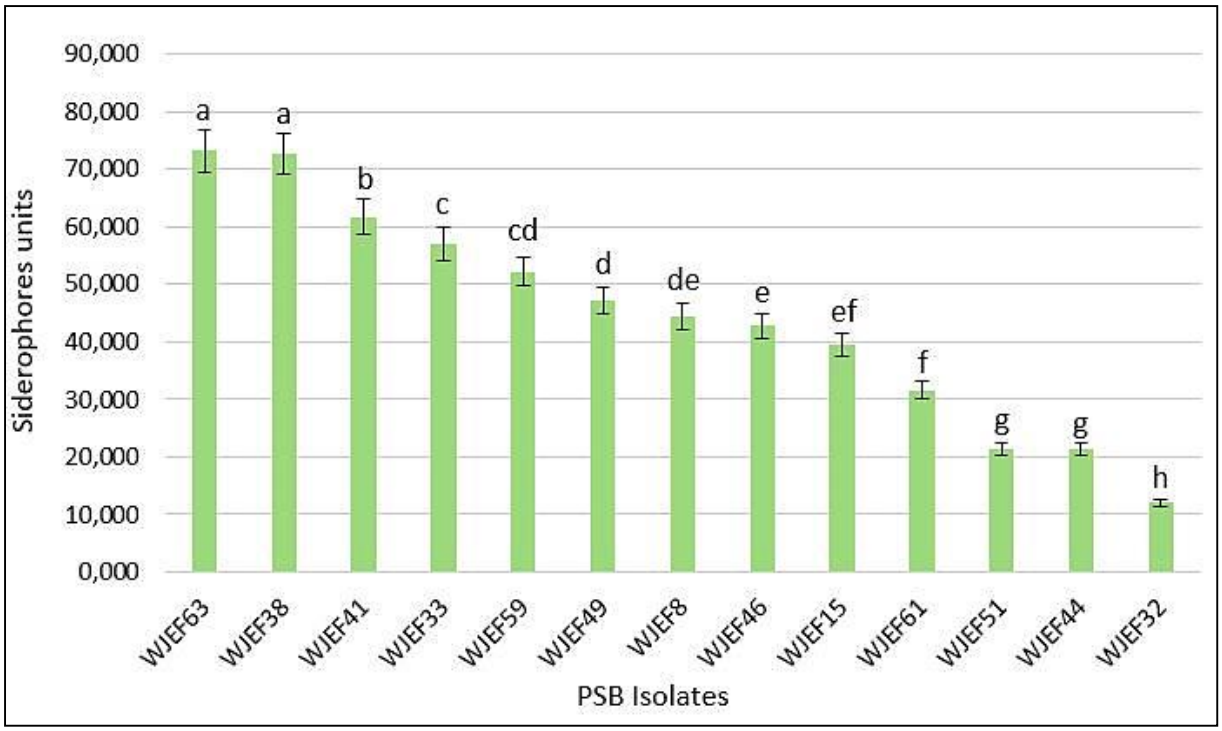


Figure 6. Siderophores levels produced by bacterial isolates. The different letters indicate a significant difference ($p<0.05$), Mean values ($n = 3$).

3.3.4. ACC deaminase activity

From the 51 PSB isolates, five (WJEF15, WJEF26, WJEF38, WJEF46, and WJEF63) were assessed to be ACC deaminase producers in which values ranged from 0.27 to 0.58 nmol α-ketobutyrate mg/protein/h) (Table 6). The highest ACC deaminase activity was exhibited by bacterial strain WJEF38 from the Ait Saleh site (0.58 nmol α-ketobutyrate mg/protein/h).

Table 6. ACC deaminase activity by PSB strains. Data represent the mean ± of three independent replicates. Values with the same superscript letter were not significantly different according to the Duncan test performed ($P < 0.05$).

Strains	WJEF15	WJEF26	WJEF38	WJEF46	WJEF63
ACC deaminase activity	0.56^{a}	0.27^{c}	0.58^{a}	0.49^{b}	0.55^{a}

3.3.5. Qualitative test for Zinc solubilising bacteria

The results on Zn-solubilization revealed that approximately 23.53% of 51 isolates could solubilize four sources of Zn: zinc oxide, zinc phosphate, zinc carbonate, and zinc sulfide. When compared to other compounds, the isolates were found to be more efficient to solubilize zinc from zinc phosphate. Bacteria from the Ait Saleh region dominate this study, accounting for 75% of all zinc-solubilizing bacteria. The halo zone of solubilization ranged from 14 mm to 31 mm, whereas ZSI ranged from 2.22 to 4, (Table 7). We noted that all zinc-solubilizing bacteria have different preferences for zinc compounds, except WJEF59, which

has a high potential to solubilize any zinc source, although this isolate is from the Ait Saleh site.

Table 7. Zinc-solubilization index of PSB isolates after 7 days on Tris-minimal medium supplemented with insoluble zinc compounds; values followed by the same letter a, b, c, d, e, f, g, h, i, in the same column show no statistical significant differences ($p < 0.05$). Mean values ($n = 3$).

Isolates	Zinc oxide	Zinc phosphate	Zinc carbonate	Zinc sulphide
WJEF8	2,44 ± 1.578^{b}	2,28 ± .989^{a}	3,10 ± .325^{g}	3,00 ± .268^{e}
WJEF15	3,40 ± .856^{f}	2,64 ± 1.061^{c}	2,42 ± 1.035^{c}	2,41 ± 0.897^{a}
WJEF17	2,73 ± 1.247^{c}	2,64 ± 1.237^{c}	2,34 ± 1.449^{b}	2,44 ± 1.268^{a}
WJEF33	2,35 ± .707^{a}	2,92 ± .33^{e}	2,46 ± .975^{c}	3,08 ± .745^{e}
WJEF38	3,27 ± .865^{e}	2,53 ± 1.323^{b}	2,79 ± .69^{e}	3,56 ± .33^{f}
WJEF41	3,44 ± .424^{f}	3,20 ± .268^{f}	2,53 ± .865^{d}	2,64 ± 1.65^{b}
WJEF44	3,09 ± .749^{d}	3,30 ± .231^{f}	2,92 ± 1.138^{f}	2,85 ± .89^{d}
WJEF46	2,38 ± 1.106^{a}	2,77 ± .84^{d}	2,81 ± .865^{e}	3,09 ± .747^{e}
WJEF49	3,30 ± .56^{e}	3,82 ± .19^{h}	2,53 ± 1.332^{d}	2,46 ± .236^{a}
WJEF59	3,88 ± .495^{h}	4,00 ± .424^{i}	2,92 ± 1.055^{f}	3,56 ± .06^{f}
WJEF53	2,64 ± .935^{c}	3,00 ± .14^{e}	2,22 ± 1.543^{a}	2,77 ± .115^{c}
WJEF51	2,50 ± 1.138^{b}	3,56 ± .882^{g}	2,91 ± .995^{f}	2,40 ± 1.597^{a}

3.4. Identification of phosphate-solubilizing bacteria

From one hundred and sixty-four bacterial isolates, fifty-one strains isolated from four separate sites representing different colony types were prepared for phenotypic identification. The morphological characteristic of the selected isolates showed that most bacterial colonies were entire, yellow, viscid, rod, and coccobacilli shapes. Except for fifteen isolates, gram reaction indicates that all strains are gram-negative. In addition, all selected isolates were positive for cell motility test and catalase activity. Only one isolate from Ait Yaakoub and one isolate from Ait Saleh can use citrate for growth. Whereas the majority of isolates that can produce H_2S belong to the Ait Saleh site In this study, nine bacterial genera were identified based on different tests performed in specific media for the phenotypic characteristics, namely *Pseudomonas*, *Rhizobium*, *Clostridium*, *Serratia*, *Proteus*, *Burkholderia*, *Staphylococcus*, *Streptococcus*, and *Bacillus* (Tables 8 and 9).

Table 8. Morphological and biochemical identification of the isolated strains.

Sites	PSB Isolates / Characteristics	Genera	Margin	Colour	Texture	Shape	Arrangement	Gram staining	Catalase	Motility	GPA (Glucose)	GPA (Lactose)	Citrate utilisation	Starch utilisation	Gelatine hydrolysis	Urea hydrolysis	King A medium	YEM medium	MSA medium	H_2S Production
Tafrant	WJEF2	Ps	E	Y	V	R	CR	-	+	+	+	+	-	+	+	-	+	-	-	-
	WJEF3	Ps	E	Y	V	R	S	-	+	+	+	+	-	+	+	-	+	-	-	-
	WJEF4	Str	E	Y	V	R	CR	+	+	+	+	+	-	+	+	+	-	+	-	-
	WJEF4'	Bu	E	Y	M	CB	S	-	+	+	+	+	-	-	+	-	+	+	-	-
	WJEF5	Cl	E	Y	V	R	SP	+	+	+	+	+	-	-	-	-	+	+	-	-
	WJEF8	Rh	E	W	V	C	S	-	+	+	+	+	-	+	+	-	-	+	-	-
Ait Yaakoub	WJEF9	Cl	E	Y	D	R	CR	-	+	+	+	+	-	-	-	-	-	+	-	-
	WJEF10	Rh	E	W	V	CB	S	-	+	+	+	+	+	+	+	-	-	+	-	-
	WJEF11	B	E	Y	V	R	CR	+	+	+	+	+	-	+	+	-	-	+	-	-
	WJEF12	St	E	Y	M	R	CR	+	+	+	+	+	-	+	-	-	-	-	+	-
	WJEF13	Ps	E	Y	M	CB	S	-	+	+	+	+	-	+	-	-	+	+	-	-
	WJEF15	B	E	Y	V	R	S	+	+	+	+	+	-	+	+	-	-	+	-	-
	WJEF16	Se	E	Y	D	CB	S	-	+	+	+	+	-	-	+	-	-	+	-	+
	WJEF17	Ps	E	Y	V	CB	S	-	+	+	+	+	-	+	-	-	+	+	-	-
	WJEF18	B	E	Y	V	R	CR	+	+	+	+	+	-	+	+	-	-	+	-	-
Dayt Al Amira	WJEF21	St	E	Y	M	R	SP	+	+	+	+	+	-	+	-	-	+	-	+	-
	WJEF22	St	E	Y	V	R	CR	-	+	+	+	+	-	+	-	-	+	-	+	+
	WJEF24	Ps	E	Y	V	R	CR	-	+	+	+	+	-	+	+	-	+	+	-	-
	WJEF25	Rh	I	Y	M	C	S	-	+	+	+	+	-	+	+	-	-	+	-	-
	WJEF26	Ps	E	Y	V	R	CR	-	+	+	+	+	-	+	+	-	+	+	-	-
	WJEF28	Ps	E	Y	V	R	CR	-	+	+	+	+	-	+	+	-	+	+	-	-
	WJEF29	B	E	Y	M	R	S	+	+	+	+	+	-	+	+	-	+	+	-	-
	WJEF30	B	I	Y	D	R	CR	+	+	+	+	+	-	+	+	-	-	+	-	-
	WJEF31	Ps	E	Y	V	R	CR	-	+	+	+	+	-	+	-	-	-	+	-	-
	WJEF32	Rh	E	Y	V	R	CR	-	+	+	+	+	-	+	+	-	-	+	-	-
	WJEF33	Rh	E	Y	V	R	CR	-	+	+	+	+	-	+	+	-	-	+	-	-
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Ait Saleh	WJEF38	St	I	Y	M	C	SP	+	+	+	+	+	-	-	-	-	-	+	+	+
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	WJEF49	Ps	E	Y	M	R	CR	-	+	+	+	+	-	+	+	-	+	-	-	-
	WJEF50	Pr	E	Y	V	R	CR	-	+	+	+	+	-	+	+	+	+	+	-	-
	WJEF51	Rh	E	OW	V	CB	SP	-	+	+	+	+	-	+	-	-	-	+	-	+
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	WJEF53	Rh	I	OW	V	CB	S	-	+	+	+	+	-	+	-	-	-	+	-	-
	WJEF54	Ps	E	Y	M	R	SP	-	+	+	+	+	-	-	+	-	+	-	-	-
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	WJEF59	B	I	Y	M	CB	S	+	+	+	+	+	-	+	+	-	-	+	-	-
	WJEF61	R	E	OW	V	CB	S	-	+	+	+	+	-	-	-	-	-	+	-	-
	WJEF62	Ps	E	W	V	R	CR	-	+	+	+	+	-	+	+	-	+	-	-	-
	WJEF63	Rh	E	OW	V	CB	S	-	+	+	+	+	-	+	+	-	-	+	-	+
	WJEF64	Rh	E	Y	M	CB	S	-	+	+	+	+	-	+	+	-	-	+	-	-

Tested positive utilized as substrate; tested negative not utilized substrate. Colony morphology: margin E: entire / I: irregular; color, Y: yellow / W: white / OW: off-white; texture, D: Dry / V: viscid /M: mucoid. Morphology: shapes; R: rod / C: cocci / CB: coccobacilli; arrangement, SP: single pairs / CR: chain rods / S: solitaire. B: Bacillus, Ps: Pseudomonas, Se: Serratia, Str: Streptococcus, Bu: Burkholderia, Rh: Rhizobium, Cl: Clostridium, St: Staphylococcus, Pr: Proteus.

3.5. Relationship between the potential and diversity of PSBs and environmental conditions of the sampling sites

The association between the PSBs load, PSBs potential, soil properties, and environmental variables was studied by a dimensional analysis (Principal component analysis (PCA)) (Fig 7) to identify how these variables are correlated with the sampling sites. According to the PCA analysis, the first synthetic component (axis x) corresponds to the studied parameters, while the second synthetic component (axis y) corresponds to the site's distribution. The studied parameters (environmental condition, soil properties, PSBs load, and PSBs potential) explained 98.5 % of the total variation in the distribution of four studied sites.

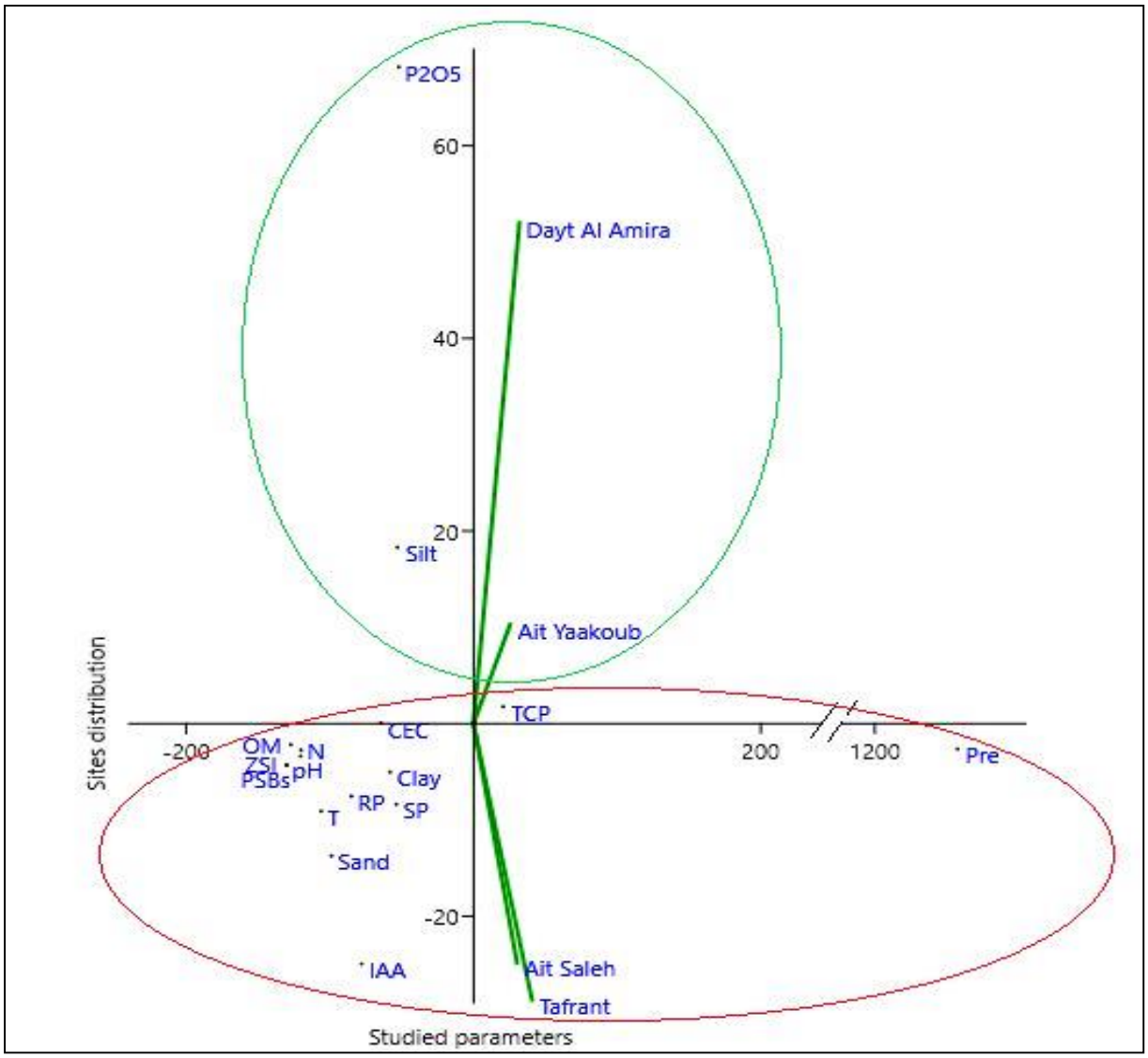


Figure 7. Sites sample distribution using principal component analysis of the various parameters studied. P_2O_5: phosphorus pentoxide, TCP: [C] concentration of solubilized tricalcium phosphate, RP [C]: concentration of solubilized rock phosphate, Pre: precipitation, T: temperature, CEC: Cation exchange capacity, OM: organic matter, N: nitrogen mineral, ZSI: zinc solubilization index, SP: siderophores production, IAA [C]: Auxins produced concentration.

The Figure 7 shows that Dayt Al Amira and Ait Yaakoub sites are heavily clustered with the silt and soil P_2O_5 concentration and they are in the opposite direction of all studied parameters, where a negative correlation is found. While Tafrant and Ait Saleh sites are positively correlated with all studied parameters except silt and soil P_2O_5 concentration. From our results, we can note that the studied sites are subdivided into two groups, in which the soil P_2O_5 content has no impact on microbial load or their PGP potential. Whereas, sand, clay, nitrogen mineral, organic matter, pH, cation exchange capacity, and temperature, on the other hand, have a negative effect on PSBs load also their PGP capacity.

Furthermore, the correlation between the ability of isolates to solubilize phosphate on NBRIP medium and selected environmental conditions prevailing at the locations from which soils were sourced was investigated. Based on morphological diversity, Ait Saleh had the highest number of individuals (22 individuals), while Tafrant had the lowest (6 individuals) (Table 9). According to Shannon *H's* diversity and Dominance *D*, the variation among the four studied sites was somewhat important and close. Tafrant had the highest diversity evenness, while Ait Yaakoub had the lowest.

Table 9. Genera diversity indices of isolates based on morphological and biochemical characteristics.

	Tafrant	**Ait Yaakoub**	**Dayt Al Amira**	**Ait Saleh**
Taxa_S	5	6	5	6
Individuals	6	9	14	22
Dominance_D	0,2222	0,2099	0,2347	0,2066
Simpson_1-D	0,7778	0,7901	0,7653	0,7934
Shannon_H	1,561	1,677	1,512	1,68
Evenness_e^H/S	0,9524	0,8916	0,9076	0,8947
Brillouin	0,981	1,146	1,168	1,372
Equitability_J	0,9697	0,9359	0,9397	0,9379

A hierarchical heat map of the bacterial distribution at the different studied sites, classified by genus, is presented in Fig 8. The heat map graph illustrated the distribution of each bacterial genus in each site and indirectly in each climate stage. In the present study, the pseudomonas genera were mostly recorded in all sites and the highest and lowest abundance of pseudomonas was found in Ait Saleh and Tafrant, respectively. Rhizobium genera were present in all sites and the maximum abundance was found in the Ait Saleh site. In addition, Bacillus was common in all studied sites except for Tafrant. However, Streptococcus and Bulkholderia have been

signalled just in the Tafrant site. Among the sites, the highest bacterial diversity (6 genera) was identified in Ait Yaakoub and Ait Saleh.

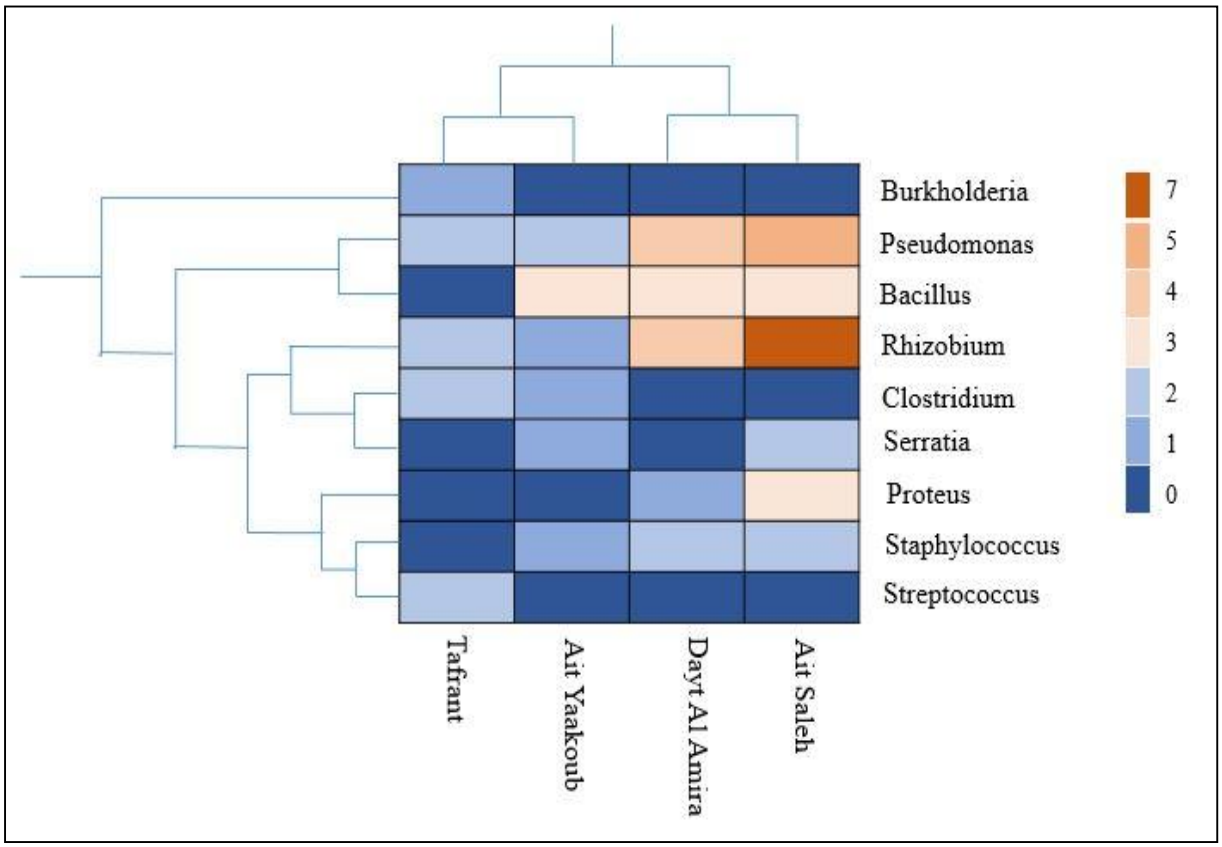


Figure 8. Hierarchically clustered heat map of the bacterial distribution of different communities from the studied rhizosphere at the genus level. A row represents the abundance of each bacterial genus from the identified groups, and a column stands for the studied site samples. The abundance for each bacterial genus was depicted by the colour intensity with the legend indicated at the right of the figure.

4. Discussion

Soil microbial communities are affected by several factors including soil conditions and environmental changes. These extreme events have serious repercussions on the structure of microbial communities, including the increase in the relative abundance of rare species and the emergence of pathogens capable of infecting plants. The present study focused on the effect of climate and soil conditions on the abundance, potential, and diversity of PSBs from the legumes rhizosphere located at different bioclimatic stages in four regions of Morocco. In addition, to assess the potential impact of these factors on the capacity of PSBs, we tested the P solubilization efficiency of isolates, evaluated their plant's growth trait potential, and assessed the impact of environmental conditions at each study site on the PSB strains. The selection of an efficient PSB is crucial, as it practically increases P in the plant rhizosphere (Aliyat et al., 2020). Phosphate solubilization capacity is considered a key factor in isolating highly efficient PSBs from agricultural soils (Aliyat et al., 2020). Among One hundred sixty-four (164) PSB

strains from different leguminous plants rhizosphere of four sites in the Fez-Meknes region, Fifty-one (51) strains show phenotypic stability of P solubilization after five successive subcultures in NBRIP-RP plates.

To date, only a few studies have focused on the population density of PSBs in different habitats and the factors that influence this. For this reason, we collected information on a place name, geographic location (latitude and longitude), mean annual precipitation (MAP) and mean annual temperature (MAT) of the study sites, soil physicochemical analysis, and the population density of the PSBs (expressed as the number of colony-forming units per kilogram of soil (CFU Kg^{-1})), pH, and total and available P of all samples. As result, all sites differed in both climatic and soil conditions. Soils in the investigated sites can be described as fertile and biologically active, with optimal properties for legume cultivation. Mineralogical compositions of different sites analyzed indicate that Al, Ca, Fe, K, Mg, Na, and P are the most important minerals in the distribution of the various sites. In addition, physicochemical characteristics revealed that the Dayt Al Amira site has the most significant values of the most minerals content with remarkable values of Al, Ca, Mg, Mn, and P. Maximum electrical conductivity, reflecting soil salinity, was recorded in the Ait Yaakoub soil sample of 300.33 ($ds.m^{-1}$) followed with Dayt Al Amira 241.2 ($ds.m^{-1}$), in addition, Dayt Al Amira registers the maximum cation exchange 37.2 ($cmol^{+}.kg^{-1}$). We focused on these geographic, climatic, and environmental parameters, as they are potentially important factors influencing the population density of PSBs in the environment (Crowther et al., 2019; J. Li et al., 2021). The population density of P-solubilizing microorganisms (PSMs) in environmental samples and its determinants is critical to understanding not only their population ecology but also their roles in regulating the biogeochemical cycling of P and mediating the plant uptake of this element (J. Li et al., 2021). As stated by Alori et al. (2017), factors influencing microbial P-solubilization abundance include vegetation extent, climatic zone, soil microorganisms interactions, ecological conditions, kinds of soil, plant types, agronomic practices, and soil physical-chemical properties such as organic matter and soil pH. Kavamura et al. (2021) report that edaphic conditions such as differences in soil physical and chemical properties drive microbiome assembly. Soil texture has also been shown to be important in structuring microbial communities as mentioned by Li et al. (2021). Microbial P-solubilization capacity is mainly regulated by N:P stoichiometry, it is concluded that long-term N inputs decreased microbial P-solubilizing and mineralizing capacity while P inputs favoured microbial immobilization via altering the microbial functional profiles (Dai et al., 2020). According to Lai et al. (2022), toxic metal pollution produces serious stress on the reproduction and growth of microorganisms in the soil, and the results of microbial

abundance showed that the total microbial biomass in toxic metal-contaminated soil was 42.86%, 53.33%, and 44.93% lower than those in non-contaminated soil. Naher et al. (2021) find that a higher number of total beneficial bacteria is associated with the highest score of soil quality index, with high OM, moderate pH value, and higher concentration of nutrients. Also, Duarah et al. (2011) reported that NPK Fertilization treatments showed a negative effect on the PSBs population, on the other hand, Jana et al. (2001) noted that the abundance of PSBs is closely related to C-N and N-P application. Likewise, He & Wan, (2022) reported that N fertilization treatment enriches culturable tricalcium PSB, phytate-degrading bacteria, and gcd and bpp abundances genes which were more abundant in silt + clay, in addition, P fractions were responsible for PSBs number and P-cycling-related gene abundance. In addition, soil rich in organic matter will favour microbial growth and therefore favour PSMs abundance (Alori et al., 2017)**.** Soils at the Ait Saleh site contained a higher content of organic matter, nitrogen, and P available than the other soils site, which showed the highest PSBs load of $3.16x10^4$ ($Cells.Kg^{-1}$ of soil), likely resulting from primary soil properties related to their different pedogenesis such as texture and nutrient abundance. However, research by Hoeber et al. (2020) showed that climatic factors may significantly determine the rate of decomposition of leaf litter which can also be an important source of soil organic matter (Koczorski et al., 2021). The significant positive relationships between soil PSB population densities and available P, NO_3-N, and dissolved organic carbon in the soil suggest functional couplings between soil PSBs and microbes responsible for soil nitrification and organic matter degradation. In terms of soil pH, it was close to neutral or slightly basic (pH = 8.23 on average), and the soil was moderately calcareous, which probably does not favour the bioavailability of P. Phosphate-solubilizing activities of PSBs are negatively affected by many factors such as temperature, pH, salinity, and dissolved oxygen (Rizvi et al., 2021). Soil pH is classically considered to be the major variable of soil chemistry due to its profound impact on countless chemical reactions (Ducousso-Détrez et al., 2022). Changes in chemical properties of rhizosphere soil, such as pH and nutrient availability can impact bacterial communities because soil pH is the main driver of microbial community structure including archaeal, bacterial, and fungal members (Kavamura et al., 2021). Soil pH values between 5.5 and 7.5 are the best for P availability, as they avoid P being fixed by aluminium, iron, or calcium, and therefore usable by plants (Alori et al., 2017)**.**

Besides soil properties, several abiotic factors can affect microbial communities (Kavamura et al., 2021). Abiotic stresses, including warm- or low-temperature environments, adversely affect the structure, composition, and physiological activities of soil microbes. Indeed,

temperature is the most vital environmental variable which adversely affects the composition, diversity, community structure, and microbial biomass, and decreases the soil nutrient pool (Rizvi et al., 2021). PSMs tend to be present at higher population densities in hot and humid regions than in dry and cold regions (J. Li et al., 2021). Except that this was not the case in our study where we found the lowest load of PSBs in the Tafrant site. In this paper, we noted that PSBs abundance was significantly different between the four sites and this was likely a result of differences in the environmental conditions; temperature, humidity, and precipitation. Phosphate solubilizing bacteria surviving at mesophilic, thermophilic, or psychrophilic temperatures release plant-available P from both inorganic and organic P stores. Therefore, they may serve as sustainable and inexpensive alternatives to high-cost and environmentally hazardous chemical P fertilizers (Rizvi et al., 2021). Therefore, the effort is directed to develop a pool of P-solubilizing soil bacteria that grow in the root region and are customized according to the crops and environmental conditions thus releasing plant-accessible P. Likewise, water is one of the most limiting factors for plant development and agricultural losses. Water affects microbial dynamics in three fundamental ways: as a resource, as a solvent, and as a transport medium (Tecon & Or, 2017). The improved soil water and heat status can stimulate soil microorganisms and soil enzymatic activity, influencing the long-term fertility of the soil (X. Wang et al., 2021). The increased water availability may favour the survival of microbes during dry periods by providing a better habitat, leading to enhanced microbial activities, respiration, and growth (Palansooriya et al., 2019). Kavamura et al. (2021) investigated the influence of soil water stress history, wheat genotypes with differences in their drought tolerance, and short-term decrease in soil water content on microbial communities of wheat. It was found that the water regime was the main driver of bacterial and fungal community structure in the rhizosphere and root samples of wheat. According to our findings, the Tafrant site has the maximum precipitation but is accompanied by a maximum annual temperature, which directly affects its climatic position in the Emberger diagram. The analysis of this climagram reveals that the station of Tafrant is the only site subjected to the humid bioclimatic stage but warm winter with Q_2 being approximately 172.57. On the other hand, the Ait Yaakoub region is placed at the limit of Sub-humid and Semi-arid climate with also warm winter and Q_2 close to 101.23, whereas the two other stations Dayt Al Amira and Ait Saleh classified as Sub-humid climate in soft winter, with Q_2 close to 126.89 and 126.04, respectively. This explains why the latter two regions have a high abundance of PSBs. Instead, Kałużewicz et al. (2018) noted that lack of water strongly influences the ability of crops to exchange gases, causing stomatal closure (Del Buono, 2021). Rising temperatures could make some large agricultural areas unsuitable for

cultivation over time (Rakkammal et al., 2023), and this could be due to increased evapotranspiration and reduced soil microbial quality. We suggest that evapotranspiration is the most important factor shaping microbial diversity. This interference impairs the photosynthetic activity and evapotranspiration of plants, thus affecting plants' biomass production (Bulgari et al., 2019) and microbial biomass diversity. Del Buono, (2021) showed that particular CO_2 enrichment fertilization in the open air can stimulate daily C uptake, plant growth, above-ground biomass production, microbial communities, and soil C, by reducing evapotranspiration for stomatal closure. Similarly, reductions in evapotranspiration cause impairment in the plant's capacity to uptake nutrients, and indirectly to a lack of sufficient microbial population resources, which explains the high PSBs load in the Ait Saleh site, given the favourable conditions. These results provide further evidence of the functional coupling between PSBs and their geographical location, whereby edaphic and climatic conditions significantly affect their occurrence.

The solubility index on TCP-NBRIP medium was in agreement with previous studies conducted on different plants' rhizosphere, where the PSBs solubility index ranges from 2 to 3.84 (Sadik et al., 2013; Rfaki et al., 2014; Manzoor et al., 2017). The PSB-isolated strains (from the four sites) used in this work were able to increase P availability in an NBRIP liquid medium containing either RP as the only P source or TCP. Our results support the idea that PSBs are characterized by their ability to easily and efficiently solubilize inorganic P. Soluble P concentration in the medium containing RP was between 18.69 $mg.L^{-1}$ and 40.43 $mg.L^{-1}$, while the medium containing TCP was between 71.71 $mg.L^{-1}$ and 94.54 $mg.L^{-1}$. We noted that strains from the Ait Saleh site contain the highest value of solubilization with either TCP or RP. These results are in agreement with other studies done by Elhaissoufi et al. (2020), who report that PSBs can solubilize insoluble inorganic P compounds, such as TCP and RP. Phosphate solubilization is accompanied by a pH medium decrease when compared to control, both in media containing RP and TCP. Furthermore, we reported that as pH values decrease, the P concentration increases. This is noted by Batool & Iqbal, (2019) who indicate a strong correlation between P solubilization and pH decrease. The acidic (lowering of pH) environment of the medium suggests the release of organic acids that occurs on the outer face of the cytoplasmic membrane via the direct oxidation pathway (Goswami et al., 2019). Many reports have established a positive correlation between pH drop and soluble P concentration in the liquid culture medium (Rizvi et al., 2021). In addition, we noted that strains isolated from Ait Saleh and Dayt Al Amira sites (classified as Sub-humid climates in soft winter) were the most efficient PSBs regarding their P solubilizing capacity, which also explains the PSBs load in

these regions taking into account the availability of P and the pH value. We hypothesize that the available P in soil may influence PSBs communities in the rhizosphere to some extent. Li et al. (2021) reveal that total P rather than pH regulate the population density of PSMs in continental and global environmental samples. Bodenhausen et al. (2019) found marked root microbiota changes in Arabidopsis between low-P and high P-fertilized treatments, while Huang et al. (2017) indicated that soil microbes were insensitive to an elevated P availability in a subalpine spruce plantation. However, our analysis suggests that climate (temperature and precipitation) interact with soil physicochemical conditions to affect soil P availability and indirectly PSBs occurrence and diversity. Although P-solubilization ability is common in the genus Bacillus and Pseudomonas, various species and strains differ in their capacity to mobilize P due to differences in the secretion of organic acids, phosphatases, and phytase or other P-solubilizing mechanisms (Kirui et al., 2022)**.** Several studies use Bacillus (Maougal et al., 2014) and Pseudomonas (Linu et al., 2019) species as bioinoculants that improve P availability for various plants. In our study, we found that Pseudomonas is the dominant genus of PSBs in the group of isolated strains, followed by the Bacillus genus. Less frequent isolates were of the *Streptococcus* and *Burkholderia* genera. In contrast, the most efficient P-solubilizing strain was the Rhizobium genus. This effect might be based on the varying site-specific environmental conditions and their interactions with the genotypes. Zhang et al. (2019) mentioned that soil pH, soil OM, fluorescein diacetate hydrolase activity, and soil bioavailable Cd were the dominant environmental factors that significantly influenced the microbial relative abundance and explained most of the variance in the species-environment correlations. Zhu et al. (2022) pointed out that organic matter content, oxygen content, pH, and total phosphorus were identified as important environmental factors determining the functional diversity of bacterial communities and survival, while pH influences species evolution.

Among the PGP traits, auxins are powerful growth hormones produced naturally by plants. They are found at the tips of shoots and roots and support cell division as well as stem and root growth. They can also influence plant orientation by directing cell division to one side of the plant in response to sunlight and gravity (G. L. B. Gomes & Scortecci, 2021). Indole-3-acetic acid (IAA) is the most common naturally occurring plant hormone of the auxin class; thus, in our next study, we anticipate verifying and quantifying specifically the IAA production using High-performance liquid chromatography HPLC to provide additional details on the potency of PSBs concerning their source samples. The results show the capacities of PSBs to solubilize zinc, production of auxins and siderophores, and ACCD activity, which were positively correlated with PSB's load and site location from which the strains were isolated. However,

this result alone did not demonstrate whether the ability of isolates to enhance PGP traits would assist the bacteria themselves to withstand the stress conditions. These suggest that water stress and low soil fertility with higher levels of Cu, Mn, and Zn at the source site can result in the selection of rhizobacteria with higher IAA production potential. Likewise, Malhotra & Srivastava, (2009) noted that nitrogen starvation that suppresses cell viability can increase IAA production by *Azospirillum brasilense SM* in vitro. Zinc solubilization results show a wide variation by isolates recovered from rhizosphere soil samples collected from different agricultural and soil management practices on nutrient agar medium. The isolates exhibited a significant variation in zinc solubilization. Maximum zinc solubilization was observed for zinc phosphate followed by zinc oxide. These findings are in accordance with Ramesh et al. (2014) who reported maximum solubilization for zinc phosphate but contrary to Yasmin et al. (2021) who found maximum solubilization for ZnO. An increase in available zinc in the medium from the Zinc-P compound was also accompanied by a reduction in pH that could be correlated with organic acids production such as gluconic acid, in which, acidification appears to be the main strategy followed by zinc-solubilising strains (R. Yasmin et al., 2021). Organic acids or exudative chelating agents can be said to be acting as agents causing zinc solubilization and an important phenomenon in metal solubilization. Carbon and N sources play a modulating role in the synthesis of secondary metabolites and the release of Zn from insoluble Zinc-P compounds (S. K. Sharma, 2012). Although some contribution may also come from the excretion of other metabolites, siderophores and CO_2 from respiration, the importance of all these processes is variable and dependent on the organisms and growth conditions (Ramesh et al., 2014). The ability of bacteria to solubilize all four inorganic zinc compounds might be interesting as zinc is known to occur in soils in discrete chemical forms varying in their solubility and availability to crop plants (Ramesh et al., 2014). Bacteria from the Ait Saleh region dominate this assay and represent 75% of the total zinc-solubilizing bacteria. This can be attributed to location specificity in terms of varying organic carbon content, and species and strains variability (S. K. Sharma, 2012)**.** In addition, the production of siderophores is a PGP feature that may play an important role in biocontrol and mobilizing phosphorus by mineral chelation (Hamdali et al., 2008)**.** Among the role of siderophores, they can solubilize and sequester iron from the soil, but they may also play an indirect role in plant growth promotion (Schindler et al., 2017)**,** which explain the high Fe values in the Dayt Al Amira and Ait Saleh regions, whose PSBs load is the highest. Among the more current methodology, the use of natural plant biostimulants like PSB strains has been proposed to improve plant resistance to abiotic environmental stresses. The advantage of using these substances is due to their effectiveness in improving crop productivity

and quality (Del Buono, 2021). Thus, adapted bacteria can be used as biofertilizers, biocontrol agents, and bioremediation for augmenting the growth and yield of crops grown in different ecological areas.

5. Conclusion

This research work provides valuable insights into the PSB's density in different habitats. The main objective of this study was to evaluate the effect of climate and soil conditions on the abundance and diversity of PSBs in four varied regions in Morocco. In addition, we tested the P solubilization efficiency and the PGP traits of isolates to assess the potential impact of the cited factors on the competencies of PSBs. The density of the PSBs population was significantly different between the four sites due to varied environmental conditions: temperature, humidity, and precipitation of the selected sites. The abundance of PSBs and PGP traits were positively correlated as the Ait Saleh site, the most PSBs load, show 70% and 69% of auxins and siderophores production, respectively. Based on the mentioned results, the climate appears to exert the most selective pressure on PSBs phenotypes such as P solubilizing competence. In addition, we noted that climate influences the structure of PSBs communities in the studied sites. In general, this study makes a significant contribution to the sustainable agriculture field by conducting a thorough investigation of PSBs' performance associated with the environmental conditions of their soil source. We suggest extending the field of work and investigating different regions that are more likely to contain efficient PSBs with traits and ecological mechanisms that can improve agricultural production as well as directing research toward appropriate pathways.

Chapitre III

Isolement et caractérisation des bactéries solubilisant le phosphate et colonisant naturellement la rhizosphère des légumineuses au Maroc

Isolement et caractérisation des bactéries solubilisant le phosphate et colonisant naturellement la rhizosphère des légumineuses au Maroc

Synthèse

Traditionnellement, les engrais organiques et inorganiques appliqués aux champs agricoles ont été utilisés pour corriger les carences en éléments nutritifs et maintenir leur équilibre. Selon la FAO, plus de 175,5 millions de tonnes d'engrais chimiques sont utilisés pour obtenir le meilleur rendement des cultures afin de répondre à la demande croissante des ménages. Le phosphore (P), l'un des principaux macronutriments essentiels à la croissance et développement des plantes, se caractérise par sa mobilité restreinte par rapport à d'autres éléments nutritifs importants tels que l'azote ou le potassium. Par ailleurs, les modèles de nutriments végétaux montrent depuis longtemps que la diffusion lente du phosphate inorganique (Pi) est une limitation majeure de l'acquisition du P par les plantes. Même si des engrais chimiques sont apportés aux sols, les plantes ne peuvent utiliser que de petites quantités d'engrais P en raison de sa complexité avec le calcium dans les sols calcaires, l'aluminium et le fer dans les sols acides, environ 80% du P appliqué devient ainsi indisponible pour la plante. Ceci montre que les plantes ont besoin de mécanismes supplémentaires pour acquérir le Pi dans des conditions de faible teneur en P. Néanmoins, l'impact négatif sur l'environnement pourrait résulter d'une application excessive et irrationnelle d'éléments nutritifs chimique dans les agroécosystèmes.

La plupart des engrais phosphatés sont fabriqués à partir de phosphate naturel (RP), principale source de P_2O_5. Alors que la demande ne cesse d'augmenter, l'offre de RP est limitée dans le monde entier. Le Maroc couvre une part importante des réserves mondiales de P ; il contient 75% du P planétaire et est le premier exportateur de P et de ses dérivés. La contribution de ce pays au marché mondial est donc supérieure à 30 %. Cette position avantageuse du Maroc sur les plans commercial et technique de l'extraction du P reste très éloignée d'une bio-industrie qui accompagne le secteur de la biotechnologie. Cette dernière pourrait ouvrir des possibilités de valorisation maximale du P du sol. Cependant, les pratiques agricoles peu coûteuses et respectueuses de l'environnement ont fait l'objet d'une attention croissante ces dernières années. Le développement d'inoculant microbiens contenant des bactéries solubilisant le P représente une solution biologique émergente pour améliorer la disponibilité du P dans la rhizosphère. Plusieurs études ont rapporté l'utilisation de micro-organismes pour solubiliser des composés phosphatés insolubles comme stratégie alternative aux engrais chimiques. En effet, l'utilisation de ces souches telluriques compétentes est une stratégie agro-écologique pour améliorer la fertilité agricole et réduire la dépendance des agriculteurs à l'égard des engrais inorganiques

afin d'améliorer la santé des sols. Cependant, la capacité des PSB à solubiliser le P du sol ou des activités de fertilisation dans de nombreux champs agricoles dépend fortement de leur sécrétion d'acides organiques. De même, bien que les PSB puissent apporter des bénéfices agronomiques, une compréhension des mécanismes microbiens est essentielle et devient une condition préalable à l'utilisation durable de ces biofertilisants dans l'agriculture.

Compte tenu des faits susmentionnés, la présente étude vise à explorer les souches de PSB isolées de sols situés à différents étage bioclimatiques au Maroc et présentes dans la rhizosphère de diverses légumineuses afin d'améliorer l'efficacité des engrais microbiens agronomiques. Il s'agissait de tester la capacité de ces souches à améliorer l'activité de solubilisation du P dans un milieu de croissance au phosphate de l'Institut national de recherche botanique (NBRIP) avec du P tricalcique (Ca_3 $(PO_4)_2$) (TCP) et du RP séparément, ainsi que leur combinaison (RP/TCP) selon un plan d'expérience à deux niveaux (2^2 = 4 expériences). Les souches bactériennes présentant un indice de solubilité du P élevé ont été sélectionnées, caractérisées et comparées à un contrôle commercial (*Bacillus subtilis*). La méthode vanadate-molybdate a été utilisée pour estimer l'activité de solubilisation du P. De plus, nous avons caractérisé les PSB isolés et détecté leur tolérance aux différentes conditions de croissance et de stress environnemental (salinité, pH, sécheresse et température), en plus de leur activité antifongique.

Parmi toutes les souches isolées (64), 12 présentent un intérêt biotechnologique prometteur en raison de leur solubilisation du P et de leur bonne résistance à différentes conditions drastiques. En outre, la souche WJEF15 a montré la plus grande efficacité de solubilisation du P en milieu solide NBRIP avec un indice de solubilisation du P de 4.1 ; tandis que la souche WJEF61 a été localisée comme la souche la plus efficace en milieu liquide NBRIP-TCP en libérant 147.62 $mg.l^{-1}$ de P soluble. En revanche, dans le milieu NBRIP-RP, la souche WJEF15 a présenté une solubilisation maximale avec 25.16 $mg.L^{-1}$. La méthode des plans d'expériences a montré qu'une combinaison de RP et de TCP avec le niveau maximum augmente progressivement la solubilisation du P de 20.58%, tandis que la souche WJEF63 a la concentration la plus efficace de 102.69 $mg.l^{-1}$. En effet, nous constatons que les souches PSB isolées ont un effet antifongique significatif allant jusqu'à 49.15% d'inhibition de la croissance mycélienne des champignons testés. Ainsi, les résultats révèlent un effet potentiel des PSB pour soutenir les cultures en tant que rhizobactéries favorisant la croissance des plantes (PGPR). En analysant les différentes orientations de cette étude, nous recommandons l'utilisation de souches de PSB à fort potentiel comme engrais microbiens dans le domaine agronomique. Afin d'exploiter le plein potentiel de ces bactéries telluriques, d'autres recherches innovantes devraient être menées

au niveau des mécanismes génétiques et se concentrer sur les concepts d'application d'inoculant microbiens en agriculture afin d'identifier davantage de PSB et d'avoir des inoculant microbiens compétents dans des systèmes de production de cultures durables dans différentes conditions.

Isolation and characterization of phosphate solubilizing bacteria naturally colonizing legumes rhizosphere in Morocco

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Abstract

Low-cost and environmentally friendly agricultural practices have received increasing attention in recent years. Developing microbial inoculants containing phosphate (P) solubilizing bacteria (PSB) represents an emerging biological solution to improve rhizosphere P availability. The present study aims to explore PSB strains isolated from soils located at different bioclimatic stages in Morocco and present in various legumes rhizosphere to improve agronomic microbial fertilizer's effectiveness. It was also aimed to test the isolated strains for their ability to solubilize P in NBRIP medium with Tricalcium P (Ca_3 $(PO_4)_2$) (TCP), rock phosphate (RP), and their combination as a source of phosphorus, by (22) experiment design. Bacterial strains with a high P solubility index (PSI) were selected, characterized, and compared to commercial control. The vanadate-molybdate method was used to estimate P solubilization activity. Stress tolerance to salinity, acidity, drought, and temperature was tested. From all isolated strains (64), 12 were screened as promising biotechnological interest because of their P solubilization and their good resistance to different drastic conditions. Besides, the strain WJEF15 showed the most P solubility efficiency in NBRIP solid medium with a PSI of 4.1; while the WJEF61 strain was located as the most efficient strain in NBRIPTCP liquid medium by releasing 147.62 $mg.l^{-1}$ of soluble P. In contrast, in the NBRIP-RP medium, the strain WJEF15 presented maximum solubilization with 25.16 $mg.l^{-1}$. The experiment design showed that a combination of RP and TCP with max level progressively increases P solubilization by 20.58%, while the WJEF63 strain has the most efficient concentration of 102.69 $mg.l^{-1}$. Indeed, among the selected strains, four strains were able to limit tested fungi growth. Thus, results reveal a potential effect of selecting PSBs to support cropping cultures as plant growth-promoting rhizobacteria (PGPR).

Keywords: microbial fertilizers, rock phosphate, stress tolerance, sustainability, antifungal activity, TCP.

1. Introduction

Phosphorus (P) is one of the main essential macronutrient compounds for growth and plant development. It is involved in central plant metabolic processes such as cellular energy storage, photosynthesis, and respiration (Balemi & Negisho, 2012; H. Malhotra et al., 2018; Razaq et al., 2017; Sanz-Sáez et al., 2017). Depending on the environmental and biological factors, it can be the primary growth-limiting nutrient factor (Alok et al., 2013; Rajkumar & Kurinjimalar, 2021). However, the amount of soluble P in soil is generally low (0.4 - 1.2 $g.kg^{-1}$) (V. Fernández et al., 2014; Joe et al., 2018). Most parts of soil P (approximately 95 – 99%) are present in insoluble forms, and therefore they cannot be easily used by plants (Mara et al., 2014). Traditionally, organic and inorganic fertilizers applied to farm fields have been used to correct nutrient deficiencies and maintain nutrient balances (Liu et al., 2020; Tiwari et al., 2020). Soil P is characterized by its restricted mobility when compared to other important nutrients such as nitrogen or potassium and plant nutrient models have long shown that slow diffusion of inorganic phosphate (Pi) is a major limitation to P acquisition by plants (Barber, 1995). This suggests that plants need additional mechanisms to acquire Pi under low P conditions, as their roots have access to a rather small fraction of total soil P. Even if chemical fertilizers are supplied to soils, plants can only use small amounts of P fertilizer because of P complexities in growing weak soil structures. A large quantity of P applied as chemical fertilizer goes out of plant-soil system through complexity with Ca^{2+} in calcareous soils, Al^{3+} and Fe^{3+} in acidic soils (Gyaneshwar et al., 2002; Izhar Shafi et al., 2020), approximately 80% of applied P so become unavailable to plant (Salvagiotti et al., 2017). Nevertheless, the negative environmental impact could be resulted from excessive and irrational nutrient application in agroecosystems, which has attracted attention from stakeholders along food value chain (Choudhury & Kennedy, 2005). According to FAO, more than 175.5 million tons of chemical fertilizers are used in agriculture to obtain highest crop yield to meet increasing habitat demands.

Most phosphate fertilizers are manufactured using rock phosphate (RP) as principal source of P_2O_5. While demand continues to increase, the supply of rock phosphate is limited worldwide (Suleman et al., 2018). The extremely poor solubility of RP is the only constraint in its direct use as a soil amendment (Baig et al., 2012). However, insoluble P forms such as tricalcium P ($Ca_3 (PO_4)_2$), aluminium P ($AlPO_4$), and iron P ($FePO_4$) can be solubilized by organic acids, phosphatase enzymes, and complexing agents produced by soil microorganisms inhabiting different soil ecosystems (Vaishampayan et al., 2001). Several studies have reported the usage of microorganisms to solubilize insoluble phosphate compounds as an alternative strategy to phosphate fertilizers (Adnan et al., 2020). Indeed, using these telluric competent strains is an

agroecological strategy for improving agricultural fertility and reducing farmers' dependency on inorganic fertilizers so enhance soil health (Janati et al., 2021). For several decades, agricultural microbiologists have studied the ability of certain bacteria to dissolve P fertilizers and P chelated in soil according to sustainability tendency. For this purpose, Behera et al. (2017) noted that PSB's ability to solubilize P from soil or fertilization activities in many agricultural fields is highly dependent on their secretion of organic acids such as citric, formic, oxalic, lactic, acetic, and malic acids. Moreover, PSBs can increase plant P availability by modifying soil processes in the rhizosphere, providing essential nutrients to plants, and generating other regulators growth in various production systems (Adnan et al., 2020; Islam et al., 2021). Likewise, PSBs can produce growth-promoting hormones such as Gibberellins and Indol Acetic Acid (IAA), which are beneficial for plant growth (Bahadur et al., 2016; Linu et al., 2019; Khan et al., 2020; Kour et al., 2020). Currently, most bacteria classified as PSBs belong to *Pseudomonas*, *Serratia*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *bacillus*, *rhizobia*, *Micrococcus*, *Aerobacter*, *Flavobacterium*, *Acinetobacter*, and *Pantoea* genera (Castagno et al., 2011; A. Chauhan et al., 2015; Adnan et al., 2019; Seenivasagan & Babalola, 2021).

Morocco covers a significant share of world P reserves; it contains 75% of planetary P and is the leading exporter of P and its derivatives. So the global market country's contribution is more than 30% (Hakkou et al., 2016). This advantageous position of Morocco on commercial and technical levels of P extraction remains very far from a bio-industry that accompanies the sector of biotechnology. The latter could open up possibilities for the maximum valorization of soil P. Although PSBs can provide agronomic benefits, they are not always abundant enough in soil to compete with other indigenous microorganisms (Acevedo et al., 2014). For this reason, the telluric bacteria that should be promising at the same time crop productivity and economic-environmental sustainability need to be selected, characterized, and identified to establish sustainable cropping systems.

Considering the above facts, the present study focused on the effect of several tolerant PSBs on different abiotic stresses to promote P solubilization from RP and TCP as the only P source in the context of biological fertilization and sustainable agricultural strategy. We isolated and characterized PSBs from legumes rhizosphere located at various bioclimatic stages and we detected their optimal resistance under different growth and environmental stress conditions (salinity, pH, drought, and temperature). The findings of this study could provide an effective approach for agronomic improvement of microbial inoculants to enhance soil P mobilization for plant growth, either in intensive production or in agroecological applications in Morocco.

2. Materials and methods

2.1. Soil sample collection and analysis

Soil samples were taken from different rhizospheres of cultivated legumes, cereals**,** and spontaneous plants from different localities in Fes - Meknes regions. Plantations located at different bioclimatic stages in four stations, named Tafrant, Ait Saleh, Dayt Al Amira, and Ait Yaakoub. These locations are characterized by:

- Tafrant: Climate is warm and temperate, with less rainfall in winter. Average annual temperature is 18.1 °C, average annual rainfall is 866 mm.
- Ait Saleh: Climate is warm and temperate. Rain falls mainly in winter, with relatively little rain in summer. It has an average annual temperature of 15.5 °C and an averages annual rainfall of 651 mm.
- Dayt Al Amira: Climate is warm and temperate. Rainfall is high even in the driest months. Average annual temperature is 12.1 °C and average annual rainfall is 683 mm.
- Ait Yaakoub: Climate is warm and temperate. In winter, rainfall is much higher than in summer. Average annual temperature is 17.8 °C and average annual rainfall is 549 mm. (Climate-data.org, 2022).

Rhizospheric soils samples were carefully collected, placed in labelled sterile plastic bags, analysed for physico-chemical characteristics and stored at -20°C before rhizobacteria isolation.

2.2. Isolation and purification of phosphate solubilizing bacteria

A soil suspension was prepared to isolate PSBs from a dilution cascade. For this purpose, ten grams (10 g) of soil were suspended in 100 mL of sterile phosphate-buffered saline (PBS) solution (pH 7.2) and shaken at 190 rpm for 45 min. Subsequently, serial dilutions were prepared to 10^{-6}. 100 µL of each dilution was placed on NBRIP agar plates (National Botanical Research in P medium), which has as composition : glucose 10 g, $(NH_4)_2SO_4$ 0.1 g, $MgSO_4.7H_2O$ 0.25 g, KCl 0.2 g, and agar 15 g, supplemented with 5 g RP from Khouribga phosphate mine, and dissolved in 1000 ml distilled water medium is stabilized to pH 6.8 (Nautiyal 1999). The NBRIP medium was then complemented with cycloheximide to inhibit fungal growth. NBRIP agars plates were incubated for six days at 28 ± 2 °C. During this incubation, clear zones of solubilization surrounding colony allowed detection of PSBs. Every different morphological colony types were isolated, purified, and retested by restricting them from single colony (five times) for purification on NBRIP plates and finally preserved in Tryptic Soy Agar (TSA) medium prior to cryopreservation in sterile glycerine (20%) at -80 °C.

2.3. Quantitative estimation of phosphate solubilisation index

Colony diameter and visible halos were evaluated between three and nine days after inoculation to calculate P solubilization index (PSI) (Nautiyal 1999). Halo and colony diameters were measured after nine days of incubation on NBRIP plates' at 28 ± 2 °C. Bacteria ability to solubilize insoluble P was described by the solubilization index formula (Equation 1):

$$PSI = \frac{CD + HD}{CD} \quad (1)$$

CD: Colony Diameter. HD: Halo zone Diameter.

2.4. Quantitative estimation of phosphate solubilization using TCP, RP, and their combination

2.4.1. Phosphate solubilizing assessment

Phosphate solubilizing bacteria's quantitative solubilization capacity of TCP, RP, and their combination was done in a liquid NBRIP medium. For this purpose, overnight pre-culture in TSA medium was prepared, then, moved to NBRIP medium. After 7 days incubation at 28 °C under shaking to 150 rpm, 20 ml of bacterial suspensions was taken and centrifuged (12000 g for 15 min). Supernatants were recovered and analyzed for their assimilable P content and pH measurements. Assimilable P content was carried out by ascorbic acid colorimetric method (Murphy & Riley, 1962). One mL of supernatant is mixed with 160 µL of a reaction solution and the OD (optical density) was then measured at 880 nm after a few minutes of incubation at room temperature. Phosphate soluble content was calculated based on standard P solutions of KH_2PO_4 (concentrations ranging from 0 to 1 $mg.l^{-1}$).

2.4.2. Optimisation study of combined RP and TCP solubilization system

Optimisation strategy to reveal and quantify the influence of PSBs effect on solubilization process of two combined phosphate sources (RP and TCP) was conducted following a two-level experimental design ($2^2 = 4$ experiments). Experimental domain of both factors (X_1 = (RP) and X_2 = (TCP)) are presented in Table 1. The experimental design was developed to study the effects of the two factors (X_1 and X_2) and their interaction (X_1*X_2), on the concentration of P solubilized and pH medium (Table 2).

Table 1. Factors levels (X1 and X2) involved in P solubilisation.

	Min level	**Max level**
	-1	+1
X_1 : RP	1%	2.5%
X_2 : TCP	1%	2.5%

Table 2. Experimental design developed to study the effects of factors (X1: RP and X2: TCP) and their interaction (X1*X2) on pH medium and P concentration.

Experiment	X_0	X_1	X_2	X_{1*2}	$Y_{P[C]}$	Y_{pH}
1	+1	-1	-1	+1	-	-
2	+1	-1	+1	-1	-	-
3	+1	+1	-1	-1	-	-
4	+1	+1	+1	+1	-	-

$Y_{P[C]}$: concentration of P solubilized response function. Y_{pH}: pH medium response function.

2.4.3. Factors and their mathematical modelling interaction

The matrix analysis model of experimental design (Table 2) results in response functions indicating the coded factors effects (X1 and X2) and their interaction, which has following formula (Equation 2):

$$Y = a_0 + a_1X_1 + a_2X_2 + a_{12}X_1X_2 \quad (2)$$

a_0: Coefficient model. a_1 and a_2: Coefficients of the linear part of mathematical model. a_{12}: Interaction coefficient of the two factors.

Mathematical analysis interactions between factors (X_1 and X_2) provide response surfaces and allow us to define the domains where there is a maximum or minimum response of the studied response functions ($Y_{P[C]}$ and Y_{pH}) experimentally.

2.5. Morphological and biochemical characterization

Phosphate solubilizing bacteria's were characterized as described in Bergey's Manual (Systematic Bacteriology, 2nd ed., vol. 5, eds., 2012), cells morphology, motility test, and Gram staining were performed (Aneja, 2007). Isolates were also tested for catalase, gelatinase, and urease activities (Aneja & Wilkes, 2003 ; Graham & Parker, 1964). Experiments were repeated three times for each isolate. To determine PSB's capability to use starch as source of carbon (Salgaonkar et al., 2018) PSBs were inoculated on starch agar media and incubated at 28°C ± 2 °C for 24h. Citrate utilization by the isolates was observed by growth on YEM agar containing sodium citrate in place of mannitol and supplemented with 1 ml of bromothymol blue (BTB) solution (1%) and incubated at 30 °C. After incubation (7 days), a distinct change in colour, from green to blue, refers to as a positive test. A glucose peptone agar (GPA) assay was performed to determine PSB's capability to use glucose as sole source of carbon and energy for growth (Amela et al., 2018). Similarly, a lactose assay was performed (Paudyal et al., 2021).

2.6. Bacterial stress tolerance

Isolates salinity tolerance evaluation was done onto liquid rich medium (TSA) containing different NaCl concentrations (0.2M, 0.4M, 0.6M, 0.8M, 1M, 1.2M, 1.6M, and 2M). Incubation was carried out at 28°C ± 2 °C for 48h. Then, absorbance was measured at 620 nm (R. P. Singh et al., 2015). As solubilization is strongly related to pH medium changes, isolates were inoculated on a solid TSA medium at different pH values starting from 3 to 13 and incubated at 28°C ± 2 °C for 48h. This test allows isolates selection that can grow over a wide range of soil pH (acid, neutral and alkaline). Phosphate solubilizing bacteria isolates were also cultured onto a solid rich agar medium (TSA) and incubated at 37°C, 48 °C, and 55 °C for 48h to assess isolates' temperature tolerance through bacterial growth. In addition, drought tolerance of PSBs was assessed on liquid medium (TSA) containing different concentrations of polyethylene glycol 6000 (PEG) (10%, 20%, and 30%). Incubation was carried out at 28°C ± 2 °C for 24h, after that absorbance assessment was done at 620 nm.

2.7. Antifungal effect of PSB strains

All 12 bacterial isolates were tested for their antagonistic effect against six fungal strains isolated from strawberries fruit and grown in a PDA medium. To assess antagonism, a 5 mm block (growing mycelium + medium) was placed in the middle of the Petri dish. Tested bacteria were placed in four different positions around the block. After incubation at 26 °C ± 2 °C for 24 hours, a zone of inhibition between colonies and fungal strains indicates antagonism presence. Finally, isolates producing more than 15 mm of inhibition zone were selected. Inhibition rate of fungal growth was determined using the percentage inhibited radial growth (% IRG) according to the following formula (Equation 3):

$$\% \text{ IRG} = \frac{R1-R2}{R1} * 100 \qquad (3)$$

R_1 radial growth of control mycelium. R_2 radial growth of treated mycelium.

2.8. Statistical analysis

Data were analyzed using SPSS software, and the results were expressed as the means ± standard deviation of three replicates. Data were examined by ANOVA I analysis, and mean comparison was performed by Duncan's multiple range test at $p \leq 0.05$. Dimensional analysis done by principal component analysis (PCA) has been used to give a summarising view of obtained results. For design of experiment trial, all experiments were done in four replicates and data were analysed using JMP 4 software. Response surface of experimental design response functions (Y_{pH} and $Y_{P[C]}$ were done by Maple 9 software.

3. Results

3.1. Soil physicochemical analysis

Soil samples' physico-chemical and PSBs content are listed in Table 3. Agricultural legume soils analysed contained, on average, 4.4% of Organic Matter, 7.9 mg.Kg^{-1} of nitrogen, 44.65 mg.Kg^{-1} of P_2O_5, 660.5 of K_2O mg.Kg^{-1}, 7406.25 of CaO mg.Kg^{-1}, and 848.83 mg.Kg^{-1} of MgO. Maximum electrical conductivity, reflecting soil salinity, was recorded in Ait Yaakoub soil sample with an average of 300.33 ds.m^{-1}. In terms of soil pH, it was close to neutral or slightly basic (pH = 8.2 on average).

Table 3. Physico-chemical and PSBs analysis of the studied soil samples.

Sites	**Tafrant**	**A. Yaakoub**	**D.A. Amira**	**A. Saleh**
Coordinates	**Y** -5.112751, **X** 34.615013	**Y** -4.950365, **X** 33.975209	**Y** -5.019709, **X** 33.692496	**Y** -4.992072, **X** 33.791152
Altitude (m)	312.5	566	1548	922
T.avg/year (°C)	18.1	17.8	12.1	15.5
P.avg/year (mm)	866	549	683	651
PSBs load (Cells.g^{-1} of soil)	$5.95*10^3$	$1.98*10^5$	$1.04*10^6$	$3.16*10^7$
pH-water	8.26	8.323	8.067	8.273
Electrical conductivity 1/5 (ds.m^{-1})	187.033	300.333	241.233	173.2
Cation exchange capacity ($cmol^+.kg^{-1}$)	40.987	32.28	37.227	31.563
Organic matter (%)	2.573	5.103	5.64	4.38
N total (%)	0.126	0.255	0.324	0.174
$N\text{-}NH_4$ + $N\text{-}NO_3$ (mg/kg N)	7.383	8.867	8.167	7.4
P_2O_5 (mg.kg^{-1})	10	46.7	98.7	23.3
K_2O (mg.kg^{-1})	432	846.3	990.7	373
CaO (mg.kg^{-1})	9450	8550	5250	6375
MgO (mg.kg^{-1})	410.667	854	1213.333	917.333

The mineralogical compositions of different analyzed sites with ICP are presented respectively in (Figure 1). We notice that Al, Ca, Fe, K, Mg, Na, and P are the most important minerals in studied sites. Based on Principal component analysis of different sites in terms of mineralogical composition we find that sites S9 (Dayt Al Amira) and S10 (Ait Saleh) are controlled both to Mg and to Ca minerals, while all the other sites are controlled by Al, Fe, K, and Na minerals.

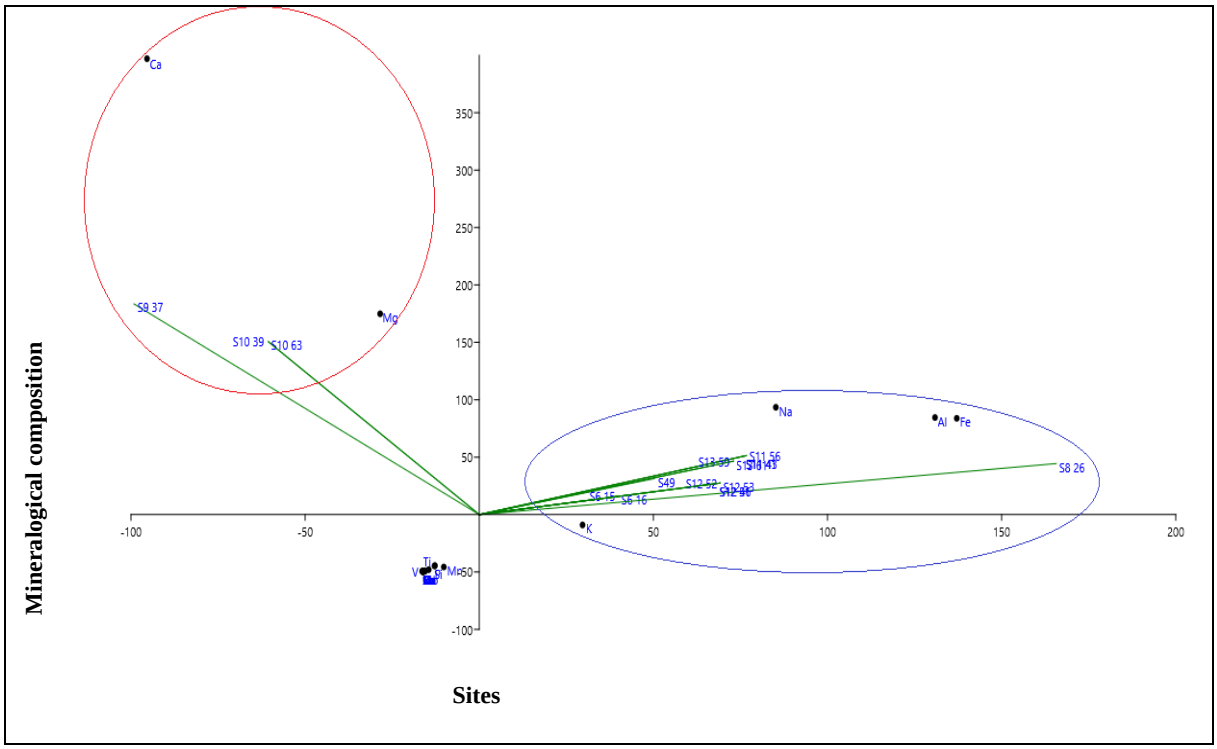


Figure 1. Principal component analysis of different soil in the function of mineralogical composition.

3.2. Selection and purification of phosphate solubilizing bacteria

Soil samples collected from different sites were used to study the PSB bacteria. Strains were selected based on colonies morphological difference and halo resulting from TCP solubilization. Although halo formation around colonies provides the first qualitative indication of PSB. Colonies that grew without forming a halo were also isolated to test their solubilization capacity. Initially, 134 isolates were selected; these isolates were transplanted onto NBRIP medium 3 times to guarantee their efficiency and stability. Among 134 isolates, 64 were designated as PSB based on their solubilization of P on NBRIP liquid medium, and only twelve isolates were screened as promising biotechnological interest.

3.3. Quantitative estimation of phosphate solubilisation index

The appearance of halo zone in all strains was noticed after 4th day of incubation, solubilization index ranged from 3.14 cm to 4.1 on average. Isolate WJEF15 showed a maximum solubilization index with PSI = 4.1 (Figure 2). Thus, a significant difference was found for all strains tested. We notice the existence of correlation between incubation time and halo zone size, whereby the halo zone size of each isolate increases with incubation time.

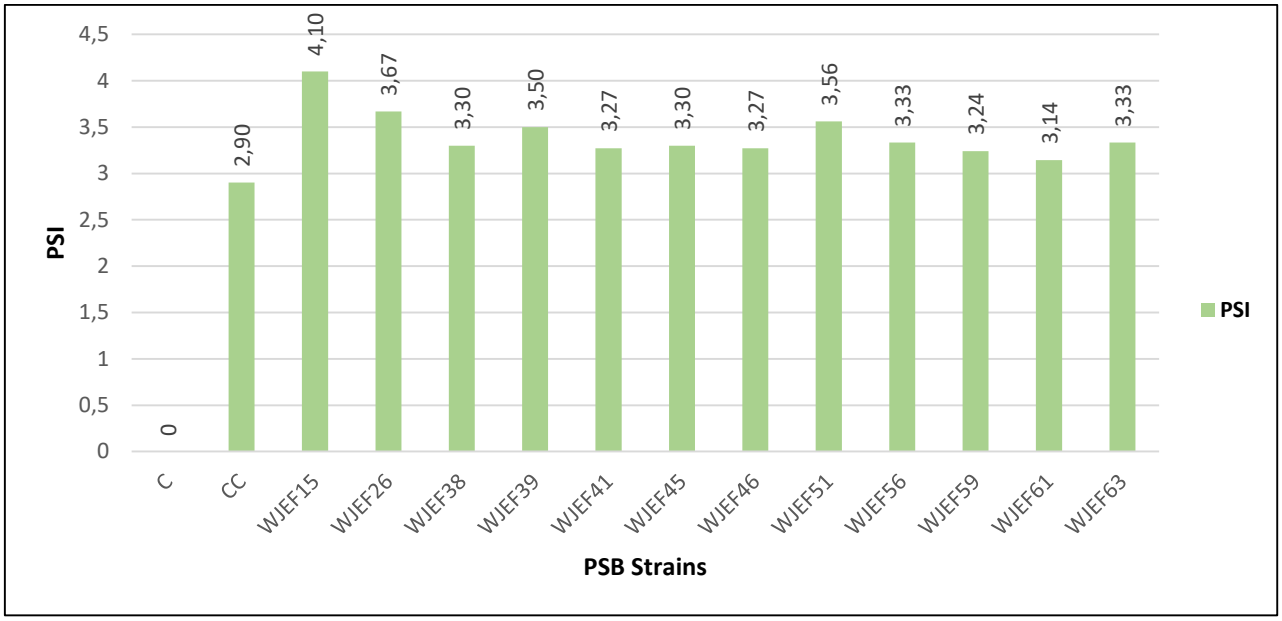


Figure 2. Phosphate solubilisation index (PSI) of isolated strains on solid NBRIP medium.

3.4. Quantitative estimation of phosphate solubilization using TCP, RP

Soluble P and pH variation are presented in (Figure 3). The solubilization of TCP, RP, and their combination in liquid NBRIP medium by tested strains was accompanied by a significant pH decrease. NBRIP-TCP soluble P concentration varied between 75.61 $mg.l^{-1}$ and 147.62 $mg.l^{-1}$, whereas NBRIP-RP soluble P concentration varied between 15.41 $mg.l^{-1}$ and 25.16 $mg.l^{-1}$ with significant variations between all strains when compared to control ($p \leq 0.05$). However, maximum solubilization in NBRIP-TCP liquid medium was observed for isolate WJEF61 (147.62 $mg.l^{-1}$). In contrast, for NBRIP-RP medium, strain WJEF15 presented maximum solubilization with 25.16 $mg.l^{-1}$. On the other hand, commercial control solubilized only 36.05 $mg.l^{-1}$ of P with TCP, and 3.39 $mg.l^{-1}$ with RP. Besides, NBRIP medium pH inoculated with selected strains strongly decreased from 7.06 to 4.145 (TCP) and from 7.97 to 4.28 (RP) after seven days of incubation. Compared to the commercial control, which decreased to 5.74 and 7.26 respectively.

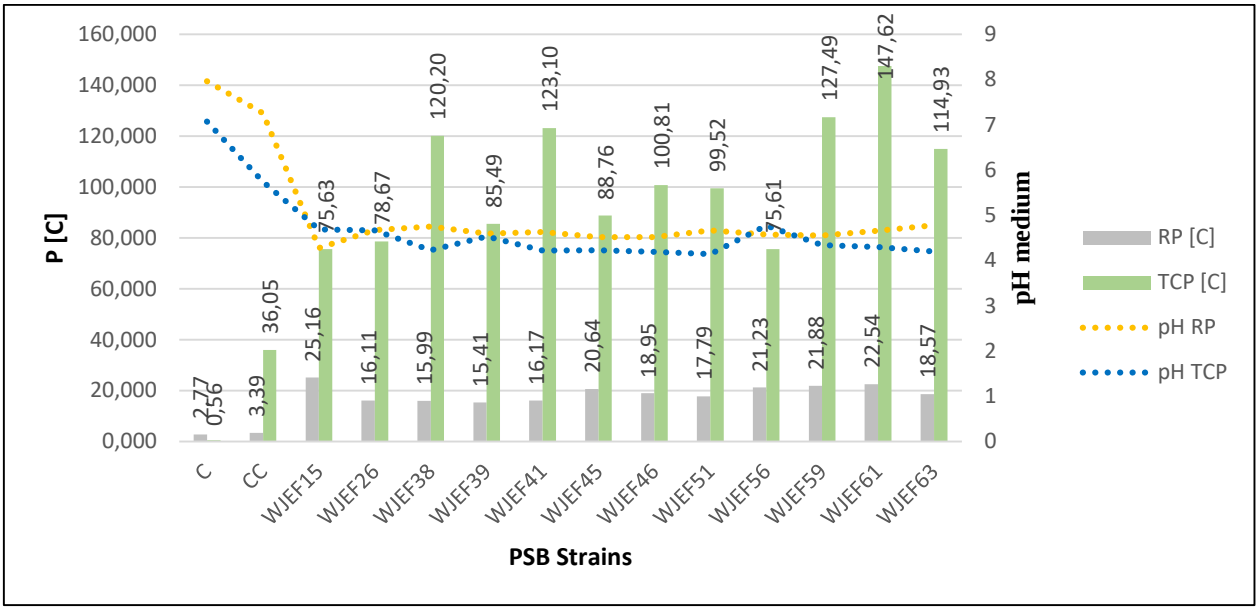


Figure 3. PSBs solubilization capacity of TCP and RP after 7 days in liquid medium.

3.5. Correlation between pH medium and solubilization concentration

Correlation between P[C] and pH was positive in all strains with high P solubility. Correlation coefficients (R) were greater than 0.84 for P [C] versus pH medium. However, in several applications, regression equations for P [C] versus pH medium were linear model (Figure 4).

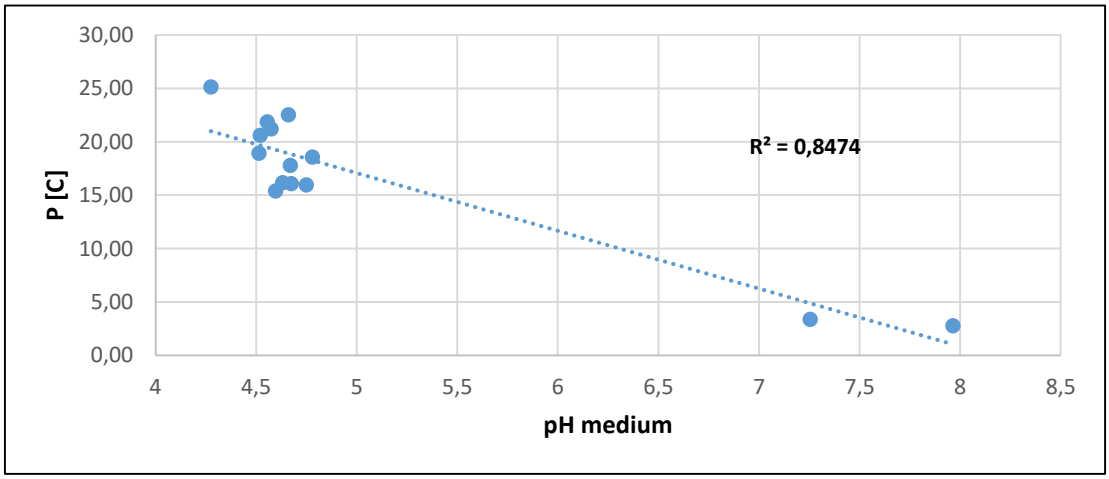


Figure 4. Negative correlation between pH medium and solubilization concentration.

3.6. Design of experiments method to study TCP and RP interaction

Experimental matrix analysis design (Table 4 and 5) results (response functions) presenting coded factors effects (X_1 and X_2) and their interaction (X_1*X_2) (equation 2). The control (non-inoculated) was introduced to evaluate the chemical reaction of medium.

Table 4. Effect of phosphorus source (RP and TCP) on culture medium acidification.

Isolates	Model Coefficient	RP Effect	TCP Effect	Interaction RP/TCP
Coefficients	a_0	a_1	a_2	a_{12}
Control	1.931	0.013	0.055	-0.016
Commercial Control	0.335	-0.025	-0.079	0.016
WJEF15	3.278	-0.034	0.029	0.047
WJEF56	2.793	0.042	-0.019	-0.101
WJEF26	2.921	-0.020	-0.138	-0.019
WJEF38	3.237	0.063	0.047	-0.129
WJEF41	3.193	0.020	0.075	-0.143
WJEF45	3.222	0.011	0.146	-0.123
WJEF46	3.209	0.057	0.104	-0.076
WJEF51	3.261	0.009	0.101	-0.149
WJEF59	3.089	0.084	0.100	-0.115
WJEF61	3.237	0.051	0.092	-0.109
WJEF63	3.199	0.085	0.140	-0.161

Table 5. Impact of phosphorus source (RP and TCP) on P solubilization in culture medium.

Isolates	Model Coefficient	RP Effect	TCP Effect	Interaction RP/TCP
Coefficients	a_0	a_1	a_2	a_{12}
Control	0.481	-0.055	-0.031	-0.049
Commercial Control	1.076	-0.030	0.178	-0.037
WJEF15	14.637	-0.598	8.050	-0.405
WJEF56	10.102	0.228	4.623	-1.197
WJEF26	10.865	-2.116	3.722	-0.898
WJEF38	15.491	-1.406	7.430	-0.873
WJEF41	14.208	0.570	8.394	-2.034
WJEF45	14.513	0.912	8.628	-1.993
WJEF46	14.738	0.966	8.768	-1.774
WJEF51	16.397	-0.182	8.601	-1.119
WJEF59	16.196	0.008	7.785	-1.589
WJEF61	16.802	0.713	8.019	-1.779
WJEF63	16.574	1.452	8.743	-1.094

Phosphate solubilization and pH medium results obtained by WJEF26 isolate according to the experimental design are summarised in the following table (Table 6). The values correspond to the mean of four replicates.

Table 6. Experimental design results done after P solubilization by isolate WJEF26.

Experiments	X_1	X_2	X_{12}	Y_{pH}	$Y_{P[C]}$
1	-1	-1	+1	4.3	33.442
2	+1	-1	-1	4.7725	23.696
3	-1	+1	-1	5.1175	70.405
4	+1	+1	+1	5.165	46.289

Y_{pH}: Function response of pH medium during P solubilization. $Y_{P[C]}$: Function response of P solubilization ($mg.l^{-1}$).

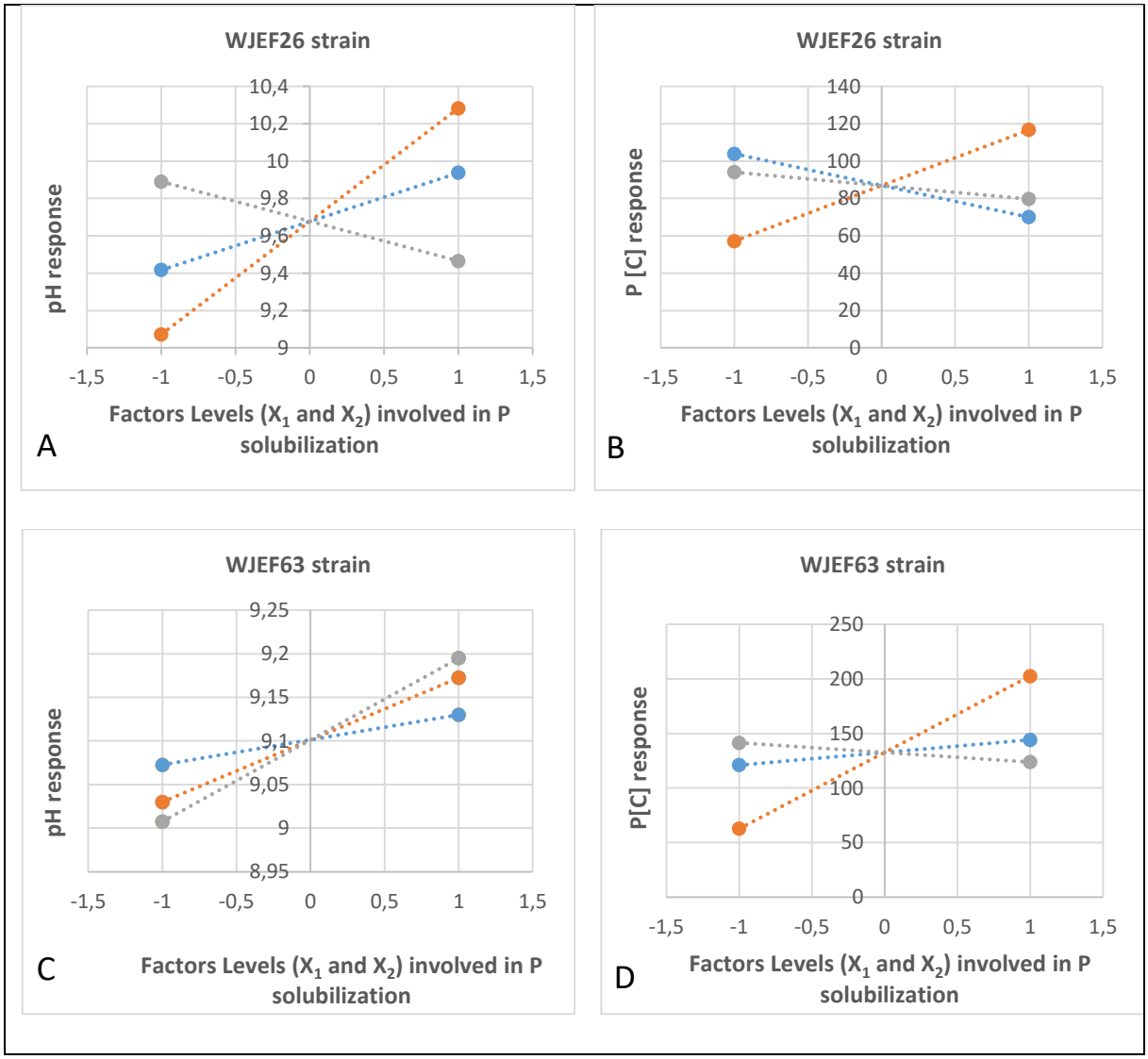


Figure 5. A) Effects of studied factors (X_1 and X_2) and their interaction on pH medium in the presence of WJEF26 strain. B) Effects of studied factors (X_1 and X_2) and their interaction on P[C] in the presence of WJEF26 strain. C) Effects of studied factors (X_1 and X_2) and their interaction on pH medium in the presence of WJEF63 strain. D) Effects of studied factors (X_1 and X_2) and their interaction on P[C] in the presence of WJEF63 strain.

Main factors effects (X_1 and X_2) and their interaction (X_1*X_2) of WJEF26 strain for example are summarized in the following figure (Figure 5 (A, B)). We notice that factors interaction on pH medium and P [C] have a positive effect in comparison to each factor alone.

For the strain WJEF63, P solubilization and pH medium results obtained according to the designed experiment are summarised in the following table (Table 7). Values correspond to four replicates mean.

Table 7. Experimental design results done after P solubilization by isolate WJEF63.

Experiments	X_1	X_2	X_{12}	Y_{pH}	$Y_{P[C]}$
1	-1	-1	+1	4.547	21.144
2	+1	-1	-1	4.482	41.505
3	-1	+1	-1	4.525	99.836
4	+1	+1	+1	4.647	102.699

Y_{pH}: Function response of pH medium during P solubilization. $Y_{P[C]}$: Function response of P solubilization (mg.l^{-1}).

Factors effects (X_1 and X_2) and their interaction (X_1*X_2) of WJEF63 isolate are summarized in the following figure (Figure 5 (C, D)). We notice that factors interaction on P [C] have a positive effect in contract of each factor alone. On the other hand, the effect of factors alone or in interaction on pH medium have the same activity.

Factor interaction is presented in the following figure (Figure 6) where we observe the response surface according to coded variables (RP medium and TCP) which delimits experimental domain. Figure (A) shows that pH medium is maximal when RP is at the lowest level and TCP is at the highest level (1% and 2.5% respectively). In the opposite, Figure (B) shows that P[C] is maximal when both factors X_1 and X_2 are at the highest level (2.5% and 2.5% respectively). In the second example done with WJEF63 strain. The interaction of studied factors is presented in the following figure (Figure 6 (C, D)) where we observe the response surface according to coded variables (RP medium and TCP) which delimits experimental domain. Figure shows that pH medium and P [C] is maximal when RP is at the lowest level and TCP is at the highest level (1% and 2.5% respectively). From response surface function, we notice that strains metabolic response depend on the nature of used microorganisms.

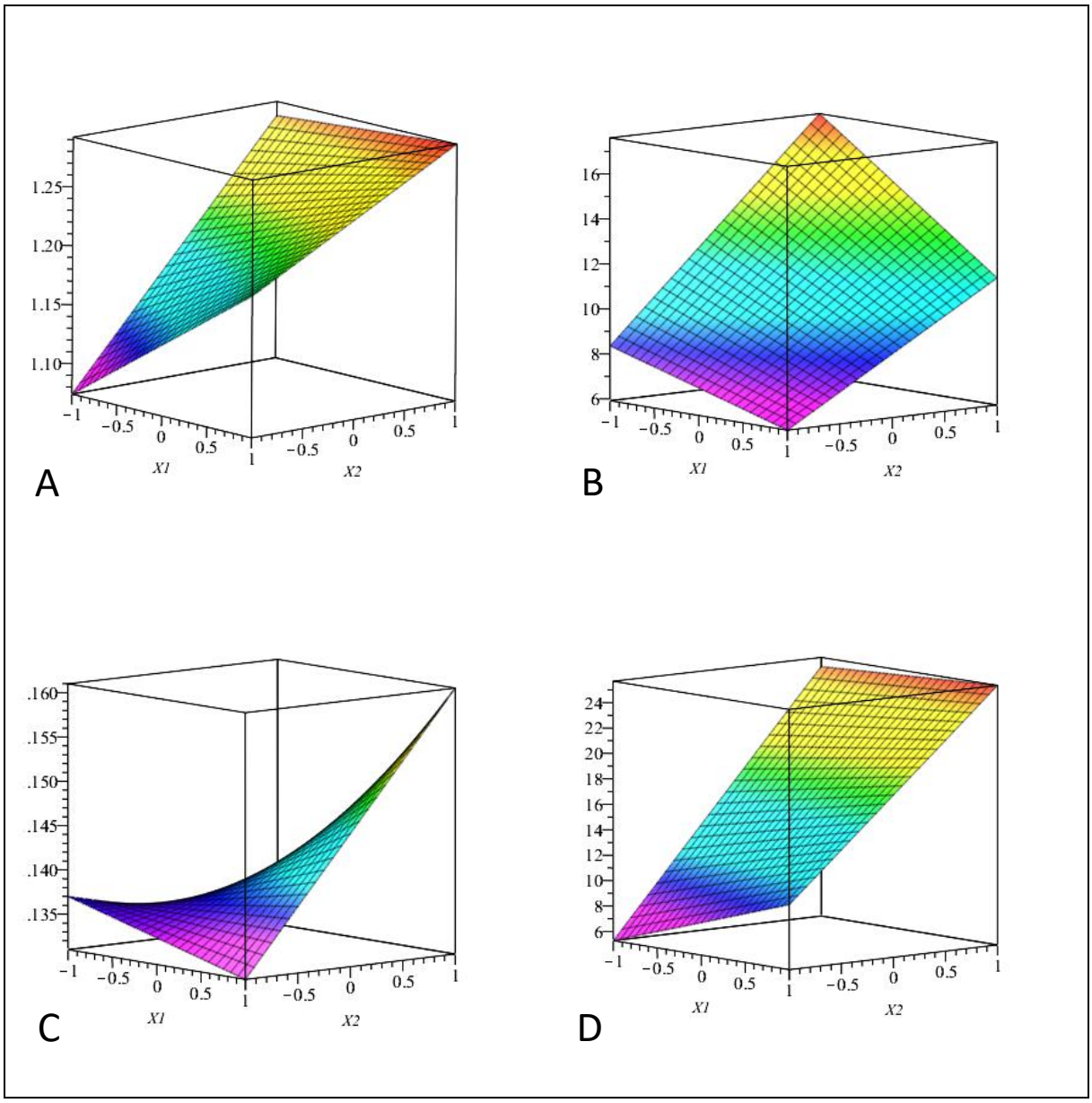


Figure 6. A) The effect of studied factors (X_1 and X_2) and their interaction on pH surface response by WJEF26 strain. B) The effect of studied factors (X_1 and X_2) and their interaction on response surface of P[C] by WJEF26 strain. C) Effect of studied factors (X_1 and X_2) and their interaction on response surface of pH with WJEF63 strain. D) Effect of studied factors (X_1 and X_2) and their interaction on response surface of P[C] with WJEF63 strain.

3.7. Morphological and biochemical characteristics

The morphological profile of studied strains shows a whole appearance; irregular edges, white, off-white or yellow colour, and a mucoid or viscous texture. Cell structures show rod and coccobacillus shapes with an arrangement of single pairs, chain rods, and solitaires. Gram reaction indicates that all isolates were gram negative with the exception of five strains (WJEF15, WJEF38, WJEF41, WJEF45, and WJEF59). Cell motility test and catalase activity is positive for all selected strains (Table 8). Similarly, all strains grew on GPA with glucose or lactose as source of carbon. The same results were obtained from starch hydrolysis test expect

for WJEF61, WJEF52, and WJEF56 strains. After submitting inoculated plates to iodine vapour, clear areas around colonies were observed and the colonies turned yellow, while blue colour occurs on growth-free areas. This indicates that two strains (WJEF56 and WJEF61) have the potential to hydrolyse starch present in medium.

Table 8. Morphological and biochemical characteristics of selected strains.

PSB strains / Characteristics		Commercial Control	WJEF15	WJEF26	WJEF38	WJEF39	WJEF41	WJEF45	WJEF46	WJEF51	WJEF56	WJEF59	WJEF61	WJEF63
Colony Morphology	Margin	I	E	E	E	E	I	E	I	E	I	I	E	E
	Color	W	Y	Y	OW	Y	OW	OW	OW	OW	Y	Y	OW	OW
	Texture	D	V	V	V	V	M	V	M	V	M	M	V	V
Cells Morphology	Shape	R	R	R	C	R	C	C	C	C	C	C	C	CB
	Arrangement	S	S	CR	S	S	S	S	S	SP	S	S	S	S
Physiological tests	Gram staining	+	+	-	+	-	+	+	-	-	-	+	-	-
	Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+
	Motility	+	+	+	+	+	+	+	+	+	+	+	+	+
	Gelatin hydrolysis	-	+	+	-	+	-	-	-	-	+	+	-	-
	Urea hydrolysis	-	-	-	+	-	-	-	-	-	-	-	-	-
	GPA (Glucose)	+	+	+	+	+	+	+	+	+	+	+	+	+
	GPA (Lactose)	+	+	+	+	+	+	+	+	+	+	+	+	+
	Starch	-	-	-	-	-	-	-	-	-	+	-	+	-

Tested positive utilized substrate; tested negative do not utilized substrate. E: entire; I: irregular; Y: yellow; W: white; OW: off-white; D: Dry; V: viscid; M: mucoid; R: rod; C: cocci; CB: coccobacillus; SP: single pairs; CR: chain rods; and S: solitaire.

3.8. Bacterial tolerance to stresses

According to results (table 9), it is evident that selected strains are tolerant to considerable variations in terms of alkalinity, acidity, salinity, dryness, and temperature. All strains present tolerate temperatures between 24 °C and 37 °C, except the strain WJEF56. In addition, strains WJEF45, WJEF46, WJEF51, WJEF61, and WJEF63 were able to grow at temperatures up to 55 °C. In addition, these isolates were able to grow and survive over a wide pH range (3 - 13). Moreover, among all tested isolates, only WJEF45, WJEF51, WJEF59, and WJEF61 were able to successfully resist to higher NaCl concentrations (2M) $g.L^{-1}$ (Table 9).

To quantify tested PSBs tolerance to high salt concentrations, we incubated PSBs in a nutrient liquid medium, and after 24 hours, we measured biomass formation expressed by absorbance. Log (AB) as a function of salt concentrations revealed that all strains significantly decreased at

1.5M NaCl concentration, except WJEF15, WJEF26, and WJEF39 strains, which resist and show positif growth (Figure 7).

Table 9. Physiological characteristics of selected isolates under temperature, pH, and salinity pressure.

	PSB strains / Pressures	Commercial Control	WJEF15	WJEF26	WJEF38	WJEF39	WJEF41	WJEF45	WJEF46	WJEF51	WJEF56	WJEF59	WJEF61	WJEF63
GT	24°C	1	1	1	1	1	1	1	1	1	1	1	1	1
	37°C	1	1	1	1	1	1	1	1	1	-1	1	1	1
	48°C	1	-1	-1	1	1	1	1	1	1	1	1	1	1
	55°C	-1	-1	-1	-1	-1	-1	1	1	1	-1	-1	1	1
G pH	pH 3	-1	1	1	1	1	1	1	1	1	1	1	1	1
	pH 5	1	1	1	1	1	1	1	1	1	1	1	1	1
	pH 7	1	1	1	1	1	1	1	1	1	1	1	1	1
	pH 9	1	1	1	1	1	1	1	1	1	1	1	1	1
	pH 11	1	1	1	1	1	1	1	1	1	1	1	1	1
	pH 13	-1	1	1	1	1	1	1	1	1	1	1	1	1
GS	1.6M	1	1	1	-1	1	1	1	1	1	-1	1	1	1
	2M	-1	-1	-1	-1	-1	-1	1	-1	1	-1	1	1	-1

GT: growth temperature. GpH: growth pH. GS: growth salinity. Tested positive/utilized as substrate; tested negative/not-utilized substrate.

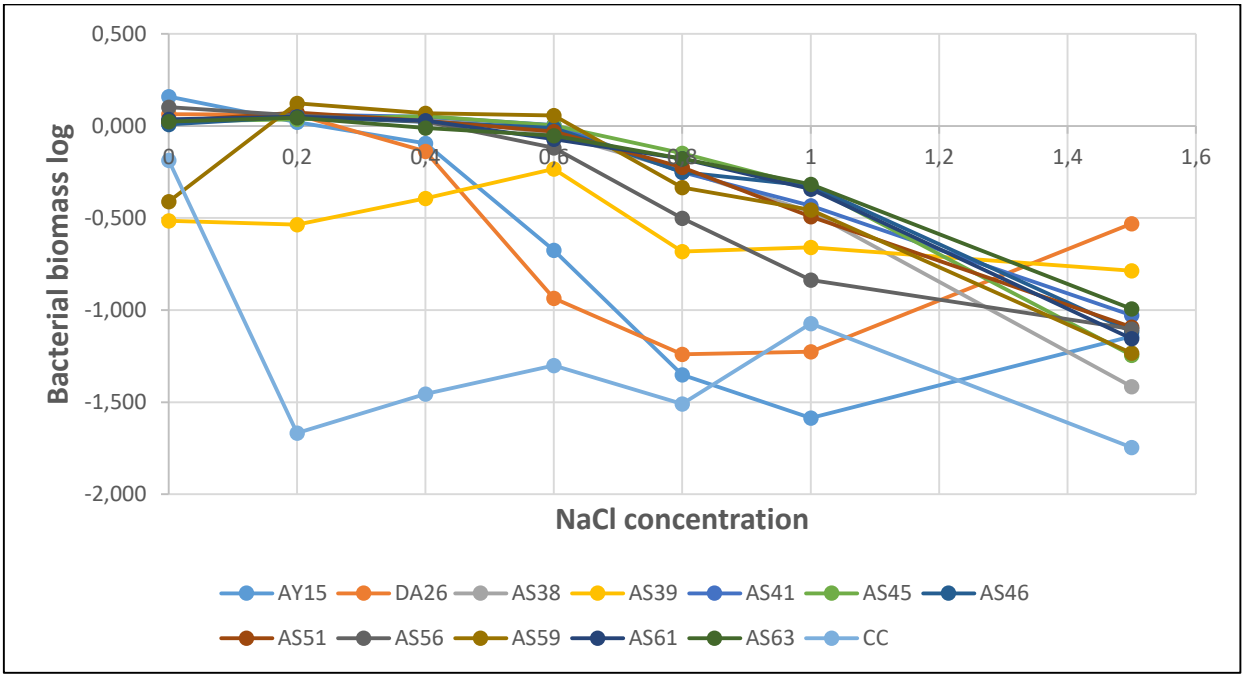


Figure 7. Biomass in function of salt concentrations $g.L^{-1}$.

Regarding drought tolerance, all twelve isolates show the same behaviour under increasing PEG-6000 concentration (10%. 20%. and 30%). PSB isolates exhibit a decline in cell numbers and absorbance value. However, the WJEF61 strain presented an optimal resistance, with an

absorbance values higher than those of the other isolates and commercial control to all PEG levels (Figure 8).

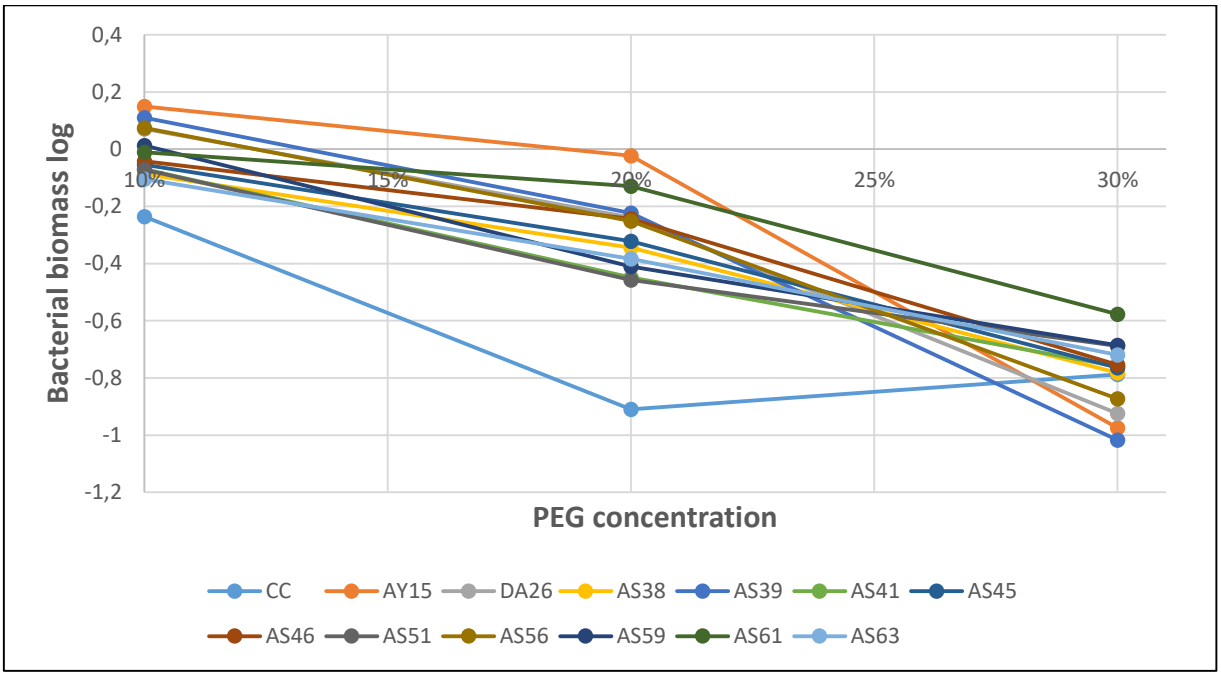


Figure 8. Biomass in function of PEG concentrations.

Based on principal correspondence analysis of tested strains on physicochemical pressure we notice that we have two major groups of strains, the first one are correlated with temperature and the second group are correlated with alkalinity, acidity, and salinity (Figure 9).

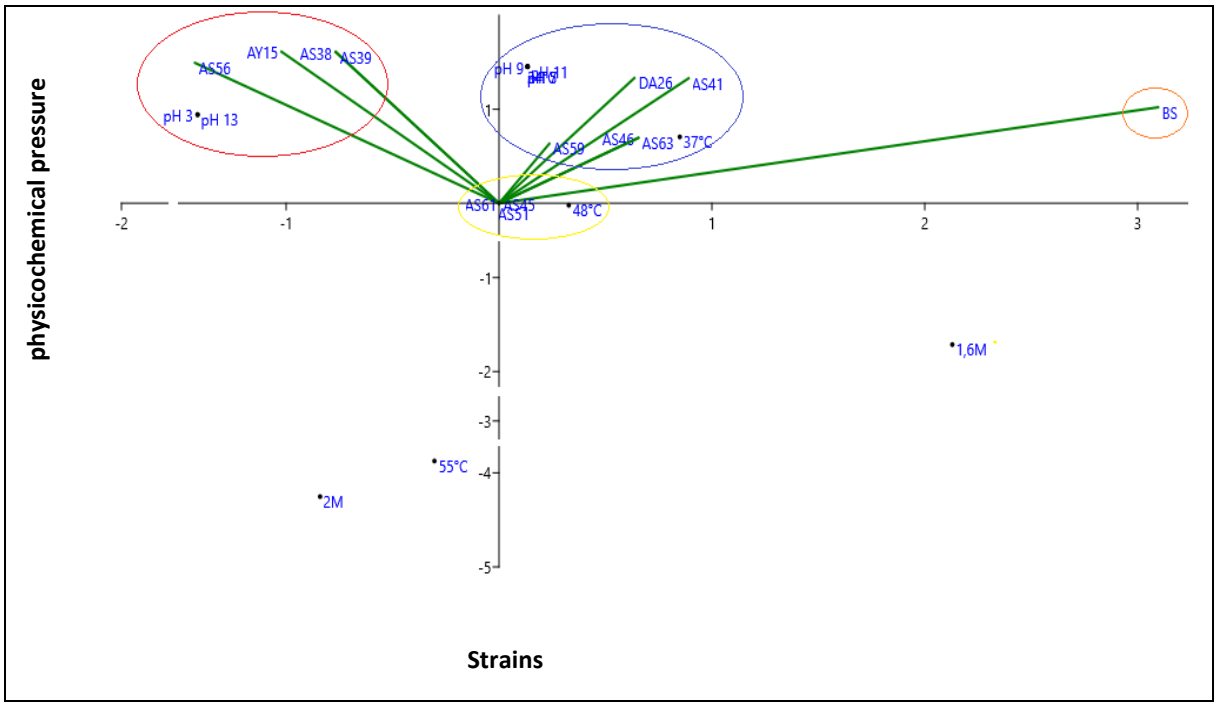


Figure 9. Principal correspondence analysis of tested isolates on physicochemical pressure.

3.9. Antifungal effect of PSB strains

Fungal growth inhibitory of isolated PSBs bacteria was tested on six plant pathogenic fungi: *Aspergillus Niger*. *Fusarium Culmorum*. *Fusarium Oxysporum*. *Penicillium Digitatum*. *Penicillium Notatum*, and *Rhizopus Stolonifer* (Table 10). Antifungal activity showed that from twelve bacteria, three (WJEF26, WJEF56, and WJEF61) were able to inhibit fungal growth of at least four fungi tested (Figure 10). In addition, best antagonist against tested fungi was located in WJEF61 strain with an IRG percentage of 49.15%, 23.51%, 32.76%, and 32.92% against *Aspergillus Niger*, *Fusarium Culmorum*, *Fusarium Oxysporum*, and *Rhizopus Stolonifer*, respectively. For the WJEF59 strain has an antagonistic effect only against *R. Stolonifer* with a % IRG of 36.54% which present a specific character (Table 10).

Table 10. Fungal inhibited radial growth behaviour of tested PSBs bacteria.

PSB strains / Fungal strains	**Commercial Control**	**WJEF15**	**WJEF26**	**WJEF38**	**WJEF39**	**WJEF41**	**WJEF45**	**WJEF46**	**WJEF51**	**WJEF56**	**WJEF59**	**WJEF61**	**WJEF63**
A. Niger	21.31	-	23.2	-	-	-	-	-	21.4	-	49.1	-	-
F. Culmorum	-	20.4	-	18.9	-	-	18	24	18.3	-	23.5	22.4	-
F. Oxysporum	18.64	-	21.1	-	22.9	-	-	16.6	-	-	32.7	-	-
P. Digitatum	26.92	-	-	-	-	33.8	-	-	20.3	-	-	-	-
P. Notatum	25.53	-	16.0	-	-	-	-	-	18	-	-	-	-
R. Stolonifer	-	23.0	21.1	17.3	26.9	30.7	25	-	-	36.5	32.9	19.2	21.4

4. Discussion

The present study focused on the effect of several tolerant PSBs on different abiotic stresses to promote P solubilization from RP and TCP as the only P source in the context of biological fertilization and sustainable agricultural strategy. Increasing soil P concentrations via inorganic and organic soil P mobilization pools involves changes in pH and the release of P-mobilising compounds such as carboxylates and phosphatases, which are exuded by the roots themselves or by microorganisms in the rhizosphere. However, the selection of an efficient PSB is crucial, as it practically increases P in the plant rhizosphere (Aliyat et al., 2020). Phosphate solubilization capacity is considered a key factor in isolating highly efficient PSBs from agricultural soils (Aliyat et al., 2020). Among one hundred and thirty-four (134) PSBs strains from different leguminous plants rhizosphere in the Fez-Meknes region, only twelve (12) PSBs

show phenotypic stability of P solubilization after five successive subcultures in NBRIP-RP plates.

Solubility index (Figure 2) indicates an PSI of TCP in agreement with previous studies conducted on different plants rhizosphere, where PSBs solubility index ranges from 2 to 5.1 (Sadik et al., 2013; Rfaki et al., 2014; Manzoor et al., 2017). The PSB isolated strains used in this work were able to increase P availability in NBRIP liquid medium containing RP or TCP as source of P, also a combination of RP * TCP. Our results support the idea that PSBs are characterized by their ability to easily and efficiently solubilize inorganic P (Elkoca et al., 2008; Anand et al., 2016.; Adnan et al., 2019). This is confirmed with the use of *Bacillus subtilis* DSM 10, which improved P nutrition of common beans in hydroponic experiments when plants were inoculated with *Rhizobium tropici* and grown with one of the two P sources (Pi or phytate) and co-inoculated with *B. subtilis* (Maougal et al., 2014). Our results show that soluble P concentration in the medium containing RP was between 16.11 $mg.l^{-1}$ and 25.16 $mg.l^{-1}$, while the medium containing TCP was between 75.61 $mg.l^{-1}$ and 147.62 $mg.l^{-1}$ (Figure 3). These results are in agreement with other studies done by Zaidi & El Naqa, (2010) and (Anand et al., 2016), who report that PSBs can solubilize insoluble inorganic P compounds, such as TCP, dicalcium P (DCP), hydroxyapatite, and RP. Phosphate solubilization is accompanied by pH medium decrease when compared to control, both in media containing RP and TCP. Furthermore, we note that as pH values decrease, the P concentration increases. This is noted by Batool & Iqbal, (2019) who indicate a strong correlation between P solubilization and pH decrease. Changes in pH value in the rhizosphere, caused by roots or microorganisms, can influence the surface charges on metal oxide particles and thus Pi availability (Hinsinger et al., 2009), ultimately leading to Pi release that is available to plant. P mobilization in the rhizosphere is largely due to pH changes induced by roots and/or associated microorganisms and to carboxylates exudation (Hinsinger et al., 2011). Soil pH is critical in determining the availability of inorganic forms of P (Hinsinger et al., 2009; Adnan et al., 2018), and it can also influence the fate of organic P through its impact on enzymatic activities. In neutral to alkaline soils, Pi occurs largely in various forms of Ca phosphate (mainly apatites) whereas, in acidic and deeply weathered soils, such as those abundant in the tropics, much of the soil Pi occurs as bound to and/or occluded in Fe and Al oxy(hydr)oxides (Jones & Oburger, 2011). As the positive charges on these minerals become increasingly positive with decreasing pH, greater amounts of P can be adsorbed at low rather than high pH. In addition, Ca-phosphates solubility is strongly increased with decreasing pH. (Devau et al., 2011) showed that both increasing and decreasing pH could lead to an increase in soil P availability, which was consistent with the

findings of previous studies, as reviewed by (Hinsinger et al., 2009). Similarly, Alori et al. (2017) observed a negative correlation between the amount of P solubilized by *B. cepacia* (SCAUK0330) and decreasing pH, leading to increased solubilization of P. As proposed by Batool & Iqbal, (2019), the decrease in pH can be explained by organic acid production and H^+ secretion. Several studies support this argument and indicate that the main mechanism of mineral P solubilization is lowering soil pH by microbial organic acid production (Rodríguez & Fraga, 1999; M. S. Khan et al., 2010; Sharma et al., 2013; Anand et al., 2016). The excretion of organic acids that promote Pi solubilization is a trait found in many P-solubilising microorganisms. Rodríguez et al. (2007) listed several bacterial genes involved in gluconic acid oxidation, which is a major mechanism of mineral P solubilization in Gram-negative bacteria (Chauhan et al., 2015).

Experimental design results of P solubilization and pH medium are highly significant, and show that pH medium and P[C] is maximal when RP and TCP are at highest level (2.5% and 2.5%) (Table 6 and 7). However, Bashan et al. (2013) pointed out that most commonly used source of insoluble Pi, tricalcium phosphate (TCP), is unreliable and insufficient. Suggested that a combination of two or three metal-P compounds is used together or in tandem, depending on the type of soil and final use of screened bacteria. This would result in a significant reduction in potential PSBs and would maximize chances for selecting most efficient strains capable of contributing to plant P nutrition. Our experiments show an increase in P solubilization accompanied by a decrease in pH, increasingly as RP and TCP concentration increase (Figure 5 and 6). Similarly, Yadav et al. (2017) use organic compost with RP at different concentrations to optimize P solubilization by PSBs. They found that the 75:25 ratio of RP and compost showed maximum soluble P accompanied by higher plant growth when compared to plants fertilized with normal compost and rock phosphate. Our result can explain Pi solubilization process by microorganisms due to carboxylates and organic acids production. Concerning the free extracellular phosphatase enzymes, although it is difficult to differentiate it origin (Burns et al., 2013), several examples show that microbial enzymes are predominant in soil (Gao et al., 2019), and these present a higher efficiency for Pi release (Adnan et al., 2019). Different types of phosphatases can be found, including phosphomonoesterases, which have been most studied, especially for P-solubilising microorganisms (Jones & Oburger, 2011).

On the other hand, as discussed by (Khan et al., 2010), although there is clear potential for the development of inoculants with PSBs, their widespread application remains limited by a poor understanding of microbial ecology, dynamics population in soil, and inconsistent performance in a range of environmental factors. However, although PSBs are present in the majority of

soils, their performance is strongly influenced by environmental factors such as soil type, temperature, salinity, cation exchange capacity, pH, organic matter, and availability of substances in soils (Rfaki et al., 2018; Masdariah et al., 2019). Similarly, Namli et al. (2017) stated that resistance to extreme environmental conditions is an important characteristic that allows inoculant bacteria to survive and maintain optimal population numbers throughout specific crop life cycle. Resistance to intrinsic and extrinsic stresses is an important factor for proliferation, development, and survival of microorganisms in rhizospheric soils. In our study, and to have a more appropriate approach for competent strains selection of PSB that could increase the chances of successful field inoculation and significantly improve plants P status. All selected strains showed good tolerance to various environmental stresses such as increased temperature from (37 to 55 °C), salinity ranging from (0.2 to 2 M), pH between 3 and 13 (Table 9), and drought variation of PEG-600 from (10 to 30%) (Figure 8). Therefore, the microorganisms that survive under drastic conditions could be used in different cultural practices to improve agricultural production. Moreover, isolated PSBs showed a strong ability to inhibit different fungal species (Table 10). These results, were confirmed by the findings of Muleta et al. (2009), who reported that several *Pseudomonas* strains which could inhibit phytopathogenic strain *Fusarium oxysporum*, possess the ability to produce HCN, siderophores, and antibiotics.

Furthermore, Gram staining indicates a group of Gram-negative and Gram-positive strains, with an advantage for Gram-negatives (Table 8). Besides, previous observations have shown that several plants rhizosphere selects more Gram-negative than Gram-positive rhizobacteria (Muleta et al., 2009). Cell motility test indicates that all selected strains were motile (Table 8). Moreover, bacterial motility is considered to be an essential element for successful root colonization and P transfer in soil (Shahid et al., 2015). In addition, it has been reported that motile bacteria have better access to root exudates in rhizospheric zone through chemotaxis (Shahid et al., 2015; Manzoor et al., 2017). Motility can potentially enhance the rhizoplane competence in terms of movement, both from bulk soil to root and along roots (Dutta & Podile, 2010), leading to heterogeneous colonization of host plant roots by the PGPR. After root surface colonization, PGPR can stimulate plant growth through several mechanisms impacting plant nutrition and, in some cases, also improving its resistance to pathogens (Pérez-Montaño et al., 2014).

However, PSB biotechnology offers an excellent opportunity to develop durable biofertilizers to be used as supplements and/or alternatives to chemical fertilizers to overcome agricultural challenges imposed by an ever-increasing food demand (S. B. Sharma et al., 2013). Moreover,

with readily available carbon resources, free-living soil bacteria can rapidly mineralize P from soil organic matter and incorporate it into their biomass. Microbial P thus represents not only an important P sink in soil and terrestrial ecosystems but also a possible source of available P for plants (X. Xu et al., 2013). In addition to chemical fertilizers, PSBs application as biofertilizers could also promote plant growth with large amounts of soluble P (Pi, polyphosphates, and organic P) released from lysed bacterial cells following rewetting of dry soils or during freeze-thaw cycles at the beginning of growing season. For example, (Turner et al., 2003) showed that the potential contribution of bacterial cell lysis to water-extractable phosphorus following soil drying varied between 88% and 95% in two Australian pasture soils. In general, the selection of a competent strain of PSBs as an inoculant and their qualification as a biofertilizer should not only be based on laboratory and greenhouse experiments but also on field experiments.

5. Conclusion

The main objective of this study was to assess several PSB strains' ability to enhance solubilization activity from RP, TCP, and their combination. As a problem, Inorganic P fixation into insoluble complexes renders these compounds inaccessible for plant absorption. The use of biofertilizers such as microorganisms can help in this regard by sustainably enhancing plant growth. Based on the mentioned results, we find that the isolated PSBs strains have an antifungal effect up to 49.15% inhibition as well as a high capacity to resist intrinsic and extrinsic stress. In addition, we note that P solubilization significantly increased by 20.58% when RP and TCP percentages in combination are at the max level. By analyzing all the different orientations of this study, we recommended the use of PSBs strains with high potential as microbial fertilizers in the agronomic field. To exploit the full potential of these telluric bacteria more studies need to be investigated on the genetic mechanisms level and microbial-mineral interfaces. Further innovative research should focus on the application concepts of microbial biotechnology in agriculture to identify more PSBs for their usage in a consortium to have competent microbial inoculants in sustainable cultures production systems under various conditions.

Chapitre IV

Stimulation de la croissance de deux légumineuses (*Vicia faba et Pisum sativum*) par inoculation de bactéries solubilisant le phosphate

Stimulation de la croissance de deux légumineuses (*Vicia faba et Pisum sativum*) par inoculation de bactéries solubilisant le phosphate

Synthèse

La culture des légumineuses joue un rôle clé dans la sécurité alimentaire et la durabilité des agroécosystèmes en fournissant plusieurs services au niveau des systèmes alimentaires et de production. Différentes variétés de légumineuses constituent une source majeure d'alimentation humaine et animale d'origine végétale. Ainsi, ces cultures peuvent fournir de nombreux autres bienfaits (fixation biologique de l'azote, atténuation des émissions de gaz à effet de serre, lutte contre les mauvaises herbes et les maladies des plantes, etc.). En fait, les cultures de légumineuses jouent un rôle clé dans la gestion intégrée de la fertilité des sols en raison de leur capacité à fixer l'azote atmosphérique en association avec les rhizobia, ce qui peut conduire à une augmentation de la fertilité des sols et à une diminution de la dépendance à l'égard des engrais chimiques dans les systèmes agricoles à faible apport d'intrants. Néanmoins, la population mondiale croissante ne cesse de demander de nourriture et les rendements agricoles diminuent en raison de facteurs de stress abiotiques tels que la pénurie de nutriments, la sécheresse, la salinité et la température, qui sont les principaux obstacles à l'agriculture durable dans le monde entier.

Ces dernières années, il est devenu de plus en plus important de répondre à la demande alimentaire sans mettre en danger les écosystèmes. En raison des pertes importantes et des fuites dans l'environnement résultant de divers processus, tels que la dénitrification dans le cas des engrais azotés et l'eutrophisation dans le cas des engrais phosphorés. Cependant, les applications d'engrais organiques et inorganiques dans les champs agricoles sont fréquemment controversées. L'application intensive d'engrais chimiques pour la croissance et la protection des plantes entraîne une dégradation des sols et par conséquent la destruction de l'environnement et des écosystèmes. Les chercheurs se sont intéressés de près à ces circonstances, qui nous obligent à améliorer la fertilité des sols en mettant en œuvre une agriculture écologique et durable.

Le phosphore (P) est essentiel au métabolisme des plantes et à leurs multiples fonctions, notamment le développement, l'anatomie et la densité des racines qui augmentent de manière significative le rendement des cultures et la résistance des plantes à diverses maladies. Au niveau cellulaire, le P est impliqué dans la division cellulaire, la croissance des tissus et la structure des acides nucléiques, qui régulent la synthèse des protéines, le transfert d'énergie et la photosynthèse. Hormis le fait que le P existe sous diverses formes dans le sol, il est absorbé

que sous forme d'anions orthophosphates monovalents ($H_2PO_4^-$) et bivalents (HPO_4^{2-}) dans des proportions relatives au pH du sol. En conséquence, on estime que moins de deux milliards d'hectares de terres agricoles dans le monde sont affectés par une faible disponibilité de P, et que la carence en P entraîne directement une réduction significative (5-15%) du rendement des cultures. L'utilisation des microorganismes du sol à des fins agricoles pourrait améliorer les nutriments du sol tout en réduisant la dépendance des agriculteurs à l'égard des engrais inorganiques.

L'exploitation des bactéries solubilisant le phosphate (PSB) est une solution biologique récemment développée pour améliorer la disponibilité du P dans la rhizosphère car elles peuvent convertir le P insoluble en formes disponibles pour les plantes. Outre l'augmentation de la disponibilité du P pour les plantes, les PSB contribuent également à la production de sidérophores, d'acide indole acétique et de gibbérelline, ainsi que d'antibiotiques, de métabolites secondaires et d'enzymes tels que 1-aminocyclopropane-1carboxylate (ACC) désaminase. L'inoculation de PSB augmente significativement la biomasse végétale aérienne et souterraine (poids frais et sec), la teneur en N et en P des plantes. De même, les graines sous inoculation de PSB expriment un taux de germination plus élevé et une croissance plus forte. Par conséquent, l'utilisation des PSB représente une question de promotion pour augmenter la croissance des plantes et la disponibilité du P dans le sol.

Étant donné que l'effet de ces bactéries sur la qualité du sol et les différentes phases de la croissance des plantes reste mal connu, nous avons abordé la question suivante : les mécanismes de performance et de promotion de la croissance des plantes par les PSB sont-ils associés à l'espèce cultivée ? En se basant sur l'hypothèse que l'inoculation des semences de légumineuses pourrait renforcer la fertilité du sol et améliorer le rendement des cultures, cette étude vise à évaluer l'impact de cinq souches de PSB isolées de la rhizosphère de légumineuses, sur la croissance de deux légumineuses (*Vicia faba* et *Pisum sativum*) et sur la qualité du sol. Les souches efficaces de PSB utilisées sont caractérisées par la solubilisation du P, l'activité ACC désaminase, la fixation de N_2, la production des auxines, de l'HCN, et des siderophores. L'activité de ces bactéries est testée *in vitro* et *in vivo* en conditions contrôlées supplémentées du P naturel (RP). Plus précisément, nous évaluons les effets de l'inoculation de PSB sur les propriétés chimiques du sol telles que le pH, la conductivité électrique (EC), la matière organique, la teneur en N, et en P en plus des caractéristiques biologiques comme la teneur en protéine, la teneur en amidon, l'activité protéasique, la respiration basale du sol de la rhizosphère, l'activité de la phosphatase alcaline, et l'activité de la β-glucosidase. En outre,

différents paramètres de croissance ont été évalués tels que le taux de germination, la teneur en nutriments, la biomasse végétale, la longueur des tiges et de racines, et la nodulation des racines des fèves et des petit pois.

Les expériences sur la germination des graines montrent que les souches de PSB peuvent stimuler de manière significative la croissance précoce des plantes tout en augmentant le taux de germination des deux plantes étudiées. Les graines traitées avec les PSB ont un taux de germination plus élevé et une longueur d'enracinement plus longue que les témoins non traités. Toutes les souches de PSB ont surpassé le contrôle en termes d'amélioration de la croissance des légumineuses testées (fèves et des petit pois) avec un pourcentage allant de 77,78% à 88,88%, respectivement. Les résultats ont montré que tous les traitements ont amélioré de manière significative les paramètres des plantes comme leur teneur en azote et en P des plantes (67,50 %, 23,11 %), respectivement. On note également une augmentation des poids frais et secs des biomasses aériennes (41,17%, 38,57%) et souterraines (56,6%, 42,28%), respectivement. Par rapport au contrôle, l'inoculation des PSB montre une augmentation de 72% de la longueur des racines, de 40,91% du poids sec de la plante et de 40,07% du poids frais. Le sol rhizosphérique des traitements PSB présentait des niveaux élevés de N, P et de matière organique. Tous les traitements ont montré des niveaux significativement plus élevés de phosphatase alcaline, de respiration basale du sol et d'activité β-glucosidase que le contrôle. Les résultats des expériences en serre ont également permis de montré que l'inoculation de PSB pouvait améliorer l'activité de la phosphatase acide du sol et le P disponible. Nos recherches montrent que les PSB de la rhizosphère des légumineuses peuvent produire les auxines, des siderophores, de l'HCN et des producteurs d'ammoniac qui soutiennent la croissance de la fève et du petit pois, en plus de solubiliser le P et de sécréter de la phosphatase acide pour dissoudre le P insoluble.

Sur la base de ces résultats, nous concluons que les souches testées sont des souches potentielles solubilisant le P et favorisant la croissance des plantes qui peuvent compléter les besoins en P de la fève et du petit pois. Il est donc appuyé que les PSB compétentes peuvent être une alternative à l'utilisation d'engrais chimiques pour améliorer la qualité du sol et la croissance des plantes. Les résultats de l'étude offre une méthode pratique pour améliorer d'une manière agronomique les inoculant microbiens afin d'augmenter la mobilisation du P du sol pour la croissance des plantes hôtes dans la production intensive ou les applications agro écologiques.

Growth stimulation of two legumes (*Vicia faba* and *Pisum sativum*) using phosphate-solubilizing bacteria inoculation

This part of the work was published in the journal *"Frontiers in microbiology"*. (Q1 - IF: 6.064).

Abstract

The application of chemical fertilizers for plant growth and protection is one of the reasons for the environment and ecosystem destruction, thus, sustainable agriculture is gaining popularity in research and among farming communities. Although most soils are high in total phosphorus (P), a large portion is unavailable to plants and regarded as a growth-limiting factor. P-solubilizing bacteria (PSB) exploitation is a newly developed bio-solution for enhancing rhizosphere P availability; however, the effect of these bacteria on soil quality and the different phases of plant growth remains unknown. This study aims to evaluate the impact of five strains of PSB, isolated from legume rhizosphere, on the growth of two plants (*Vicia faba* and *Pisum sativum*) and certain soil properties. The efficient strains of PSB used are characterized by the P-solubilization, the ACC deaminase activity, the fixation of N, and the IAA, HCN, and siderophores production. The activity of these bacteria is tested *in vitro* and *in vivo* under controlled conditions on the growth of the two plants supplemented with the rock P (RP). According to our findings, all PSBs strains outperformed the control in terms of enhancing the growth of the tested legumes with a percentage ranging from 77.78% to 88.88%, respectively. The results showed that all treatments significantly improved plant parameters like nitrogen-(N) and P-content in the plants (67.50%, 23.11%), respectively. In addition, an increase in the fresh and dry weights of above- (41.17%, 38.57%) and belowground biomasses (56.6%, 42.28%), respectively. Compared to the control, this leads to an increase of 72% in root length, 40.91% in plant dry weight, and 40.07% in fresh weight. Rhizospheric soil in PSBs treatments displayed high levels of N, P, and organic matter. All treatments were found to have significantly higher levels of alkaline phosphatase, basal soil respiration, and β-glucosidase activity than the control. It is concluded that multi-traits PSB can be an alternative for utilizing chemical fertilizers to enhance soil quality and plant growth. Despite the potency of PSBs, its use as a source for the development of sustainable agriculture implies focusing on crop species and adaptation, stress tolerance and climate resilience.

Keywords: alkaline phosphatase, broad bean and pea, PSBs inoculation, plant growth promotion, rock phosphate

1. Introduction

More food is needed to feed the world's growing population, but agricultural yields are decreasing due to abiotic stressors such as nutrient shortage, drought, salinity, and temperature, which are the main obstacles to sustainable agriculture worldwide (Kapadia et al., 2021). It has become increasingly important in recent years to address the food demand without endangering ecosystems. Due to the significant losses and escape to the environment as a result of various processes, such as denitrification in the case of nitrogen fertilizers and eutrophication in the case of phosphorus fertilizers, both organic and inorganic fertilizer applications to agricultural fields are frequently criticized (Namli et al., 2017). Along with ecosystem pollution, extensive use of mineral fertilizers is also leading to land degradation. Researchers have paid close attention to these circumstances, which force us to improve soil fertility by implementing eco-friendly and sustainable agriculture, which is possible solely using biofertilizer-based microorganisms. Indeed, using these soil microorganisms for agricultural purposes can improve soil nutrients while reducing farmers' reliance on inorganic fertilizers (A. Zaidi et al., 2017).

The most important nutrient for plant growth and development during an adequate rate from the earliest stages of their development is phosphorus (P), which is second only to nitrogen (N). This element is crucial to plant metabolism and multi-functions including developing roots, root anatomy, and root hair density, all of which significantly increase crop yield and plant resistance to various diseases (M. Khan et al., 2022). P is crucial at the cellular level as it is involved in cell division, tissue growth, and nucleic acid structure, all of which regulate protein synthesis, energy transfer, and photosynthesis (H. Malhotra et al., 2018). Except, P exists in various forms in the soil but a significant portion is insoluble due to fixation and precipitation by soil minerals like aluminium, iron, and calcium, it is absorbed from the soil solution largely as monovalent ($H_2PO_4^-$) and divalent (HPO_4^{2-}) orthophosphate anions in proportions relative to the soil pH (Ducousso-Détrez et al., 2022). Accordingly, it has been estimated that less than two billion hectares of agricultural land worldwide are affected by low P availability, and P deficiency directly causes a significant reduction (5–15%) in crop yields (Shenoy & Kalagudi, 2005; Elhaissoufi et al., 2022). According to Alori et al. (2017), the release of various organic acids by microorganisms causes the acidification of microenvironments, which in turn causes P ions to be replaced by cations, a process known as phosphorus solubilization (Kalayu, 2019). For this reason, P-solubilizing microorganisms can convert insoluble P into plants' P-available forms. However, given their range of activities, P-solubilizing bacteria (PSB) are the most significant soil microbes for P-solubilization. Along with increasing P availability for the plants, several PSBs likewise contribute to the production of siderophore, indole acetic acid (IAA),

and gibberellin, as well as antibiotics, secondary metabolites, and 1-aminocyclopropane-1carboxylate (ACC) deaminase enzyme (Lee et al., 2019; Mitra et al., 2020; G. Zhao et al., 2021; C. Song et al., 2022). In addition, PSBs are also believed to be effective biocontrol agents for soil-borne pathogens (A. Yadav & Yadav, 2022a). Therefore, PSB utilization represents a promoting issue to increase plant growth and P availability in soil. When compared to the control, Namli et al. (2017) found that PSB inoculation treatments significantly increased above and below-ground plant biomasses (fresh and dry weight), plant N, and P content. Likewise, Biswas et al. (2018) noted that the sterilized seeds of mung beans (*Vigna radiata*) displayed a greater germination rate and higher growth under PSBs inoculation. Additionally, according to Song et al. (2022), PSBs from intercropped soybean rhizosphere soil may secrete organic acids that increase the solubilization of unavailable P, enhance soil ACP activity and P availability, and produce IAA and siderophores that aid in the germination and growth of maize seeds. On their part, Valetti et al. (2018) presented that all the PSB strains used in their experiment were able to promote plant growth under greenhouse conditions and also increase rapeseed yield (from 21 to 44%) in field trials.

Since PSBs can play a key role in enhancing the host plant's ability to withstand stressful conditions such as nutrient deficiency, we addressed the question: are the performance and plant growth promotion mechanisms of rhizobacteria associated with the crop species? Considering the importance of using PSBs, we hypothesize that legume seed inoculation could enhance soil fertility and improve crop yields. In addition to their important role in the integrated management of soil fertility, their ability to fix atmospheric N_2 in association with rhizobia may result in improved soil fertility and reduced reliance on N fertilizers in agricultural systems (Vanlauwe et al., 2019); legumes are an essential source of vegetable protein for human and animal nutrition (Stagnari et al., 2017). This is consistent with the study's goal and justifies the choice to work on the two species of this family to examine the process by which five P-solubilizing rhizobacteria performed. Specifically, we assess the effects of PSBs applications on some soil chemical properties such as pH, electrical conductivity (EC), organic matter, N, and P in addition to the biological characteristics like rhizosphere's basal soil respiration, alkaline phosphatase activity, and β-glucosidase activity. Furthermore, different growth parameters consistently germination rate, nutrient content, plant biomass, shoot length, root depth, and yield of Broad Bean and Pea plants. The study's findings may offer a practical method for agronomically enhancing microbial inoculants to increase soil P mobilization for plant growth in intensive production or agroecological applications. This work contributes to Morocco's national strategy (GREEN GENERATION 2020-2030), which aims to achieve

sustainable development and maximise yields. This involves improving soil quality using biological methods.

2. Materials and Methods

2.1. Phosphate solubilizing bacteria isolation and P activity

Five PSB isolates based on their P-solubilizing capacities (PSC) (low, moderate, and high P-solubilizers) served as inoculants in this study to explore the above- and below-ground physiological responses of rock P (RP)-fertilized Pea and Broad Beans seeds. These isolates were among 51 PSB isolates collected from the soil rhizosphere (strongly adhering to the roots) of legumes at four agricultural production sites in the Fes-Meknes region (Tafrant, Ait Yaakoub, Dayt Al Amira, and Ait Saleh). The National Botanical Research Institute (NBRIP)-agar P growth medium was used for PSBs isolation, with either tricalcium P (TCP, $Ca_3 (PO_4)_2$, 5 g/L) or RP (5 g/L containing 28.15% of P_2O_5) as the sole source of P added with (per litre); glucose: 10 g; $MgCl_2 \bullet 6H_2O$: 5 g; $MgSO_4 \bullet 7H_2O$: 0.25 g; KCl: 0.2 g and $(NH_4)_2\ SO_4$: 0.1 g (Nautiyal, 1999). Colonies were selected according to the development of phosphate solubilization zones and quantitative analysis of P solubilization rates in NBRIP liquid medium supplemented with TCP or RP was performed after seven days of incubation at 28 °C. The available P portion was determined by a spectrophotometer at 880 nm using the ascorbic acid colourimetric method. The absorbance was measured using standard solutions of KH_2PO_4 with P concentrations ranging from 0.1 to 1 $mg.L^{-1}$ (Janati et al., 2022)**.**

2.2. Bacterial characterization for plant-growth-promoting traits

In addition to P solubilization, isolates were tested for plant growth-promoting (PGP) traits such as N-fixation **(**ammonium producers), siderophores production, indole acetic acid (IAA) production, 1-aminocyclopropane-1-carboxylic (ACC) deaminase activity, and hydrogen cyanide (HCN) production.

2.2.1. Analysis of IAA production

According to Gordon & Weber's (1951) methods, IAA production by PSBs was determined with some revisions. The strains were inoculated separately into an LB liquid medium containing L-tryptophan (1 L + 0.1 g w/v). The suspension was incubated at 28 ± 2 °C for 4 days. The broth was centrifuged at (30000xg, 10 min). 0.1 mL of the supernatant was mixed with 0.1 mL of Salkowski's reagent (4.5 g $FeCl_3$ and 587.4 mL of 98% H_2SO_4, solution) and kept in the dark for 15 min (Biswas et al., 2018). The absorbance at 530 nm was measured using

a standard solution of IAA varying in concentration from 0 to 100 μg.mL^{-1}. The content of IAA per unit volume of fermentation broth was calculated according to the standard curve.

2.2.2. ACC deaminase activity

The isolates' growth in DF minimal salts medium (Dworkin & Foster, 1958) augmented with 3 mM ACC was examined to determine whether they could use ACC as an N source. The absorbance at 540 nm, which is the -ketobutyrate produced from the enzymatic cleavage of ACC through ACCD as previously discussed by Honma & Shimomura, (1978), was used to measure the ACC deaminase activity of the isolates. By comparing a sample's absorbance at 540 nm to a standard curve of -ketobutyrate with concentrations between 0.1 and 1.0 mM, the amount of -ketobutyrate produced by this reaction is calculated (Chinnadurai et al., 2009).

2.2.3. Analysis of N-fixation

In an N-free Ashby medium made up of the following components: agar 15 g, mannitol 15 g, K_2HPO_4 0.4 g, $CaCl_2$•$2H_2O$ 0.1 g, NaCl 0.2 g, $MgCl_2$ 0.1 g, $FeSO_4$•$7H_2O$ 3.0 mg, NaMoO•$2H_2O$ 3.0 mg, free N-fixation has been proven to occur. PSB isolates grown in Ashby medium were regarded as free N-fixer isolates after 7 days at 28 ± 2 °C. Isolate's ability to produce ammonium was verified according to Geetha et al. (2014), 10% peptone water was prepared, autoclaved, and inoculated with selected strains. After 3 days of incubation, 0.5 mL Nessler's reagent was added to 1 mL supernatant. A positive test result was indicated by the development of a brown to yellow colour; the intensity of the colour revealed how much ammonia the isolates had produced.

2.2.4. HCN production

Hydrocyanic acid (HCN) production was quantified following Bakker & Schippers (1987). On tryptone soya agar plates that had been amended with 0.44% glycine, a few strains were streaked. The filter paper was placed on the agar plate, dipped in a solution of 2% sodium carbonate and 0.5 mL picric acid, sealed with para-film, and incubated for 72 hours at 37 ± 2 °C. According to the test, the isolates produce HCN because the filter paper's colour changes from yellow to reddish-brown (Lagzian et al., 2013).

2.2.5. Analysis of siderophores production

All of the tested strains were inoculated into mannitol salt agar (MSA) liquid medium and shaken for two days at a temperature of 28 ± 2 °C at 180 rpm in the incubator. The fermentation broth underwent a 6800xg, 10-minute centrifugation. Using the chrome azurol sulfonate (CAS) assay, the isolates' production of siderophores was quantitatively assessed (Shin et al., 2001).

A solution containing 10 mL of $FeCl_3 \cdot 6H_2O$ solution was added to a solution containing 60.5 mg CAS in 50 mL of deionized water. Additionally, to make CAS' final volume of 100 mL, 72.9 mg of HDTMA (hexadecyltrimethylammonium bromide), dissolved in 40 mL of deionized water, was added. After thoroughly combining 4 mL of the CAS solution with 4 mL of the culture supernatant, the mixture was incubated for 1 hour. At 630 nm, the absorbance of each sample was determined. By measuring the percentage of siderophores units produced by various bacterial strains, siderophores production was assessed (Equation 1).

$$SUs = [(Ar - As)/Ar] \times 100 \quad (1)$$

Ar: absorbance of the reference (CAS reagent) and As: absorbance of the sample.

2.3. Plant and soil used

The effect of bacterial strains on germination and plant growth is tested on two legume crops, Feve-Aguadulce Broad Bean (*Vicia faba*) and PP-Onward Pea (*Pisum sativum*). The seeds were obtained from the agriculture plant section of A.PHY.SEM Company. The soil used in this study was collected from a field on the Science and Techniques Faculty of Fez's experimental farm. Standard procedures were followed to determine the physical and chemical characteristics of the soil before the experiment began (Pavan et al., 1992). Before and after the culture, the soil's N, P, and K contents are measured.

2.4. Controlled seed germination experiment

For seedling bioassay, five bacterial isolates with different PSC were grown in TSA agar medium (24 h at 28 ± 2°C). Broad Bean (*Vicia faba*) and Pea *(Pisum sativum)* seeds were surface sterilized with sodiumhypochlorite (2 %) and washed five times with distilled sterile water. Sterilized seeds were inoculated with bacteria using a suspension containing 10^9 bacteria.mL^{-1}, separately. The seeds are placed in Petri dishes containing sterilized filter paper and incubated for one week at 25 °C. Seeds are considered to be germinated when the radicle breaks through the seminal envelope. Germination parameters such as germination energy, seed water content, and average germination time were measured during the germination week. After seven days post-inoculation, the germination percentage was registered. The root length of each seedling was measured seven days after incubation and compared to the non-inoculated seeds.

2.5. Biological assay

After 24 hours of incubation, we carried out biological tests using kinetics. During the six days of germination, we condemned two seeds (for the two studied legumes, separately) each day for the determination of soluble proteins, protease activity and starch content. To this end, for

each sample (two Pea seeds and two Broad Bean seeds), we removed the seed coats and embryos (future seedlings) and kept the cotyledons to carry out the biological tests mentioned above. Physiologically speaking, the main proteins in legumes are reserve proteins, whose role is to be a source of nitrogen, carbon, sulphur and amino acids for the embryo's future needs. We carried out kinetics to see the effect of PSB inoculated into the seeds, which can improve the concentration of soluble proteins, which is one of the essential characteristics for efficient germination. We then analysed protease activity to validate the positive correlation with soluble protein. Starch is also required to make simpler sugars such as maltose and glucose after hydrolysis by amylase, which is necessary to maintain germination and seedling growth. For this purpose, a kinetic analysis of the starch concentration during the six days of germination was required to validate the positive effect of PSBs during the germination of the two leguminous plants tested. So we noticed the induction of proteolytic and aminolytic activities.

2.5.1. Determination of soluble proteins

For soluble proteins determination, 1 g of fresh material seeds were homogenized in an ice-cold mortar supplemented with 2 mL of phosphate buffer solution, the homogenate is centrifuged (30000xg, 10 min at 2°C), and 1 mL of the supernatant is mixed with 2 mL Bradford reagent before incubating in the dark for 15 minutes. The protein concentration is determined using a reference to a standard solution of bovine serum albumin (BSA) of known concentration. The absorbance was measured at 595 nm (Karimi et al., 2022).

2.5.2. Determination of protease activity

Protease activity was determined according to (Anson, 1938) with few modifications. 1g of fresh material seeds were homogenized in an ice-cold mortar supplemented with 2 mL of phosphate buffer solution, the homogenate is centrifuged (30000xg, 10 min at 2°C), and the supernatant (enzyme extract) is recovered. One mL of 2% casein is combined with 250 µL of enzyme extract, and the mixture is then incubated at 37 °C for 30 minutes. The reaction was stopped by adding 500 µL of 10% trichloroacetic acid (TCA). The substrate was treated as previously mentioned for the control after being precipitated with 500 µL 10% TCA before being added to the enzyme solution. After centrifugation, 300 µL supernatant was added to the 2.5 mL cu-alkaline solution and kept for 15 minutes. The mixture was then thoroughly combined with 250 µL of folin ciocalteau reagent (FCR), which had been diluted two times. Using a spectrophotometer, the absorbance was measured at 660 nm. The difference in absorbance between the test sample and the control sample was used to measure the protease activity.

2.5.3. Determination of starch content

Starch content was determined according to Allfrey & Northcote (1977) as cited by Dkhil & Denden (2010). Seeds were homogenized in an ice-cold mortar (1 g fresh material) and pestled in a volume of 10 mL 80% (v/v) ethanol. The homogenates were centrifuged (30000xg, 10 min at 2°C) and then perchloric acid ($HClO_4$; 6 ml, 30%, v/v) was added to solubilize starch from the pellet. The suspension was left at room temperature for 6 h, and starch was detected with an I_2-KI reagent. Samples of 1 mL starch solution were mixed with 1 mL I_2-KI reagent, and then were vortexed and left standing at room temperature. The standard curve of 0 to 5 $mg.mL^{-1}$ that was created using soluble starch dissolved in 30% $HClO_4$ and detected with the same I_2-KI reagent was compared to the absorbance at 620 nm.

2.6. Pots trails experiment

Five PSBs strains with different PSCs were selected for the legumes pot experiment (50 mL of bacterial suspension per pot). The experiment included pots control without inoculation and pots with strains inoculation for both Pea and Broad Beans seeds. All pots are supplemented with RP solution (5%) as a P-fertilizer. The experiment was conducted at the experimental greenhouse of the Science and Technology Faculty, Fez, Morocco, from 21 March to 21 May 2022, with an average temperature of 30 °C in the daytime and 13 °C at night. The experiment used a three-replicate per treatment, fully randomized design. Each pot contained five seeds, which received daily weight-based irrigation equal to 70% of the water-holding capacity. Plants were harvested for root and shoot analyses sixty days after germination.

2.6.1. Morphological shoots and roots traits measurement

Plants were harvested and divided into shoots and roots sixty days after germination. An analytical balance was used to calculate the fresh weights (FW) and shoots and roots highest (SH, RH). Analysis was done on plant nodulation and biomass. Before being ground to a fine powder for P and N concentration analyses, the shoots and roots of treated plants and control plants were dried in an oven at 70 °C for two days to determine their dry weights (DW).

2.6.2. Determination of P and N nutrients acquisition

The rhizosphere growth soil was obtained by carefully separating roots from the adhering soil, which was then sieved (2 mm) before measurements of P and N concentrations. Following ash solubilization in hydrochloric acid (10 N) and finely ground dried samples (0.5 g) that were dried at 100 °C for 48 h to determine the total P contents in shoots and roots. The absorbance was measured at 820 nm after the obtained filtrates (1 mL) were added to 5 mL of a reaction

mixture containing ammonium molybdate (2.5%) and hydrazine sulfate (0.15%). A total of 100 mg FW of shoots and roots were ground in an extraction solution made up of 5 mL of sodium acetate buffer (0.1 M pH 5.6) and 1 mM dithiothreitol. Homogenates were centrifuged (13000xg, 30 min at 4 °C) and aliquots of 50 µL were used for quantification of inorganic P (Pi), Shoot and root P contents were measured following the ascorbic acid method (Bargaz et al., 2017). The Kjeldahl method was also applied to the finely ground shoot subsamples (0.5 g) for total N analysis (Magomya et al., 2014). The ratio of plant P content to root dry weight was used to calculate the root P acquisition efficiency (RPAE), which measures the ability of roots to absorb P from the soil (Pan et al., 2008). According to Bargaz et al. (2017), the activity of root acid phosphatase was measured. 100 mg of root FW samples were ground in 5 mL of sodium acetate buffer (0.1 M pH 5.6) containing 1 mM dithiothreitol as part of the extraction process. 50 µl of the supernatant from the centrifugation of homogenates (13000 x g, 30 min at 4 °C) was used to measure the amount of root APase activity. The enzyme activity was measured as the amount hydrolyzing one nmol of p-nitrophenyl phosphate (pNPP) per minute per g of root FW.

2.6.3. Soil analysis after inoculation

Samples of the soil were taken from each pot after the experiment. According to Naseby & Lynch (1997) methods, the chemical characteristics of the rhizosphere soil were determined, including pH-water, electrical conductivity (EC), soil organic matter, N-total and P-available (Fernández et al., 2007), basal soil respiration (BSR), alkaline phosphatase activity (APA), and -Glucosidase activity (GA).

2.7. Statistical analysis

The IBM SPSS Statistics V. 26 software was used to analyze statistical data. The results were shown as means and standard deviations based on three replications. Before performing an analysis of variance (ANOVA), tests for homogeneity of variance and normal distribution were run, and then a Waller-Duncan (W) post hoc test was used to determine whether there was a significant difference between the treatment's mean values at a $p \leq 0.05$ significance level. Excel Office 2013 is also used for mathematical modelling and regression analysis.

3. Results

3.1. Phosphate solubilizing bacteria isolation and screening for PGP Traits

All isolates showed separate P-solubilizing halos around bacterial colonies and the P-solubilizing index varied from 1.4 to 3.84. From the 51 PSB isolates presenting different PSCs

of TCP, five PSBs were selected WJEF38 (high PSC) with 120.20 mg.L^{-1} solubilized P, 100.81 to 114.93 mg.L^{-1} for WJEF46 and WJEF63 (moderate PSC) and from 75.63 to 78.67 mg.L^{-1} for WJEF15 and WJEF26 (low PSC), respectively (Table 1). Throughout seven days of incubation, medium acidification with either RP or TCP revealed a sharp decline from an initial value of 7 to 4.195. Additionally, PSBs were determined to be isolates that produced IAA 8.426 – 91.026 µg.mL^{-1}), N_2-fixers, ammonia producers (24.43 – 98.37 µg.mL^{-1}), ACC deaminase producers (0.27 – 0.58 nmol α-ketobutyrate mg/protein/h), siderophore-producers (39.46 – 73.26 Units), and also HCN producers except for WJEF26.

Table 1. Phosphate solubilizing bacteria characteristics. Data represent the mean ± of three independent replicates. Values with the same superscript letter were not significantly different according to the Duncan test performed ($P < 0.05$).

	P-solubilization					PGP traits				
Strains	**PSI**	**P [C] mg.L^{-1}**		**pH**		**AIA µg.mL^{-1}**	**Siderophores**	**ACC deaminase**	**NH_3 µg.mL^{-1}**	**HCN**
		RP	**TCP**	**RP**	**TCP**					
WJEF15	2.46^{ab}	72.64^{b}	75.63^{d}	4.275^{d}	4.69^{a}	8.43^{d}	39.46^{c}	0.56^{a}	71.2^{c}	+
WJEF26	2.30^{b}	53.75^{c}	78.67^{d}	4.675^{b}	4.67^{a}	–	–	0.27^{c}	24.43^{e}	–
WJEF38	2.67^{a}	77.26^{a}	120.20^{a}	4.75^{a}	4.23^{b}	91.03^{a}	72.66^{a}	0.58^{a}	98.37^{a}	+
WJEF46	2.27^{b}	74.58^{ab}	100.81^{c}	4.515^{c}	4.19^{b}	59.78^{c}	42.83^{b}	0.49^{b}	61.89^{d}	+
WJEF63	2.33^{b}	76.58^{a}	114.93^{b}	4.78^{a}	4.2^{b}	71.15^{b}	73.26^{a}	0.55^{a}	85.15^{b}	+

3.2. Inoculation effect on the physical and chemical soil properties

The physicochemical properties of the soil were recorded at the beginning of the experiment. Soil data including nitrogen (N), P, and potassium (K) available can be found in Table 2. The experimental soil used in this research was silty clay loam soil with characteristics: humidity of 5%, oxidizable organic matter of 1.08%, and cation exchange capacity (CEC) of 0.19 (dS·m^{-1}).

Table 2. Physicochemical properties of soil used before and after the experiment.

	Organic matter g.Kg^{-1}	**pH**	**Total N g.Kg^{-1}**	**Available N mg.Kg^{-1}**	**Total P g.Kg^{-1}**	**Available P mg.Kg^{-1}**	**Total K g.Kg^{-1}**	**Available K mg.Kg^{-1}**
Before inoculation	12.75	7.88	1.5	18.56	0.79	6.32	9.67	42.44
After inoculation	17.88	5.09	2.4	56.96	1.04	32.14	9.94	46.23

3.3. Effects of PSBs on root growth and seedlings germination *in vitro*

A petri dish assay was carried out to study the P-solubilizing bacteria effect at the early seedling stage of Pea and Broad Bean. All strains show a significantly positive impact by promoting Pea and Broad Bean seedling growth. The germination percentage and root length compared to the non-inoculated control were increased from 77.78 to 88.88% for Broad Bean seeds and from

44.44 to 66.66% for Pea seeds. The maximum root length results were observed in seeds inoculated, with the WJEF38 strain (3.56 cm) followed by the WJEF63 strain (3.51 cm). We note that these strains also gave the best germination percentage for both plants tested, with an increase of 14.27% for beans and 50% for peas compared to the control. Besides that, the present results showed a significant response regarding germination rate and root length of Broad Bean inoculated seeds better than Pea inoculated seeds (Figure 1). Our results showed a significant relationship between root length and germination percentage of inoculated seeds. Root length increased considerably with increasing germination percentage, which explains a strong positive correlation ($R^2 = 0.9239$).

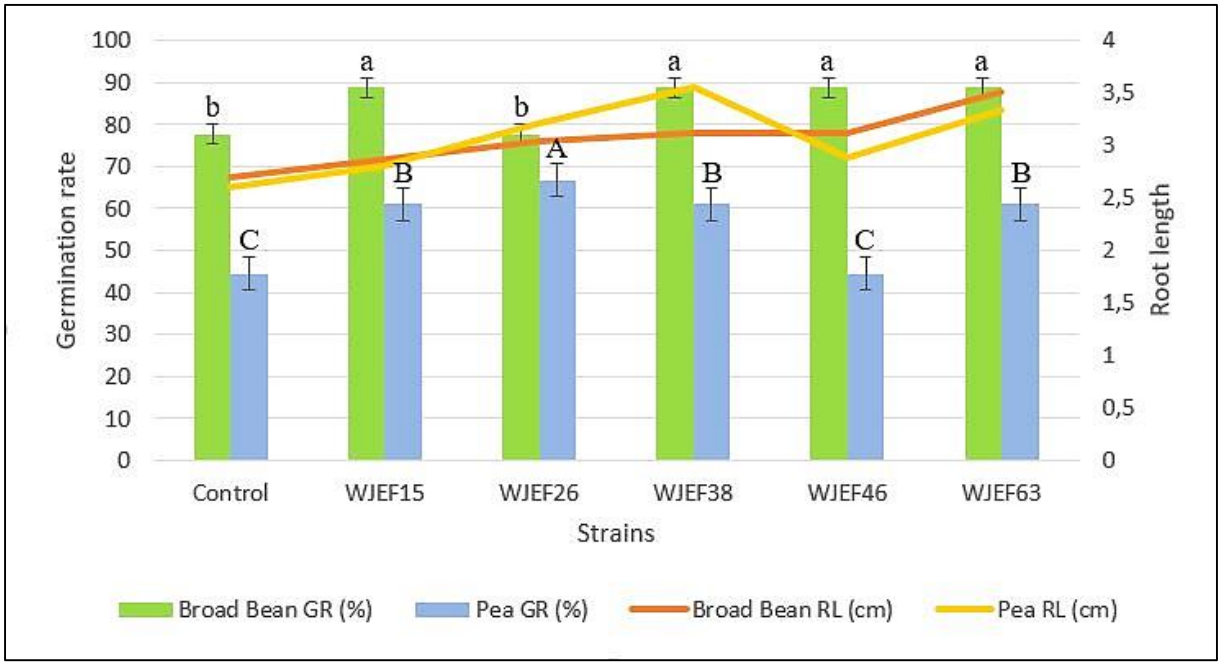


Figure 1. Germination percentage and root length of Pea and Broad Bean seeds inoculated with different PSB isolates. Values with the same superscript letter were not significantly different according to the Duncan test performed ($P < 0.05$).

3.4. Inoculation effect on the germination parameters

After seven days, post-inoculation several parameters of germination were evaluated (Table 3). The effectiveness of PSBs on germination energy, average germination time, and water content, confirms the results of germination percentage and root length. This time, as stated in the previous paragraph, the germination parameters of the Broad Bean seeds were also significantly higher than those of the Pea seeds. On the other hand, if we take the water content of the seeds and the average germination time, the increase for the peas is greater than for the Broad Bean. In this case, the efficiency of the strains is more important on Peas than on Broad Bean crops. From the results, we can see that some strains are more efficient than others in certain

phenomena. This allows us to say that strains WJEF26, WJEF38, and WJEF63 are the most competent on this level.

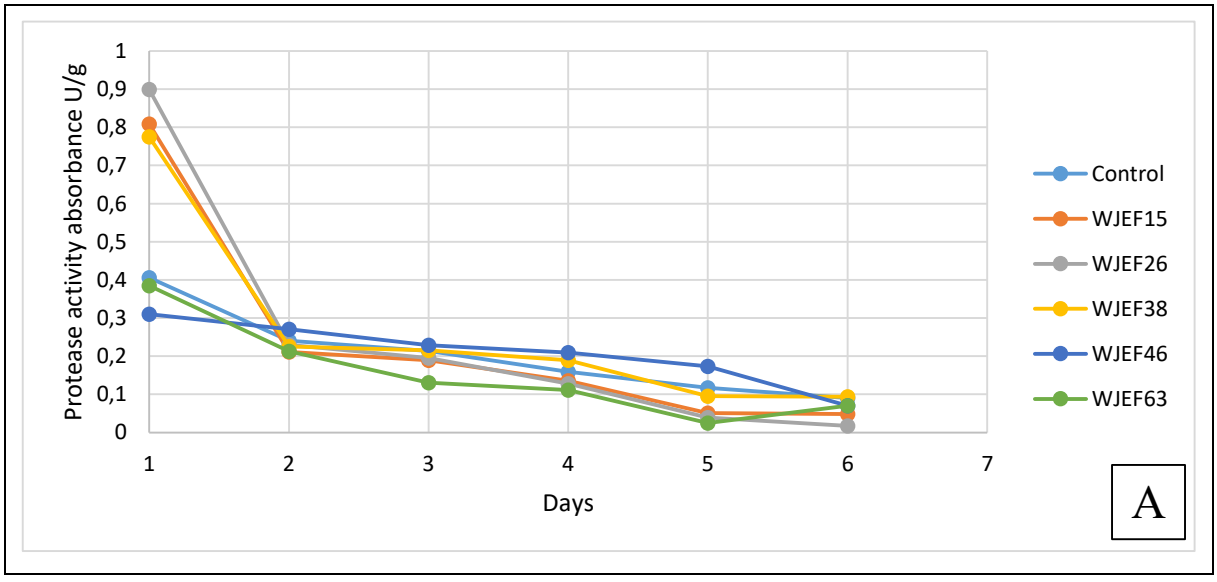
Table 3. Germination parameters of Pea and Broad Bean seeds inoculated with PSBs over one week. Data represent the mean ± of two independent replicates with five repetitions each. Values with the same superscript letter were not significantly different according to the Duncan test performed ($P < 0.05$).

Strains	Broad Bean			Pea		
	GE (%)	AGT (s/d)	WC (%)	GE (%)	AGT (s/d)	WC (%)
Control	52.77^{d}	0.350^{d}	32.233^{c}	33.33^{d}	0.518^{b}	44.789^{e}
WJEF15	50^{d}	0.378^{c}	33.592^{c}	44.44^{b}	0.480^{c}	60.186^{d}
WJEF26	44.44^{e}	0.398^{b}	38.764^{a}	44.44^{b}	0.500^{c}	64.133^{a}
WJEF38	72.22^{b}	0.411^{a}	37.739^{b}	38.88^{c}	0.519^{b}	61.556^{c}
WJEF46	61.11^{c}	0.316^{e}	37.876^{b}	38.88^{c}	0.536^{b}	63.390^{b}
WJEF63	83.33^{a}	0.421^{a}	38.337^{a}	61.11^{a}	0.662^{a}	63.333^{b}

GE: germination energy, AGT: average germination time (seeds/days), WC: water content.

3.4.1. Protease activity

The protease activity (PA) variation over one week is presented in Figure 2. The first three days show an important activity. Broad Bean seeds inoculated with strains WJEF26, WJEF15, and WJEF38 have high PA compared to the control. On the other hand, Peas seeds inoculated with WJEF26, WJEF15, WJEF38, and WJEF45 represent the higher PA compared to the control. After five days, PA decreases dramatically. Results explain the protein degradation phenomena, which occur between the first and fourth day when germination takes place and thus becomes less intense after the fifth day.



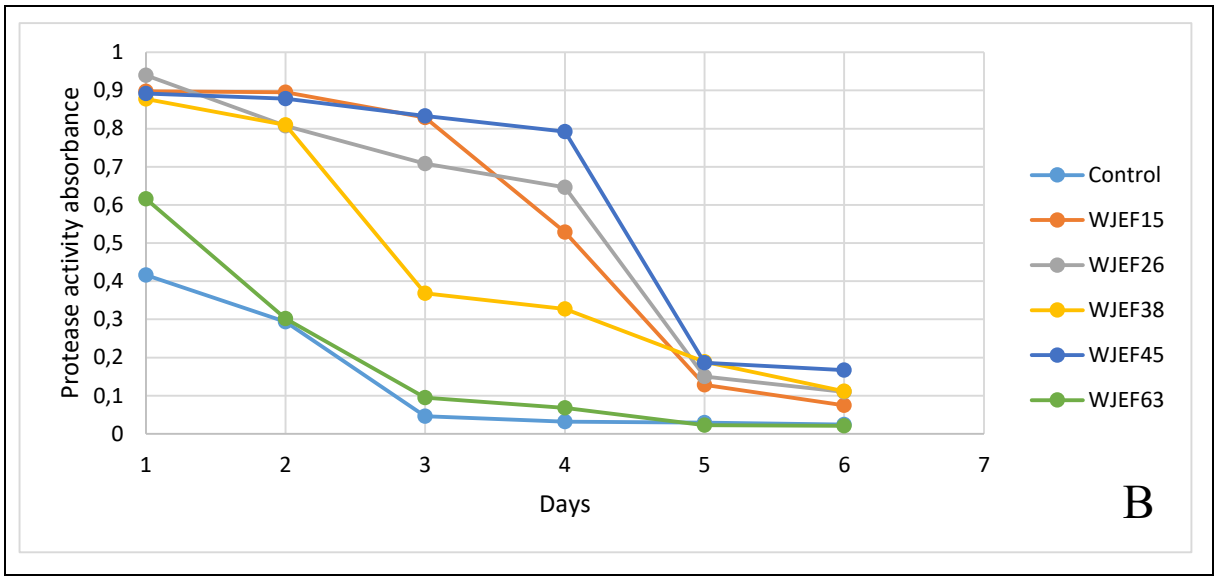
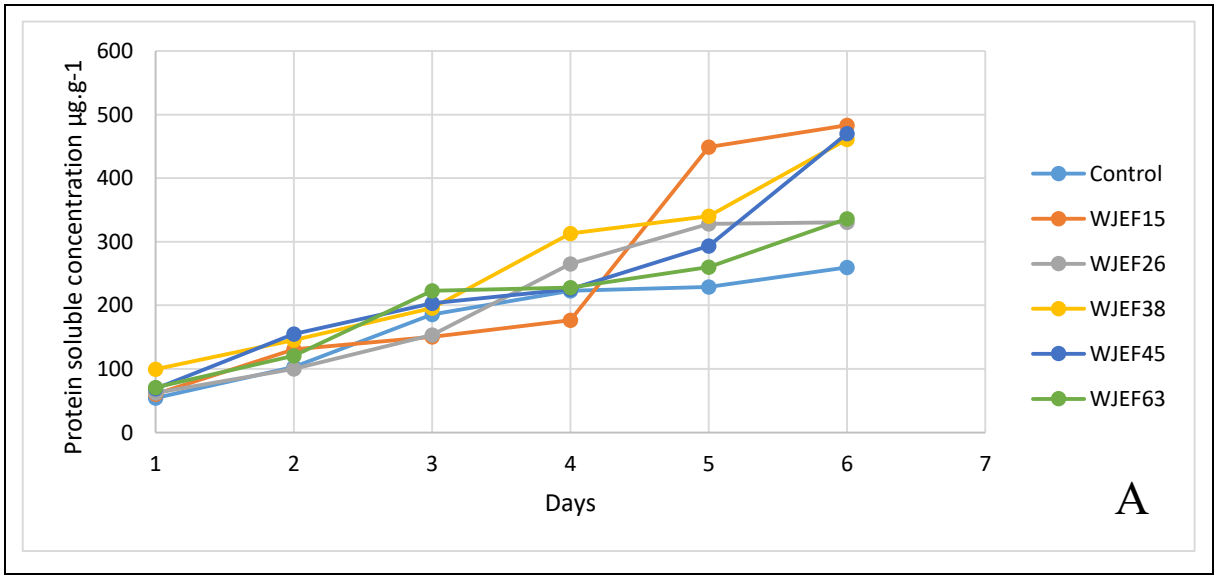


Figure 2. Kinetics of protease activity variation over one week; A) Broad Bean inoculated seeds; B) Pea inoculated seeds.

3.4.2. Protein soluble

The protein soluble (PS) concentration on the first and third day is almost low for all inoculated seeds including control not exceeding a concentration of 200 µg.g^{-1} FW. From the fourth day, all strains showed high protein content compared to the control (Figure 3). This can be explained by a protease activity that degrades proteins between the first and second day resulting in better germination. Broad Bean seeds inoculated with WJEF15 have a higher PS concentration; however, Peas seeds inoculated with WJEF45 were the most important value.



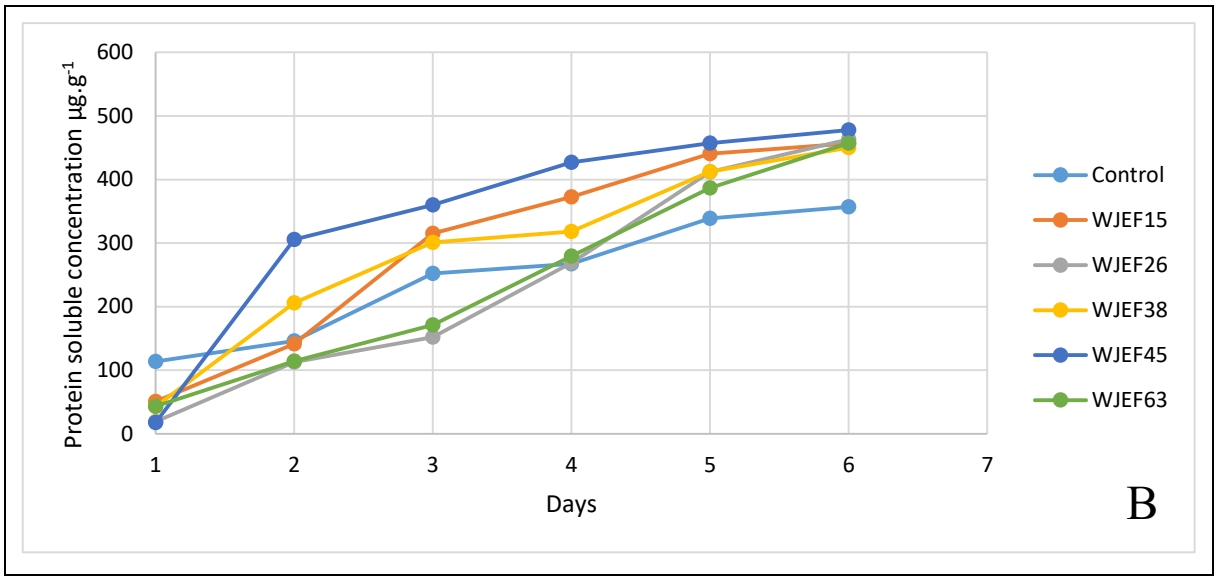
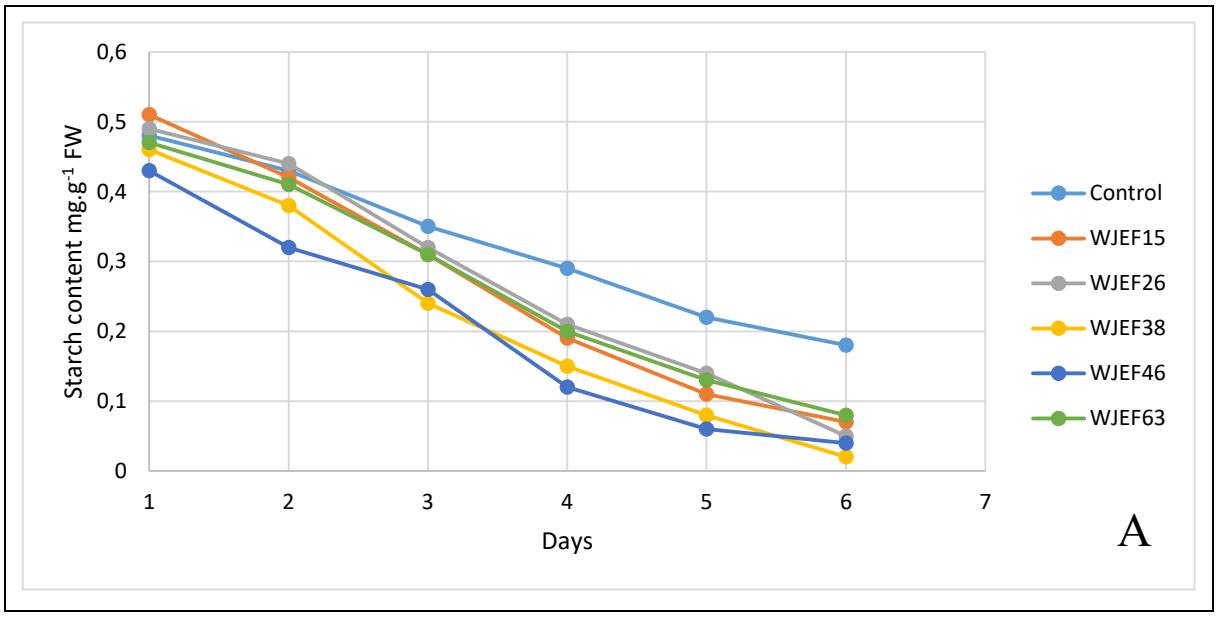


Figure 3. Kinetics of protein soluble concentration over one week; A) Broad Bean inoculated seeds; B) Pea inoculated seeds.

3.4.3. Starch content

Figure 4 shows the effect of PSB isolates on the starch level in germinating seeds Broad Bean and Pea seeds. During germination, the amount of starch decreased clearly and reached 0.02 and 0.01 mg.g^{-1} FW at Broad Bean and Pea seeds respectively. Starch level decreases more important with inoculated seeds compared to the control. A large decrease of starch was observed with seeds inoculated with WJEF38 and WJEF46 for bot legumes tested.



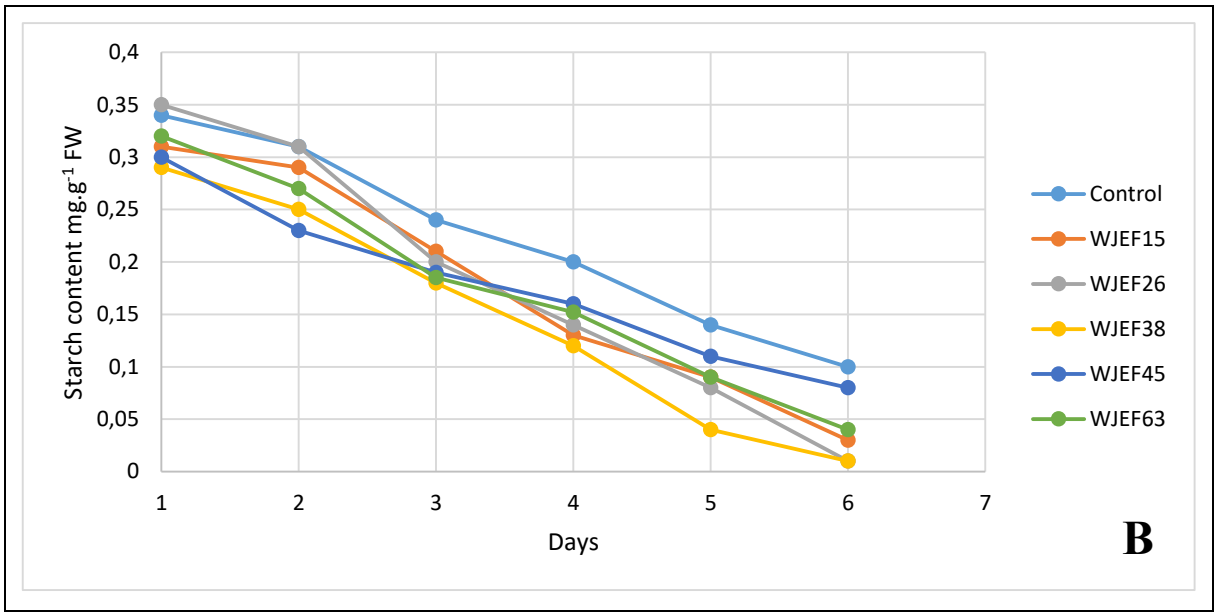


Figure 4. Kinetics of seed's starch content over one week; A) Broad Bean inoculated seeds, B) Pea inoculated seeds.

3.5. Effects of PSBs on plants growth *in vivo*

Phosphate solubilizing isolates were inoculated in Broad Bean and Pea plants to determine their capacity to promote plant growth in greenhouse trials. Eight weeks after inoculation, plants were harvested. All PSB strains showed a significant increase in growth parameters when compared to non-inoculated plants, notably SH, and RL (Table 4). In addition, there has been an increase in SFW and RFW (40% and 55.9%, respectively) in both tested legumes compared to the control. Likewise, SDW and RDW increased by 38.57% and 40.9%, respectively. However, Differences were also noted among PSB isolates, the WJEF38 strain was the significant isolate to increase growth parameters of both tested seeds, reaching either values higher than inoculated or non-inoculated plants. Otherwise, based on the results of SH, RL, SFW, and SDW, we notice that there is an affinity of the PSBs strains with the Broad Bean seeds more than the Pea seeds. On the other hand, we observe that inoculated and non-inoculated plants developed the same number of leaves despite a difference in distance between the nodes. In terms of nodulation, all PSBs strains showed a significant effect when compared to the control; furthermore, we noted a significant variation between PSB strains, whit the WJEF38, WJEF46, and WJEF63 showing the best effect.

Table 4. The differences in RP-fertilized broad bean and pea growth in response to PSB isolate inoculation versus RP treatments alone. The data are the average of two independent replicates with five iterations each. At $p < 0.05$, values with the same superscript letter did not differ significantly.

Legumes	Strains	SH (cm)	RL (cm)	SFW (g)	RFW (g)	SDW (g)	RDW (g)	Nodulation
Broad Bean	Control	$22,90^{e}$	$8,09^{d}$	$2,26^{e}$	$1,63^{e}$	$1,03^{e}$	$0,63^{e}$	+
	WJEF15	$35,68^{c}$	$13,70^{c}$	$2,99^{d}$	$1,87^{d}$	$1,43^{d}$	$0,80^{d}$	++
	WJEF26	$37,69^{c}$	$19,00^{b}$	$3,52^{c}$	$2,25^{c}$	$1,72^{c}$	$1,08^{b}$	+
	WJEF38	$51,01^{a}$	$24,38^{a}$	$4,38^{a}$	$2,62^{a}$	$2,36^{a}$	$1,15^{a}$	+++
	WJEF46	$39,5^{b}$	$20,62^{b}$	$3,92^{b}$	$2,08^{b}$	$1,99^{b}$	$0,84^{d}$	+++
	WJEF63	$31,92^{d}$	$13,00^{c}$	$3,01^{d}$	$1,89^{d}$	$1,13^{e}$	$0,90^{c}$	++
Pea	Control	$20,13^{D}$	$10,78^{F}$	$2,20^{E}$	$1,61^{D}$	$1,08^{D}$	$0,91^{D}$	+
	WJEF15	$25,52^{B}$	$12,47^{E}$	$3,59^{C}$	$2,88^{A}$	$1,59^{B}$	$1,32^{B}$	+
	WJEF26	$22,76^{C}$	$18,87^{C}$	$2,34^{D}$	$2,01^{C}$	$1,11^{D}$	$1,13^{C}$	++
	WJEF38	$36,67^{A}$	$22,66^{A}$	$5,49^{A}$	$2,83^{A}$	$2,67^{A}$	$1,54^{A}$	+++
	WJEF46	$26,33^{B}$	$16,58^{D}$	$3,78^{B}$	$2,37^{B}$	$1,63^{B}$	$1,49^{A}$	++
	WJEF63	$26,02^{B}$	$21,00^{B}$	$3,84^{B}$	$2,79^{A}$	$1,35^{C}$	$1,33^{B}$	+++

SH: shoot height, RL: root length, SFW: shoot fresh weight, RFW: root fresh weight, SDW shoot dry weight, RDW: root fresh weight.

3.5.1. P and N nutrients acquisition

The PSB inoculation's contribution was apparent in the response to P fertilization. Seed-inoculated plant parameters were higher than those from the fertilized plant alone. A high positive correlation between plant P content and root acid phosphatase activity in response to PSBs inoculation was observed (Table 5). In our study, higher acid phosphatase activities were found in the roots of seeds inoculated with PSBs, which could accelerate the decomposition of organic and inorganic P and promote plant P uptake. We noted an increase in P availability in the root and an improvement in the plant aerial surface in response to PSB inoculation. With an average increase of 67.50 and 57.24% for Broad Bean and Pea, respectively, compared to non-inoculated plants, shoot N content increased in response to PSBs inoculation. Significantly, higher than the control, a notable increase in RPAE was seen, indicating that PSB strains probably help to improve internal P use efficiency.

Table 5. Variations in P content in both root and shoot, root P acquisition efficiency RPAE, shoot total N, and root acid phosphatase activity of plants fertilized with RP and inoculated with PSB strains. The data are the average of two independent replicates with five iterations each. At $p < 0.05$, values with the same superscript letter did not differ significantly.

Legumes	Strains	P contents ($mg.g^{-1}$)		Shoot total N ($mg.g^{-1}$)	RPAE ($mg.g^{-1}$)	RAPA ($nmol{\cdot}g^{-1}{\cdot}min^{-1}$)
		shoot	root			
Broad Bean	Control	2.13^{f}	1.08^{e}	25.32^{e}	3.16^{d}	16.52^{f}
	WJEF15	3.77^{d}	2.21^{c}	32.97^{d}	7.93^{c}	18.26^{e}
	WJEF26	3.15^{e}	1.89^{d}	33.63^{c}	8.25^{c}	53.56^{d}
	WJEF38	4.87^{a}	2.45^{b}	37.51^{a}	10.87^{a}	86.89^{a}
	WJEF46	4.56^{c}	2.73^{a}	36.28^{b}	10.14^{b}	60.28^{c}
	WJEF63	4.79^{b}	2.66^{a}	37.42^{a}	10.48^{a}	70.64^{b}
Pea	Control	1.65^{E}	0.76^{E}	19.51^{E}	2.88^{E}	12.62^{F}
	WJEF15	3.11^{D}	1.34^{D}	24.07^{D}	4.41^{D}	21.73^{E}
	WJEF26	3.36^{C}	2.27^{B}	29.3^{C}	5.87^{C}	31.02^{D}
	WJEF38	4.23^{B}	2.61^{A}	31.38^{B}	8.14^{B}	64.43^{B}
	WJEF46	4.18^{B}	2.04^{C}	32.61^{B}	8.73^{A}	60.95^{C}
	WJEF63	4.49^{A}	2.77^{A}	34.08^{A}	8.96^{A}	67.2^{A}

RPAE: Root P acquisition efficiency, RAPA: Root acid phosphatase activity.

3.5.2. Rhizosphere biological properties after inoculation

The following table depicts the effects of various PSB inoculations on the biological properties of rhizosphere soils. We note that the PSBs strains application can decrease soil pH. The minimum pH value was found with the WJEF63 strain in both Broad Bean and Pea plants, which was 5.09 and 5.13, respectively. In the P-available parameter, the WJEF63 inoculation increased P-Available by 23.11% for Broad Bean and 22.77% Pea compared to the control, which confirms that the application of PSB strains was able to increase P-available in the soil. On the other hand, The PSBs inoculation had a significant impact on the rhizosphere's soil N content, ranging from 19.57% to 56.95% (Table 6). The control show the lowest N content was found, in contrast, the WJEF63 treatment show the highest one, which was not significantly different from the values of other PSBs treatments. A similar trend was also observed for soil EC, with maximum values occurring in the WJEF63 strain 0.63 and 0.68 $dS{\cdot}m^{-1}$ in Broad Bean and Pea treatment, respectively. The variation of the treatments' measured alkaline phosphatase activity (APA) ranged between 7.29 to 22.47 µg pNP g^{-1}. However, PSBs inoculation significantly affected the APA in the rhizosphere soil. The highest APA was determined in WJEF63, which was statistically different from all treatments. β-glucosidase (GA) activity varied between 1.89 and 6.2 mg pNP g^{-1}. The highest GA was observed with WJEF63 strains and it was different from the control treatment statistically. In addition, Basal soil respiration

varied between 0.75 and 1.35 mg CO_2 g^{-1} $24h^{-1}$. The values in the control treatments, which refer to the production of carbon dioxide when soil organisms respire, were typically lower than those in the other treatments.

Table 6. Mean values for soil parameters as influenced by the applied PSB and RP in legumes experiments. Data represent the mean ± of two independent replicates with five repetitions each. The data are the average of two independent replicates with five iterations each. At $p < 0.05$, values with the same superscript letter did not differ significantly.

Legumes	Strains	N Total	P Available	OM	pH	EC	BSR	APA	GA
Broad Bean	Control	21.4^{d}	7.43^{e}	1.1^{e}	7.54^{a}	0.21^{d}	0.75^{f}	7.29^{e}	2.1^{f}
	WJEF15	42.5^{c}	23.42^{c}	1.32^{d}	5.29^{bc}	0.49^{c}	1.04^{e}	21.02^{d}	4.36^{e}
	WJEF26	41.67^{c}	20.78^{d}	1.49^{c}	5.36^{b}	0.46^{c}	1.19^{d}	21.17^{c}	5.86^{c}
	WJEF38	55.83^{a}	31.82^{a}	1.92^{b}	5.23^{c}	0.58^{b}	1.35^{a}	22.31^{a}	6.01^{b}
	WJEF46	50.34^{b}	29.07^{b}	2.06^{a}	5.17^{c}	0.61^{a}	1.27^{b}	22.08^{b}	5.47^{d}
	WJEF63	51.37^{b}	32.14^{a}	1.88^{b}	5.09^{d}	0.63^{a}	1.21^{c}	22.47^{a}	6.2^{a}
Pea	Control	19.57^{E}	7.21^{E}	1.08^{D}	7.21^{A}	0.22^{E}	0.95^{E}	7.62^{E}	1.89^{E}
	WJEF15	44.69^{D}	28.36^{C}	1.54^{C}	5.79^{B}	0.57^{B}	1.02^{D}	20.73^{D}	5.73^{C}
	WJEF26	49.63^{C}	25.94^{D}	1.83^{B}	5.47^{D}	0.41^{D}	1.09^{C}	21.22^{C}	5.25^{D}
	WJEF38	52.32^{B}	30.59^{B}	2.16^{A}	5.33^{E}	0.66^{A}	1.04^{C}	21.06^{C}	5.94^{A}
	WJEF46	51.83^{B}	31.38^{A}	1.77^{BC}	5.62^{C}	0.52^{C}	1.17^{B}	22.36^{A}	5.22^{D}
	WJEF63	56.95^{A}	31.66^{A}	1.91^{B}	5.13^{F}	0.68^{A}	1.24^{A}	21.96^{B}	5.69^{B}

N total ($mg.Kg^{-1}$), P available ($mg.Kg^{-1}$), Electrical conductivity: EC ($dS{\cdot}m^{-1}$), Organic matter: OM (%), Basal soil respiration: BSR (mg CO_2 g^{-1} $24h^{-1}$), Alkaline phosphatase activity: APA (µg pNP g^{-1}), and β-Glucosidase activity: GA (mg pNP g^{-1}).

According to the regression analysis (Table 7, Figure 5), rhizosphere soil phosphatase activity (alkaline) was significantly associated with available P contents of soil, P contents in the shoot, and P contents in the root of both Broad Bean and Pea seeds. A polynomial model and exponential model resulting from the regression analyses of soil phosphatase activity and the other parameters.

Table 7. Mathematical modelling of P parameters in response to soil phosphatase activity.

P-Parameters	Equation	Mathematical model	R^2*
Broad Bean Soil P Available	$y = 0{,}418x^2 - 10{,}795x + 63{,}916$	polynomial	0,9832
Broad Bean Shoot P Content	$y = 0{,}0614x^2 - 1{,}6448x + 10{,}856$	polynomial	0,9437
Broad Bean Root P Content	$y = 0{,}0234x^2 - 0{,}5902x + 4{,}141$	polynomial	0,9204
Pea Soil P Available	$y = 3{,}3392e^{0{,}1015x}$	exponential	0,9886
Pea Shoot P Content	$y = 1{,}014e^{0{,}0621x}$	exponential	0,8951
Pea Root P Content	$y = 0{,}4199e^{0{,}076x}$	exponential	0,7624

R^2: Weighting coefficient.

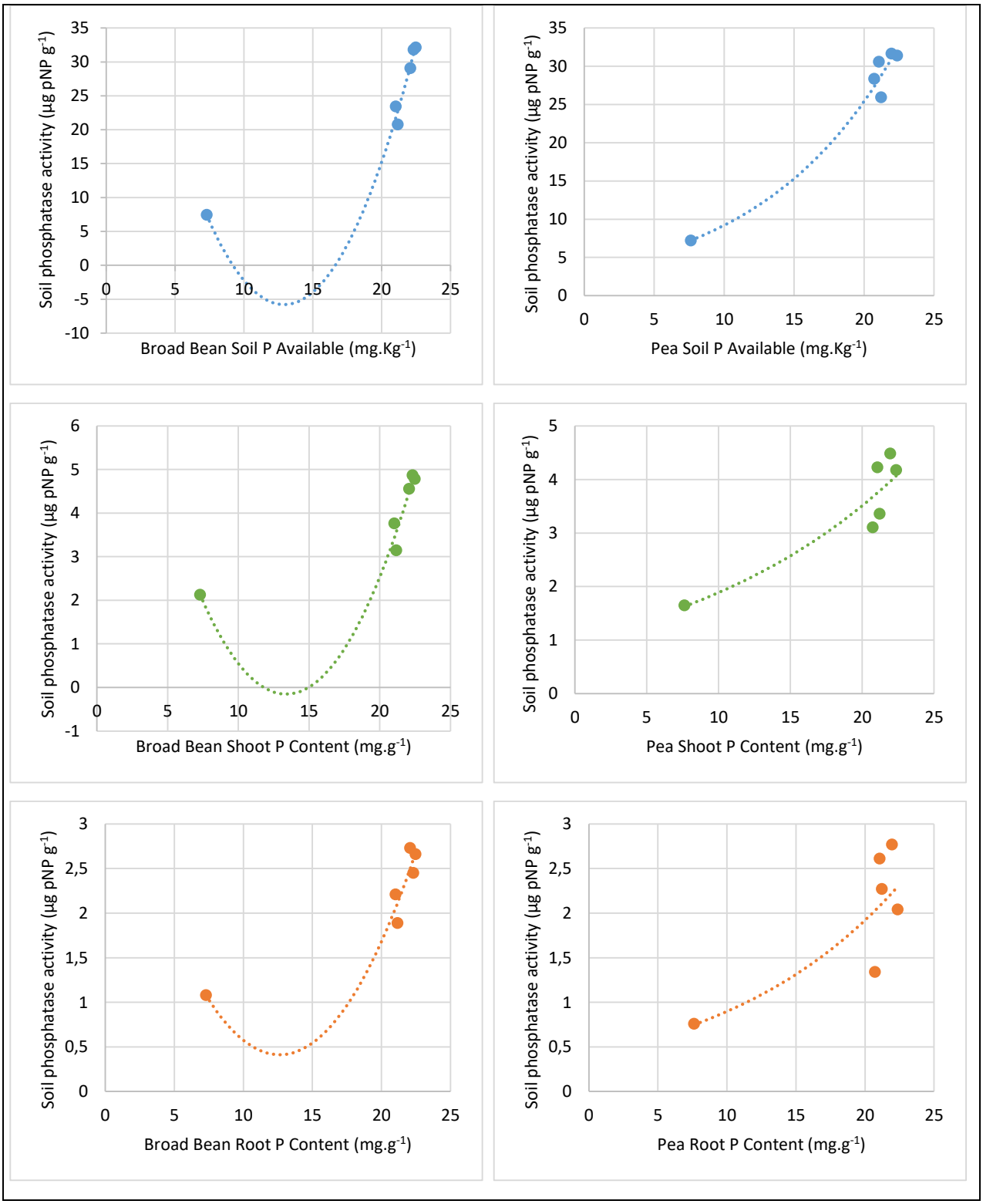


Figure 5. Correlation between rhizosphere phosphatase activity with soil available P, Soot P content, and Root P content.

4. Discussion

The majority of studies that were used to evaluate the effects of PSB inoculation were conducted in carefully monitored environments. The bacteria that perform the best in these studies are assessed in the field. The type of soil, the amount of nutrients present, and the species

of the host plant are some variables that can influence how bacteria behave in soil. Field tests were conducted to assess different PSB strains that might help in the growth of a variety of crops, including chickpeas, wheat, corn, and barley (Masdariah et al., 2019; Elhaissoufi et al., 2020). However, few studies have been conducted to evaluate PSB inoculation in Broad Beans and Peas. The current study raises the level of knowledge on the PSB-legumes interaction under insoluble P, specifically variations in root growth, as well as the associated rhizosphere modifications and above-ground growth related to P-use efficiency were studied using Broad Bean and Pea crops as tested legumes.

Soil bacteria are an important component of the agroecosystem playing an important role in soil nutrient cycling, pesticide degradation, soil health maintenance, and crop productivity (Meetei et al., 2022; Tarafdar, 2022). By increasing crop nutrient uptake and facilitating the efficient recycling of poorly soluble P in the soil, inoculating PSB strains increases the availability of poorly soluble P in the soil, which can reduce the need for P fertilizer application (Bargaz et al., 2021; Ajibade et al., 2023). In our study, a total of 51 PSBs were isolated from the rhizosphere soil samples under legume cropping systems at four experimental bases Tafrant, Ait Yaakoub, Dayt Al Amira, and Ait Saleh. Among them, five strains that had different RP-solubilizing capacities, with values ranging from 53.75 to 76.58 $mg.L^{-1}$, were chosen as inoculum. These findings are consistent with those of Elhaissoufi et al. (2020) who determined the soluble RP range between 6.41 and 66.02 μgmL^{-1}.

Additionally, PSBs' numerous positive effects are frequently cited as important elements that encourage plant growth and raise soil P availability. The production of indole3-acetic acids (IAA), minocyclopropane1-carboxylate (ACC) deaminase, siderophores, ammonia, and P solubilization by these bacteria of various genera have been reported to promote plant growth (Sagar et al., 2020; Kapadia et al., 2021). According to studies, IAA can help crops grow and develop as well as absorb and use nutrients by encouraging the growth of root hairs and root elongation (Harahap et al., 2022; C. Song et al., 2022). the majority of the microorganisms isolated from the rhizosphere of a variety of crops can synthesize and release auxins as secondary metabolites (Gusain & Bhandari, 2019). All tested strains produce IAA except for WJEF26, the amount of IAA secreted by the WJEF38 strain was as high as 91.03 μgmL^{-1}. Iron is one of the essential nutrients that, along with IAA, can restrict biotic metabolism and growth. By making available iron to host plants or by reducing available iron to pathogens in the environment, rhizospheric bacteria that produce siderophores may decrease pathogen competitiveness, promoting the growth, and development of plants (Elnahal et al., 2022). Siderophores are low-molecular-weight compounds that can bind Fe^{3+} and deliver nutrients

directly to microbial cells. All isolated strains produced siderophores in this study except for WJEF26, with the activity of the four PSB strains ranging from 39.46 to 73.26 units. Tested strains were characterized by other bacterial traits such as HCN and ammonium-producing strains (equivalent to 98.37 μgmL^{-1}) and were likely involved in non-symbiotic N_2 fixation during plant growth. According to several studies (Elhaissoufi et al., 2020; Khoshru et al., 2020; Mahmud et al., 2021) ammonia production, medium acidification, and HCN production may all help seedling robustness, including crucial early-stage root nutrient absorptive capacity that could enhance RP solubilization and subsequent utilization.

Root germination and growth are known to be significantly influenced by PGP traits such as IAA, siderophores, and ACCD secreted by rhizosphere bacteria (Kapadia et al., 2022). Therefore, the possibility of treating seeds with the fermentation broth of particular strains was explored. It is evident from the seed germination experiment that PSBs helped Broad Bean and Pea seeds grow. The findings showed that a strong capacity for IAA production in conjunction with a high capacity for P solubilization can increase the percentage of seeds that germinate, significantly influencing the regulation of endogenous IAA levels with favourable effects on P uptake and plant physiological status. On their part, Yañez-Yazlle et al. (2021) SA211 is a significant producer of IAA, which primarily affects rooting, the growth of secondary roots, and the development of root hairs. Excess production of this auxin may be the root of the anomalous growth. In our study, which was based on the legumes pot experiment, higher acid phosphatase activities were found in the rhizosphere soil of Broad Bean and Pea roots that had been inoculated with PSBs. This may have the effect of accelerating the decomposition of organic P and inorganic P that is not yet available, which would further encourage legume plants to absorb P. Similar to this, Song et al. (2022) found higher acid phosphatase activities in the rhizosphere soil of maize roots inoculated with PSBs, which may hasten the decomposition of organic P and unavailable inorganic P, further promoting P uptake by maize plants. The P-solubilizing, IAA-synthesizing, acid phosphatase activity, and root colonization properties of the PSB strains may be responsible for the significant ($P \leq 0.05$) increase in the tested legumes' growth parameters, including plant P contents, following inoculation (Tables 4 and 5). Since RP was used as an insoluble P source in the pot experiment, there may be a strong correlation between the rise in legume-P contents and the solubilization of RP. Similarly, Elhaissoufi et al. (2020) pointed out that the development of wheat seedlings may be related to the multiple properties of PGPs (i.e., IAA, siderophore, $NH4^+$, etc.), which likely contribute to a further improvement in growth, in addition to the increased biosolubilization of P in the rhizosphere and changes in root morphology. Additionally, the fact that PSB strains have a relatively higher

capacity to produce IAA may be the cause of their significant impact on root length and root fresh and dry weight. By increasing P-uptake from the soil and its transport to plant shoots, the inorganic P-solubilization and IAA production abilities of PGPR are known to enhance plant growth (V. Gupta & Buch, 2019; C. Wang et al., 2021). Following inoculation with PGPR strains, the promotion of root colonization and plant growth has also been previously discussed (Elhaissoufi et al., 2020; Khourchi et al., 2022). Under field circumstances, our study's findings were incredibly inconsistent. When compared to non-inoculated plants, inoculated plants treated with RP fertilizer showed a significant ($P \leq 0.05$) increase in plant height, N, and P contents. This has been approved by H. Khan et al. (2022) noted that soil inoculation of PSB with mineral P improved soil fertility post-harvest by increasing soil organic matter from 0.61% to 0.70%, lowering pH from 7.74 to 7.68, and increasing total soil nitrogen from 0.04 to 0.09%. The fact that sterilized soil was used in the pot experiment and, as a result, there was no competition between the inoculated bacteria and the native microflora, is one explanation for the effect of the PSB strains. Although RP-non-inoculation could not compete with RP-PSB-fertilizer treatments, significantly higher P contents were found in inoculated plants. This could be because the inoculated strains solubilized P.

The majority of earlier studies (Elhaissoufi et al., 2020; He & Wan, 2022) primarily discussed the direct effects of PSBs on plant growth based primarily on P solubilization and plant growth. Other studies have also documented significant positive effects on a small number of root parameters without a clear relationship to parameters related to aboveground P. This study, however, offered fresh proof that PSB effects extend beyond P-solubilization alone and include numerous known and unidentified indirect effects on the morphological shoot and root traits, enhancing both the acquisition and internal utilization of P. In this article, multiple differential responses at the level of PSB-plant interactions are reported. Of particular note is the significant rise in rhizosphere P availability because of PSB inoculation (Table 1), which may provide a sufficient amount of P for roots to grow, subsequently, better plant growth, and P nutrition, which is presumably guaranteed for later growth stages. Elhaissoufi et al. (2020) also show that P biosolubilisation by rhizosphere bacteria probably stimulates positive surface and subsoil interactions. These authors state that PSBs could play a key role in plant growth by promoting root development in 15-day-old seedlings more than P solubilisation, which appeared to be pronounced earlier on the seventh day after germination and tended to decrease in 15-day-old and 42-day-old wheat seedlings. In addition, we suggest that a successful growth and development phase after germination gives a strong impetus to the whole life of the plant. High germination accompanied by strong seedling cell development leads to high plant vigour, and

vice versa. The small quantities of monomeric sugars released into the soil serve as a substrate for soil microorganisms, which move from a solitary to a symbiotic lifestyle. All this explains the importance of bacteria in the plant's life cycle and development, and indirectly bacterial inoculations improve the plant's biomass.

Because of the increased root absorption capacity and the potential internal remobilization of cellular P pools caused by increased root APase activity, PSB also tended to stimulate both root APase activity and RL (Elhaissoufi et al., 2020; Timofeeva et al., 2022). This is in line with earlier research that found PSB strains could produce APase to enhance P nutrition (Khourchi et al., 2022). Additionally, compared to non-inoculated plants, inoculated plants demonstrated a noticeable improvement in aboveground physiological traits, as evidenced by higher P and N levels. All of the treatments significantly increased the nitrogen and phosphorus contents in the above-ground parts of legume plants, and the results were consistent with those of earlier studies by Majeed et al. (2015, 2022). Because ammonium must be in a reduced state for the plant to use it, ammonia is preferable to nitrate or nitrite in the soil. The soil's rhizoflora then transforms ammonia into a usable state. When incubated on N-containing media in the current study, nearly all of the isolates produced a significant amount of ammonia, suggesting that the isolated microbes were engaging in PGP activities to obtain and supply nitrogen to the plant rhizospheres and increase biomass (Emami et al., 2018). The multiple functions of PSBs also help plants better absorb nutrients, which increases the amount of nitrogen and phosphorus in aboveground plant parts. Better nutrient uptake by the plants is also a result of root growth in response to PSBs inoculation. Crop plants' overall growth, development, and health are dependent on the availability of adequate nutrients, and they are controlled by their microbiome's capacity to produce a range of traits that enhance plant growth. Multiple nutrients are provided to the plants through the inoculation of diverse rhizobacteria, aiding in plant growth (S. Gupta & Pandey, 2020). As shown in this study, two crops responded to PSBs by increasing their root and shoot biomass.

Different PSB properties that promote plant growth are associated with improved plant growth in legume plants. According to Gianfreda (2015), measurements of soil enzymes also indicate the health of the plants. In our findings, we note that all tested strains show secretion of ACCD ranging from 0.27 to 0.56 nmol α-ketobutyrate mg/protein/h. PGPR has many direct and indirect pathways for improving crop yields in stressed conditions, including the production of ACCD (Sagar et al., 2020). The enzyme ACCD reduces the level of ACC in root exudates; a low level of ACC reduces the concentration of ethylene in plant roots, which aids in root length and thus nutrient absorption (Sagar et al., 2022). The enzyme ACCD converts the ACC secreted

by stressed plants into ammonia and -ketobutyrate, which has a significant impact on the physiology, growth, and development of the plant (Saleem et al., 2018). In such a study, Aneurinibacillus aneurinilyticus and P aenibacillus sp., which secrete ACCD, were inoculated in consortia and increased the weight and biomass of Phaseolus vulgaris seedlings under saline-stressed conditions (S. Gupta & Pandey, 2020). Soil enzyme activities and respiration provide information about the microbial population and their activities in the soil. Similar to the findings of this study. Gianfreda (2015) found that soil enzyme activity is higher in the rhizosphere when compared to bulk soil. Similar findings were made by K. Wang et al. (2023) who found that soils that had microbes added in addition to NPK had higher enzyme activities than sterile soils. According to Bargaz et al. (2021) the presence of insoluble P, for which PSB can produce enzymes and organic acids to solubilize this unavailable phosphorus, is what accounts for the higher alkaline phosphatase enzyme activity. Timofeeva et al. (2022) determined that the use of chemical fertilizers and PSBs activates soil enzymes at various levels. We determined that adding both chemical- and biological-fertilizers activates and boosts soil biological activity. Additionally, we can say that every strain originated from the rhizosphere, which explains their significant effect on the soil characteristics and, consequently, on the plant growth parameters. Agriculture and food production may benefit from the use of PSBs. Using these bacteria can lessen the need for P-fertilizers, which can be expensive and have negative environmental effects like polluting groundwater. Through the P- and nutrient cycle, they can also improve soil quality and raise crop yields. Future research is anticipated to strengthen the mathematical framework connecting the PSB's potential and its contribution to the advancement of modern agriculture.

5. Conclusion

Experiments on seed germination demonstrate that PSB strains can significantly encourage early plant growth while raising the germination rate of the two plants under study. Seeds treated with PSB had a higher germination rate and longer rooting length than the untreated controls. The pot experiment further demonstrated that PSB inoculation could enhance soil acid phosphatase activity and available P, as well as plant P uptake. Our research shows that PSBs from the rhizosphere soil of legumes can produce IAA, siderophores, HCN, and ammonia producers that support the growth of Broad Bean and Pea in addition to solubilizing P and secreting acid phosphatase to dissolve insoluble P. It is possible to conclude that these species have a high potential for reducing the use of chemical P fertilizers and promoting long-term agricultural development. Based on these findings, we conclude that PSB-tested strains are

potential P-solubilizing and plant-growth-promoting strains that can supplement Broad Bean and Pea P requirements. Before using the five strains as commercial inoculants for improving Broad Bean and Pea crops, we recommended being thoroughly tested and identified. To understand the existence of microbial communities in different conditions and their effect in field conditions, a consortium of PGPs and their interactive effect must be studied at the molecular level. Further research into the mechanisms and experiments with these organisms in different plants would lead to the effective use of these PGPRs as bioinoculants for various soil properties. Many such studies and experiments conclude that PSBs strains are a viable alternative for plants to withstand abiotic stress and enhance plant growth promoters.

Chapitre V

Amélioration du rendement de *Vicia faba* et *Pisum sativum* dans des conditions de stress par l'inoculation de bactéries solubilisant le phosphate (en consortia) et la fertilisation au phosphate naturel

Amélioration du rendement de *Vicia faba* et *Pisum sativum* dans des conditions de stress par l'inoculation des bactéries solubilisant le phosphate (en consortia) et la fertilisation au phosphate naturel

Synthèse

En raison des effets du réchauffement climatique, les plantes sont soumises à une variété de stress, comprenant à la fois des facteurs biotiques et abiotiques. La salinisation des sols est l'une des menaces environnementales les plus graves pour les cultures dans le monde entier. Au niveau mondial, le sel affecte plus de 800 millions hectares de terres agricoles. La productivité des cultures est limitée dans de nombreuses régions du monde en raison des taux croissants de salinisation des sols. Au niveau mondial, 33 % des terres agricoles irriguées et 22 % des terres agricoles cultivées sont sensibles à la salinité, qui augmente de 10 % par an. Par conséquent, le sel pourrait affecter 50 % des terres cultivables dans les années à venir, ce qui constitue une menace sérieuse pour l'agriculture mondiale. Le stress salin provoque divers effets sur la physiologie et le métabolisme des plantes, notamment un déséquilibre et une toxicité ionique. Il réduit la capacité des plantes à absorber les nutriments, perturbe les propriétés du sol et entraîne une diminution de la quantité d'eau absorbée. De même, la sécheresse est l'un des stress abiotiques les plus vulnérables et dommageables aux plantes car elle limite le développement et la productivité des cultures et affecte la sécurité alimentaire mondiale, ce qui constitue une préoccupation majeure pour l'agriculture moderne. La sécheresse chez les plantes provoque la fermeture des stomates, une fixation du CO_2, une stimulation de la photorespiration. Elle entraine également, la génération d'espèces réactives d'oxygène (ROS), qui endommagent la membrane cellulaire, les protéines, les acides aminées et affectent en fin de compte la croissance.

La détérioration des propriétés physicochimiques du sol par la salinité et la sécheresse réduit la mobilisation des nutriments, la diversité microbienne et le rendement des légumineuses en raison de l'accumulation d'ions. Les fèves et les pois, parmi les légumineuses les plus consommables, sont une source importante de protéines pour l'Homme. Ils sont principalement cultivées dans les pays en voie de développement et les pays équatoriaux. Ces légumineuses sont un aliment de base utilisé dans le monde entier, et leur demande augmente avec la croissance de la population mondiale. Tout au long de leur vie, ces légumineuses peuvent être soumises à divers facteurs de stress environnementaux, notamment la sécheresse et la salinité, qui peuvent entraver leur croissance et les empêcher d'atteindre leur optimum de croissance. Dans ce contexte, il est essentiel de mettre en œuvre des mesures de production agricole à long

terme, rentables et respectueuses de l'environnement. La création d'inoculant microbiens contenant des bactéries solubilisant le phosphate (PSB) est une nouvelle solution biologique pour augmenter la disponibilité du P dans la rhizosphère et la tolérance des plantes au stress abiotique. Ces microorganismes sont efficaces et permettent d'obtenir des rendements élevés et des produits de haute qualité en améliorant les performances des processus métaboliques de la plante et leur tolérance au stress. En outre, les interactions plantes-microbes sont essentielles à la croissance et au développement des plantes dans un environnement changeant. Plusieurs études ont été menées pour développer des bio-inoculant sous forme de consortium à base de PSB-tolérants afin d'améliorer le développement des plantes en cas de stress. L'utilisation de ces bactéries a permis d'augmenter le poids sec, la longueur des racines et des pousses, le nombre de nodules, les nutriments de la plante et les enzymes du sol, ainsi que les teneurs en chlorophylles, protéines, proline et en sucre dans des conditions stressantes.

Sur la base de ces rapports, nous émettons l'hypothèse que l'inoculation de consortia de PSB pourrait protéger les plants de légumineuses des dommages causés par la salinité et la sécheresse. Cette recherche a été conçue pour élucider le rôle des PSB compétentes et tolérantes au stress dans l'amélioration de la tolérance au stress hydrique et salin chez la fèves et le petit pois, en tenant compte des processus morphologiques, biochimiques et physiologiques des plante testées.

La présente étude a utilisé des plantes non tolérantes à la sécheresse et à la salinité pour étudier les fonctions et les mécanismes potentiels des PSB isolées en tant que consortia pour améliorer la nodulation, la croissance des plantes, l'absorption des nutriments et le rendement dans ces conditions drastiques. Après un criblage, les souches ont été sélectionnées et identifiées à l'aide de la technique de l'ARNr 16S. Cinq souches de PSB présentant des activités de promotion de la croissance des plantes, notamment la solubilisation du phosphate, la capacité à tolérer le sel et la sécheresse, l'activité antioxydante et la formation de biofilms, ont été utilisées en fonction de trois différents consortium en serre et sous des conditions non contrôlés. Les paramètres physicochimiques du sol ont été mesurés après inoculation notamment le pH, la conductivité, l’azote total et le P disponible. En plus, les teneurs des plantes en minéraux tels que l’azote, le phosphate, et le potassium ont été évalués.

Les expériences en serres montrent que les souches de PSB peuvent encourager d’une manière significative la croissance précoce des plantes tout en augmentant le taux de germination des deux plantes étudiées dans des conditions de stress par rapport aux témoins non traité par les PSB. Les graines traitées avec des consortiums de PSB présentent un taux de germination plus

élevé et une longueur d'enracinement plus longue que les témoins non traités. Les résultats obtenus dans la présente recherche ont révélé que les consortiums bactériens étudiés ont des caractéristiques qui peuvent atténuer les conditions de stress. L'inoculation des consortiums PSB peut améliorer l'activité de la phosphatase acide du sol et l'absorption des éléments nutritifs disponibles pour les plantes. Une plus grande accumulation de matière sèche et une valeur significative de chlorophylle est observée dans les plantes inoculées par rapport aux témoins.

Les isolats WJEF15, WJEF26 et WJEF38 ont été identifiés avec succès comme étant des *Pseudomonas spp*. tandis que les isolats WJEF46 et WJEF63 sont des *Bacillus spp*. Les résultats se sont avérés significatifs pour chaque traitement étudié avec un avantage du traitement T3 qui comporte les cinq souches utilisées ; il a atténué l'effet du stress salin et hydrique et a amélioré le développement de la plante. Le traitement T3 a favorisé la croissance des plantes avec une augmentation de 34,5 % et 32,45 % de la longueur des pousses, 39,58 % et 36,3 % de la longueur des racines, 46,9 % et 42,3 % de la biomasse, pour la fève et le petit pois, respectivement, par rapport au contrôle. Les attributs physiologiques des plantes ont également été améliorés par l'inoculation bactérienne, montrant une augmentation de 39,8 % et 39,8 % de la chlorophylle pour la fève et le petit pois, respectivement. En outre, le poids frais des pousses (49,72 % et 65,13 %) et le poids frais des racines (48,32 % et 56,98 %) ont été améliorés pour la fève et le pois, respectivement, par rapport au témoin. De plus, les minéraux du sol et l'accumulation de nutriments minéraux dans les pousses et les grains, tels que l'azote, le phosphate, et le potassium après l'inoculation, ont montré une augmentation significative par rapport aux témoins non traités.

Sur la base de ces résultats, nous concluons que les souches testées sont des souches potentielles de solubilisation du P et de promotion de la croissance des plantes qui peuvent compléter les besoins en P de fèves et de petit pois dans des conditions de stress salin et hydrique. Les résultats de cette étude pourraient fournir une approche efficace pour l'amélioration agronomique des inoculant microbiens afin d'augmenter la mobilisation du P du sol pour la croissance des plantes, que ce soit en production intensive ou dans des applications agro-écologiques au Maroc. Collectivement, l'application de ces consortiums de PSB tolérants aux stress abiotiques pourrait constituer une stratégie écologique pour atténuer la sécheresse et le stress salin. Cette recherche contribue à la stratégie nationale du Maroc (GREEN GENERATION 2020-2030), qui vise à atteindre un développement durable en améliorant la qualité des sols et en augmentant les rendements grâce à l'utilisation de méthodes biologiques innovantes.

Yield improvement of *Vicia faba* and *Pisum sativum* under stress conditions by inoculation with phosphate solubilising bacteria (in consortia) and rock phosphate fertilisation

This part of the work was the subject of an article submitted for publication.

Abstract

Deterioration of physicochemical properties of soil by salinity and drought reduces nutrient mobilization, microbial diversity and crop yield of legumes due to the accumulation of ions. A wide range of adaptation strategies are required to overcome this problem. This research was designed to elucidate the role of the most potent stress-tolerant-PSBs for the amelioration of drought and saline stress tolerance in broad bean and pea legumes taking into account morphological, biochemical and physiological processes of tested plant. After screening, strains were selected and identified using 16S rRNA technique. Five PSB strains exhibit plant growth-promoting activities including phosphate solubilizing, ability to tolerate salt and drought, antioxidant activity, and biofilm formation were used as three treatments as a consortium under greenhouse condition. Isolates WJEF15, WJEF26 and WJEF38 were successfully identified as *Pseudomonas spp.* while isolates WJEF46 and WJEF63 are *Bacillus spp*. The results were found to be significant with T3 treatment which mitigated the effect of salt and drought stress and improved the plant development. The T3 treatment promote plant growth with an increase of 34.5% and 32.45% in shoot length, 39.58% and 36.3% in root length, 46.9% and 42.3% in biomass, for broad bean and pea, respectively, as compared to control. Physiological attributes of plants were also improved by bacterial inoculation showing an increase of 39.8% and 39.8% in chlorophyll for broad bean and pea, respectively. In addition, shoot fresh weights (49.72% and 65.13%) root fresh weights (48.32% and 56.98%) were enhanced for broad bean and pea, respectively, as compared to control. However, the highest alkaline phosphatase activity and β-glucosidase activity in the rhizosphere soil were determined in T3 treatment in stress-free conditions with 22.97 μg pNP $g^{-1}$and 5.81 mg pNP g^{-1}, respectively for broad bean, and 22.62 μg pNP g^{-1} and 5.83 mg pNP g^{-1}, respectively for pea. The results of soil minerals and accumulation of mineral nutrients in shoot and grains such as N, P, and K after inoculation were found to be significant. Our results concluded that PSBs strains are a viable alternative to withstand salt and drought stress and enhance broad beans and peas growth development.

1. Introduction

Due to the effects of global warming, plants are subjected to a variety of stresses, including both biotic and abiotic factors. Soil salinization is one of the most difficult environmental threats to crops around the world (Thaker et al., 2021). Globally, salt has affected over 800 Mha of agricultural land (Yasin et al., 2018). Crop productivity is being limited in many parts of the world due to rising rates of soil salinization (A. Kumar et al., 2020). Worldwide, 33% of irrigated and 22% of cultivated agricultural lands are susceptible to salinity, which is increasing by 10% per year (Latif et al., 2023). As a result, salt may affect 50% of cultivable land in the coming years (Abbas et al., 2019) posing a serious threat to agriculture worldwide (Van Zelm et al., 2020). Salt stresses have a variety of effects on plant physiology and metabolism, including ion imbalance, ionic toxicity, osmotic stress, and reduced water potential (A. Kumar, Singh, Mukherjee, et al., 2021). Furthermore, higher salt concentrations in the soil reduce plants' ability to absorb nutrients, disrupt soil properties, and result in less water. These factors integrate making drought one of the most significant abiotic stresses to the plant, which is a major concern for modern agriculture as one of the most damaging environmental factors, limiting crop development and productivity and affecting global food security (Slimani et al., 2023). Drought stress in plants causes osmotic pressure, which negatively influences stomatal closure, CO_2 fixation, photorespiration stimulation, and other processes, resulting in the generation of reactive oxygen species (ROS), which damages the cell membrane and ultimately affects growth (Hossain & Dietz, 2016).

Broad beans and peas (the most important leguminous crops) are a significant source of high-quality protein for humans due to their high nutrient content (N. Singh et al., 2022). They are one of the most widely consumed staple crops, primarily grown in developing and equatorial countries (Majili et al., 2020; Boutagayout et al., 2023). These legumes are a staple food used worldwide, and their demand will rise as the world's population grows. Furthermore, cultivation of broad beans and peas has been documented to improve soil nitrogen contents (Mahgoub et al., 2021). Throughout their lives, these legumes may be subjected to a variety of environmental stressors, including drought and salinity, which can impair growth and prevent them from reaching their full growth potential (Goyal & Habtewold, 2023). However, stress conditions are causing more degraded lands to be created around the world, resulting in a decrease in agricultural productivity. As a result, many plant scientists are eager to increase its output. In this scenario, it is critical to implement long-term, cost-effective, and environmentally friendly crop production measures. Creating microbial inoculants containing phosphate (P) solubilizing bacteria (PSB) is a new biological solution for increasing rhizosphere P availability (Janati et

al., 2023) and abiotic stress tolerance (Lau et al., 2022). Plant growth-promoting rhizobacteria (PGPR) like PSBs have been shown to improve plant growth and stress tolerance by regulating physiological and biochemical features (A. Kumar, Singh, Mukherjee, et al., 2021). These microorganisms are efficient, allowing for high yields and high-quality products by enhancing the performance of the plant's vital processes (De Vasconcelos & Chaves, 2019). Creating salt and drought-tolerant crops through traditional breeding or a biotechnological method takes time.

Plant-microbe interaction is critical for maintaining microbial diversity, soil properties, and crop yield under stress (Bargaz et al., 2018). Furthermore, in nature, plant-microbe interactions are critical for plant growth and development in a changing environment. Many studies have shown that bacteria isolated from saline soil, such as *Achromobacter*, *Arthrobacter*, *Bacillus*, *Chryseobacterium Enterobacter*, *Ochrobactrum* and *Pseudomonas* isolated from saline soil can promote plant growth under saline conditions (Jha & Subramanian, 2014; S. Sharma et al., 2016; Bhise et al., 2017; Sarkar et al., 2018). Tolerant-PSB consortia, on the other hand, can be formed based on salinity and drought tolerance, as well as the complementarity of PGP activities. Similarly, several studies have been conducted to develop bioinoculants to improve plant development under stress, saline environment, and seedling development, among other things. Slimani et al. (2023) found that using bacteria increased dry weight by 140% and total chlorophyll by 141% under saline water conditions. Consortia also increased the protein, proline, and sugar content of plant shoots. When compared to the stressed control, the same treatment increased superoxide dismutase activity by 90%, catalase activity by 29%, and polyphenol oxidase activity by 37%. According to Jabborova et al. (2021), under drought conditions, co-inoculation of *B. japonicum* USDA 110 and *P. putida* NUU8 increased root length by 56%, shoot length by 33%, root dry weight by 47%, shoot dry weight by 48%, and nodule number by 17%, as well as nodulation, plant and soil nutrients, and soil enzymes. Latif et al. (2023) found that PGPR promoted the growth of common ice plant (*Mesembryanthemum crystallinum L.*), tomato (*Lycopersicon esculentum cv. Bella*), rice (*Oryza sativa*), and pea (*Pisum sativum L.*) under salinity. Based on these reports, we hypothesize that PSB consortia inoculation could protect broad bean and pea plants from saline and drought damage. The current study used drought-tolerant legumes to investigate the potential functions and mechanisms of PSBs isolated from the rhizosphere of legumes as consortia to improve nodulation, plant growth, nutrient uptake, and yield under saline and drought conditions. Our findings suggest that PSB could be useful in modulating drought and saline tolerance in broad bean and pea plants. This research contributes to Morocco's national strategy (GREEN

GENERATION 2020-2030), which aims to achieve sustainable development by improving soil quality and increasing yields through the use of biological methods.

2. Materials and methods

2.1. Plant materials

The effect of PSB inoculation on plant growth under stress conditions and RP fertilization is investigated in two legume crops: Feve-Aguadulce Broad Bean (*Vicia faba*) and PP-Onward Pea (*Pisum sativum*). The seeds were obtained from A.PHY.SEM's agriculture plant section. These plants are selected based on the most commonly grown legumes in Morocco, particularly in the Fez-Meknes region. The experimental research on studied legumes conducted in this study complies with relevant institutional, local, and national regulations.

2.2. Bacterial strains and growth conditions

In this experiment, five PSB strains (WJEF38, WJEF46, WJEF63, WJEF15, and WJEF26) with different plant growth-promoting traits were already evaluated in the Functional Ecology and Environment Engineering Laboratory. Rhizobacterial PSB strains were chosen based on their ability to solubilize P and Zn, fix N_2, produce ACC deaminase, siderophores, ammonia, HCN, and indole acetic acid (IAA) (Janati et al., 2023). The bacterial culture was grown in 5 mL of TSA in a shaking incubator for 12 h, and then 1 mL of culture from each strain was added to 100 mL of TSA broth individually and in consortia and then incubated for 24 h until the colony-forming unit was 10^9 CFU.mL^{-1}.

2.3. Screening of PSB isolates

2.3.1. Biofilm formation assay

The biofilm formation ability of PSB cells was investigated in polypropylene tubes. Isolates were grown in TSA medium and incubated for 24 hours at 28°C ± 2 °C without shaking before the cultures and the media used were discarded. The biofilm formation was quantified by the crystal violet staining method (Ghasemi et al., 2023). Each tube was filled with 1% crystal violet solution and incubated at room temperature for 15 min. After removing the solution, the tubes were then rinsed thoroughly with sterile water, and colour adherence detected biofilm. Non-inoculated tubes served as controls.

2.3.2. Stress tolerance

The salinity tolerance of the PSBs strains was evaluated using a TSA liquid medium containing NaCl concentrations ranging from 0.2 to 2 M. For 48 h, the cells were incubated at 28 ± 2 °C followed by an absorbance measurement at 620 nm (R. P. Singh et al., 2015). In addition, on

TSA liquid medium containing various concentrations of polyethylene glycol 6000 (PEG) (10, 20, and 30%), the drought tolerance of PSB strains was evaluated. After 24 h of incubation at 28°C ± 2 °C absorbance was measured at 620 nm. To select strains that can grow in a variety of soil pH conditions (acid, neutral, and alkaline) and because P solubilization is strongly related to pH changes in the medium, PSB strains were inoculated on a solid TSA medium at various pH values ranging from 3 to 13 and incubated at 28°C ± 2 °C for 48 h. For isolates' temperature tolerance through bacterial growth, PSB strains were also cultured onto TSA solid medium and incubated at 37, 48, and 55 °C for 48 h.

2.3.3. Antioxidant activity

2.3.3.1. Total antioxidant capacity

The phosphomolybdenum colourimetric method was used to measure the total antioxidant capacity (TAC). The tube samples were filled with an aliquot of 0.1 mL of the reagent (H_2SO_4 0.6 M, $NaPO_4$ 28 mM, and $(NH_4)6Mo7O_{24}\cdot 4H_2O$ 4 mM) and incubated at 95°C for 90 min. After the sample had cooled to room temperature, the absorbance at 695 nm was determined. The same conditions were used to incubate a typical blank solution, which contained 1 mL of reagent solution and the appropriate volume of the sample's solvent (Kapadia et al., 2022). The TAC was calculated using the following linear equation based on the ascorbic acid calibration curve and expressed as μg of ascorbic acid equivalent per mL of extract (μg AAE.mL^{-1}).

$$Y = 0.0013X + 0.0118 \ (R^2 = 0.9971) \quad (1)$$

Where Y is the absorbance at 695 nm and X is the ascorbic acid concentration (μg.mL^{-1}).

2.3.3.2. DPPH scavenging

Different concentrations of bacterial liquid (150 μL) were added to the DPPH· (0.1 mmol.L^{-1}) radical methanol solution (150 μL). This mixture was stirred and incubated in a dark place for 30 min and the absorbance was measured at 517 nm (E. Zhao et al., 2022). The DPPH scavenging was calculated by the equation:

$$\text{DPPH scavenging (\%)} = [1-(A_1-A_2)/A_0] \times 100\% \quad (2)$$

A_0, A_1 and A_2 represent the absorbance value without the test liquid, the sample, and without free radicals, respectively.

2.3.4. Antagonism assay

APCsalt plates were used to test the antagonistic interactions between bacteria using the double-layer agar and opposition plating methods (Yañez-Yazlle et al., 2021). For opposing trials, one strain was streaked in the centre and surrounded on both sides by other strains. Antagonism was

evident from the strains' lack of interaction. For the double-layer agar experiments, plates were filled with melted agar to half and left to partially gel. The remaining 50% of the agar was poured over the first semi-gelled layer after one strain was inoculated. After the second layer had gelled, additional strains were streaked. Antagonisms are present when a halo of bacterial growth inhibition appears.

2.4. Experiment modalities: Pot trial study

Plant growth promotion studies were carried out in triplicates using a complete randomized block design (RBD) with four treatments; T0 (control non-inoculated), T1 (WJEF38, WJEF46, and WJEF63), T2 (WJEF38, WJEF26, and WJEF15), and T3 (WJEF38, WJEF46, WJEF63, WJEF26, and WJEF15). The consortiums were selected based on the P-solubilization capacity of the PSBs and their PGP properties (Janati et al., 2023). Each treatment was subjected to three different growing conditions: salt stress, water stress (deficit) and normal environmental conditions (stress-free). The seeds were surface sterilized with 2% sodium hypochlorite and washed five times with distilled sterile water before germinating on clean straw paper moistened with salinized distilled water (0.6% NaCl) in an incubator at 28 °C for four days. When the radicle breaks through the seminal envelope, the seed is said to have germinated. The seeds were then immersed in separate PSB strain suspensions containing 10^9 CFU.mL^{-1} and then in consortia depending on the treatments used before being planted into pots. As a P-fertilizer, RP solution (5%) is added to all pots (RP is produced at the Khouribga P mine, Morocco). The experiment was carried out at the Science and Technology Faculty's experimental greenhouse in Fez, Morocco, from 13 February to 15 May 2023, with an average temperature of 28°C during the day and 12°C at night. Each pot held ten seeds, which were irrigated daily with weight-based irrigation equal to 70% of their water-holding capacity. Plants from each treatment were harvested ninety days after sowing (DAS) for analysis of plant growth parameters and soil mineral content.

2.4.1. Plant growth parameters

After 90 days of sowing, all plants in each experimental plot were harvested and randomly selected to evaluate the number and weight of grains per pot. An analytical balance was used to calculate the shoots and roots' highest and fresh weights (SH, RH, FW) by removing the intact plants from the pot. Grain protein content was determined using the Bradford method. Total chlorophyll content was measured according to Pérez-Patricio et al. (2018). Analysis was done on plant nodulation and biomass. Before being ground to a fine powder for P and N

concentration analyses, the shoots and roots of treated plants and control plants were dried in an oven at 70°C for two days to determine their dry weights (DW).

2.4.2. Soil physicochemical analysis

Samples of the soil were taken from each pot after the experiment and plants were harvested and separated into shoots and roots. The rhizosphere growth substrate was obtained by carefully separating roots from the loosely adhering soil, which was then sieved (2 mm) before mineral measurements. According to Naseby & Lynch (1997) methods, the chemical characteristics of the rhizosphere soil were determined, including pH-water, electrical conductivity (EC), soil organic matter, N-total, and P-available, (Fernández et al., 2007), basal soil respiration (BSR), alkaline phosphatase activity (APA), and -Glucosidase activity (GA).

2.4.3. Plants mineral contents

Shoot and grain's P contents were measured following the ascorbic acid method (Bargaz et al., 2017). The activity of root acid phosphatase was measured (Janati et al., 2023). The Kjeldahl method was also applied to the finely ground shoot and grains subsamples (0.5 g) for total N analysis (Magomya et al., 2014). The K content was measured according to (Fernández et al., 2009). The ratio of plant P content to root dry weight was used to calculate the root P acquisition efficiency (RPAE), which measures the ability of roots to absorb P from the soil (Pan et al., 2008). The root acid phosphatase activity (RAPA) was measured according to Bargaz et al. (2017).

2.5. Molecular identification of isolates

Before DNA isolation, PSB strains were cultivated under gentle agitation in 10 mL of Luria–Bertani broth for 24 h at 28°C. One millilitre of the bacterial culture was placed in micro-tubes and pelleted by centrifugation for 2 min. A chloroform phenol technique was used to extract DNA (Derdak et al., 2022). Quantitation of the DNA was performed using a NanoDrop Lite Spectrophotometer (Thermo Fisher Scientific). Total genomic DNA was isolated from samples and utilized as a template for polymerase chain reaction amplification of a 16S ribosomal DNA fragment (PCR). The PCR reactions have been conducted through a DLAB TC1000-G thermocycler. Two pairs of conserved universal primers were used for amplification: Fd1 5′ -AGAGTTTGATCCTGGCTCAG-3 'and RP2 5′ -ACGGCTACCTTGTTACGACTT-3′. The PCR was performed in a total volume of 15 µL composed of approximately 100 ng of genomic DNA, 0.3 µL of each 10 mM dNTP (Promega), 3 µL of 5X buffer (Promega), 0.9 µL of 25 mM $MgCl_2$ (Promega), 0.12 µL of each 10 mM primer and 0.075 µL of Taq polymerase 5U

(Promega). The PCR program used was as follow: (95 °C, 2 ’/ (95 °C, 40′ ’- 55 °C, 40′ ’- 72°C, 1′) x35; 72 °C/5 ’/ 4°C). The ExoSAP-IT purification kit (GE Healthcare) was used to purify the PCR products, and the BigDye Terminator Kit version 1.0 was used to sequence those (Applied Biosystems). The sequences obtained were corrected using BioEdit, introduced into Geneious prime and aligned via BLAST to determine the degree of similarity of our sequences with those present in the database.

2.6. Statistical Analysis

The statistical data were examined using IBM SPSS Statistics V. 26 software. Based on three replications, the results were presented as means and standard deviations. Before performing an analysis of variance (ANOVA), tests for homogeneity of variance and normal distribution were run, and then a Waller-Duncan (W) post hoc test was used to determine whether there was a significant difference between the treatment's mean values at a $p \leq 0.05$ significance level. Dimensional analysis done by principal component analysis (PCA) has been used to give a summarizing view of obtained results. Genetic Phylogenetic tree was constructed using MEGA 11 software.

3. Results

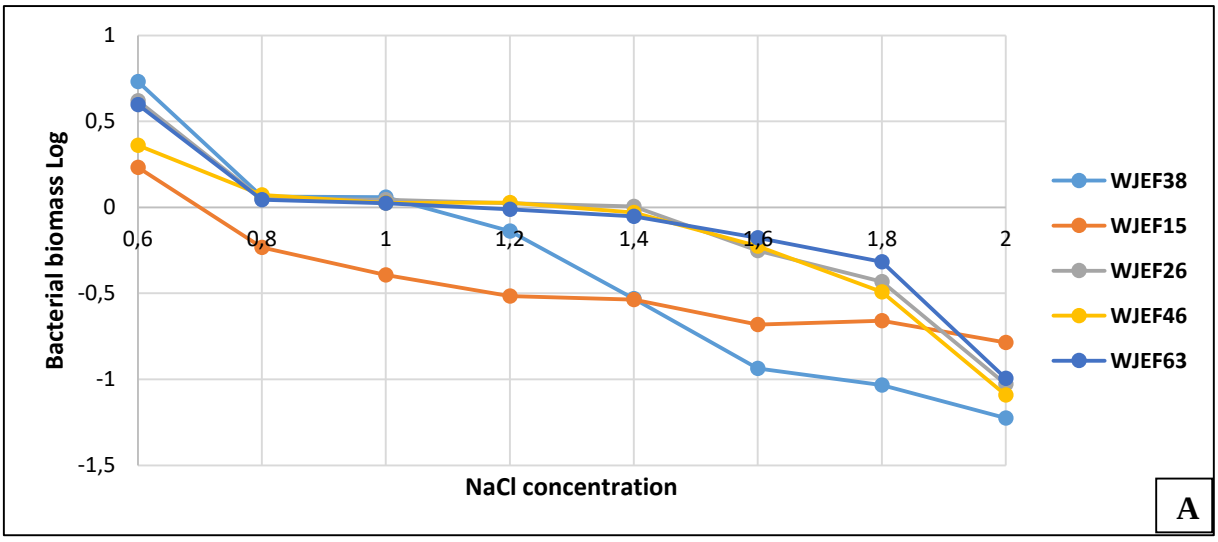
3.1. Screening of PSB strains

Among all the isolates, the strain WJEF46 was the only strain that did not produce biofilms. Strains WJEF38 and WJEF26 showed the highest production, in contrast, WJEF15 and WJEF63 showed low biofilms production. The biofilm formation is either genetically determined or depends on the culture conditions. Considering antioxidant activities, strains produced varying amounts of total antioxidant activity. Isolate WJEF26 produced a maximum total antioxidant activity of 13.36 ± 0.09 AAE.mL^{-1} compared to the other four isolates. In addition, we evaluated the antioxidant activity of PSB by evaluating their scavenging efficiency to free radical 1,1-diphenyl2-picrylhydrazine (DPPH) (Table 1). The efficiency of scavenging free radicals increases with the increase of PSB concentration, which was mainly attributed to the PSB metabolites released. In general, strains grow well together and show any antagonism when plated. Bacteria were cultured at different stress conditions. Through results, it is evident that selected strains are tolerant to considerable variations in terms of alkalinity, acidity, salinity, dryness, and temperature. All PSB strains could survive and proliferate even when the temperature was increased to 55 °C, which indicated that PSBs have relatively high heat resistance.

Table 1. Screening of PSB strains activities in vitro. Data represent the mean ± of three independent replicates. Values with the same superscript letter were not significantly different according to the Duncan test performed ($P < 0.05$).

Strains	Biofilm formation	TAC	DPPH scavenging (%)		
			10^7 CFU.mL^{-1}	10^8 CFU.mL^{-1}	10^9 CFU.mL^{-1}
WJEF15	++	11.24 ± 0.11^{c}	34	41	57
WJEF38	+++	10.13 ± 0.14^{c}	28	35	40
WJEF26	+++	13.36 ± 0.09^{d}	33	44	55
WJEF46	—	7.08 ± 0.16^{a}	21	37	52
WJEF63	+	9.37 ± 0.36^{b}	23	32	42

In addition, these isolates were able to grow and survive over a wide pH range (3–13). Moreover, among all tested isolates, only WJEF46 does not successfully resist higher NaCl concentrations (2M) g.L^{-1}. To quantify tested PSBs' tolerance to high salt concentrations, we measured biomass formation expressed by absorbance. The Log (AB) as a function of salt concentrations revealed that all strains biomass at 2M NaCl concentration resist and show growth, except the WJEF38 strain that significantly decreased at 1.6M NaCl concentration (Figure 1). Regarding drought tolerance, all isolates show the same behaviour under increasing PEG-6000 concentrations (10, 20, and 30%). PSB strains exhibit a decline in cell numbers and absorbance value. However, the WJEF46 strain presented an optimal resistance, with an absorbance value higher than those of the other isolates to all PEG levels (Figure 1).



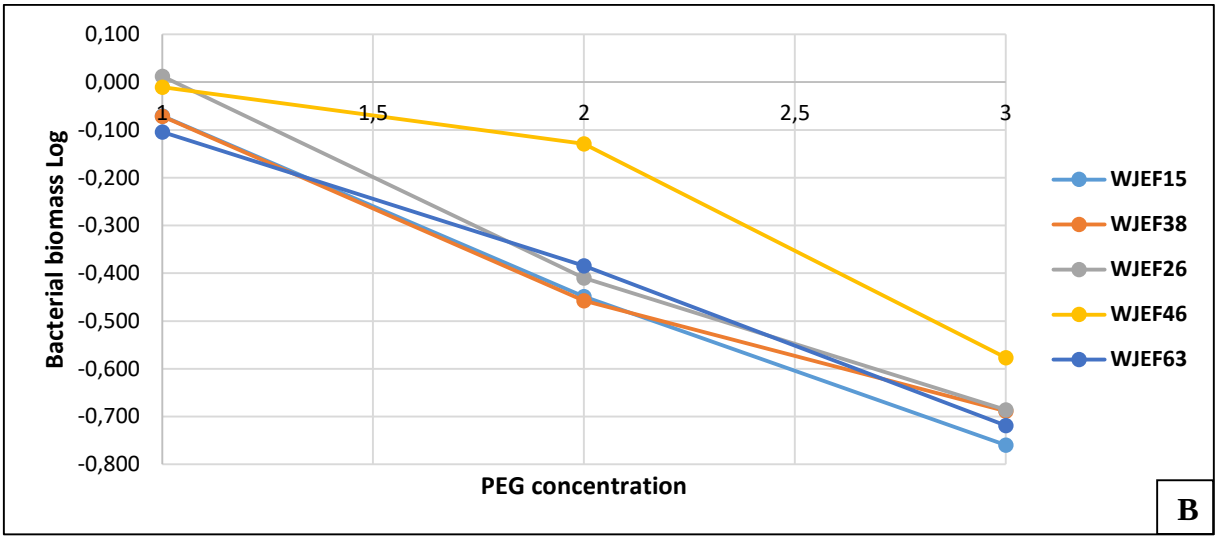


Figure 1. A) PSBs Biomass in the function of salt concentrations $g.L^{-1}$. PSBs Biomass in the function of PEG concentrations.

3.2. Effects of PSBs on plants growth

3.2.1. Agronomic and yield-attributed traits

After ninety days of inoculation of Broad Bean and Pea seeds with PSBs consortia, plants were harvested. All consortia treatments showed a significant increase in growth parameters when compared to non-inoculated plants in the three growth conditions. Data on the influence of different PSBs consortia inoculations on germination rate (GR), shoot and root fresh weight and highest (SH, RH, FW), total chlorophyll content, shoot and root dry weight (SDW, RDW) and nodulation biomass are presented in Table 2. Combinations of three or five different PSB strains significantly affected crop yield and yield components with the recommended dose of RP fertilizer, in both crop species. Inoculation of the consortia had a significant influence ($p < 0.05$) on the growth parameters of the crops used. Differences were noted among consortia treatments; the T3 was the significant treatment to increase the growth parameters of both tested seeds, reaching values higher than either inoculated and non-inoculated plants in the different growth conditions. The results show that the T3 treatment, which contains a consortium of the five strains used, gives the best results, with a remarkable difference compared with the T0 (control) on all the studied parameters. The same results were observed during the stress conditions, where T3 treatment was able to provide results more or less close to the unstressed conditions. In addition, in T3 treatment there has been an increase in SFW and RFW in both tested legumes compared to the control of each growth condition ranging from 45.42 to 49.72% and 37.06 to 48.32% for broad bean, respectively and from 34.76 to 65.13% and 46.99 to 56.98% for pea, respectively.

Table 2. The differences in RP-fertilized broad bean and pea growth in response to PSB isolate inoculation individually and in consortia under stress conditions versus RP treatments alone in natural conditions.

Legumes	Growth conditions	Treatments	SH	RH	SFW	RFW	SDW	RDW	ChC	Nodulation
Broad Bean	Stress-free	T0	19.76^{e}	6.93^{g}	1.98^{d}	1.23^{e}	0.94^{e}	0.49^{e}	7.02^{c}	+
		T1	37.35^{b}	17.62^{b}	3.21^{b}	2.03^{b}	1.52^{b}	0.98^{b}	9.53^{b}	+
		T2	34.32^{c}	11.84^{d}	2.87^{c}	1.42^{d}	1.19^{c}	0.73^{c}	7.43^{c}	++
		T3	49.71^{a}	21.78^{a}	3.89^{a}	2.38^{a}	2.02^{a}	1.27^{a}	10.14^{a}	+++
	Hydric stress	T0	13.41^{f}	3.54^{h}	0.92^{f}	0.73^{g}	0.64^{f}	0.62^{e}	2.23^{h}	-
		T1	18.31^{d}	10.31^{e}	1.33^{e}	0.96^{d}	1.07^{d}	0.57^{e}	2.74^{g}	+
		T2	15.87^{d}	9.39^{f}	1.01^{f}	0.74^{d}	0.82^{e}	0.39^{f}	2.45^{g}	+
		T3	20.27^{e}	13.52^{c}	1.83^{d}	1.16^{f}	1.33^{b}	0.87^{c}	3.14^{f}	++
	Saline stress	T0	20.64^{e}	7.3^{g}	1.43^{e}	1.03^{f}	0.92^{e}	0.63^{d}	3.96^{e}	-
		T1	31.35^{d}	11.45^{d}	2.02^{d}	1.38^{d}	1.29^{c}	0.69^{d}	4.42^{d}	++
		T2	29.41^{d}	10.69^{e}	1.82^{d}	1.14^{e}	1.02^{d}	0.61^{d}	4.75^{d}	++
		T3	37.65^{b}	11.2^{d}	2.62^{c}	1.78^{c}	1.49^{b}	0.71^{c}	5.19^{d}	+++
Pea	Stress-free	T0	17.6^{E}	8.31^{F}	2.12^{E}	1.17^{D}	0.78^{F}	0.51^{E}	8.4^{B}	+
		T1	31.84^{B}	15.31^{B}	3.73^{B}	2.37^{A}	1.36^{D}	1.12^{B}	8.32^{B}	+
		T2	28.47^{C}	14.95^{B}	2.96^{C}	1.91^{B}	1.16^{D}	0.93^{C}	8.78^{B}	++
		T3	38.39^{A}	20.52^{A}	6.08^{A}	2.72^{A}	1.87^{B}	1.44^{A}	10.44^{A}	+++
	Hydric stress	T0	11.27^{G}	7.71^{F}	1.52^{F}	0.67^{F}	0.98^{E}	0.46^{E}	3.22^{G}	-
		T1	14.73^{F}	11.74^{D}	2.25^{D}	1.22^{D}	1.83^{B}	0.94^{C}	3.4^{G}	-
		T2	12.82^{G}	12.09^{C}	2.09^{D}	1.02^{B}	1.63^{C}	0.79^{D}	3.91^{F}	+
		T3	15.03^{F}	12.40^{C}	2.57^{B}	1.49^{C}	2.03^{A}	1.17^{B}	3.65^{F}	+
	Saline stress	T0	15.32^{F}	5.36^{G}	1.82^{E}	0.97^{E}	0.58^{G}	0.39^{F}	5.05^{E}	-
		T1	19.66^{E}	9.82^{E}	2.09^{D}	1.33^{C}	1.02^{E}	0.79^{D}	6.73^{D}	++
		T2	19.57^{E}	9.73^{E}	2.31^{D}	1.09^{D}	1.12^{E}	0.58^{E}	6.21^{D}	++
		T3	21.45^{D}	9.58^{E}	2.79^{C}	1.83^{B}	1.47^{D}	0.93^{C}	7.19^{C}	+++

The data are the average of two independent replicates with five iterations each. At $p < 0.05$, values with the same superscript letter did not differ significantly. GR, germination rate; SH, shoot height; RL, root length; SFW, shoot fresh weight; RFW, root fresh weight; SDW, shoot dry weight; RDW, root fresh weight; ChC, chlorophyll content.

Likewise, an increase in SDW and RDW ranging from 38.25 to 53.46% and 11.26 to 61.41% for broad beans, respectively, and from 51.72 to 60.54 % and 58.06 to 64.58% for peas, respectively. On the other hand, there is a remarkable reduction in chlorophyll content under water stress, accompanied by reduced root and shoot height.

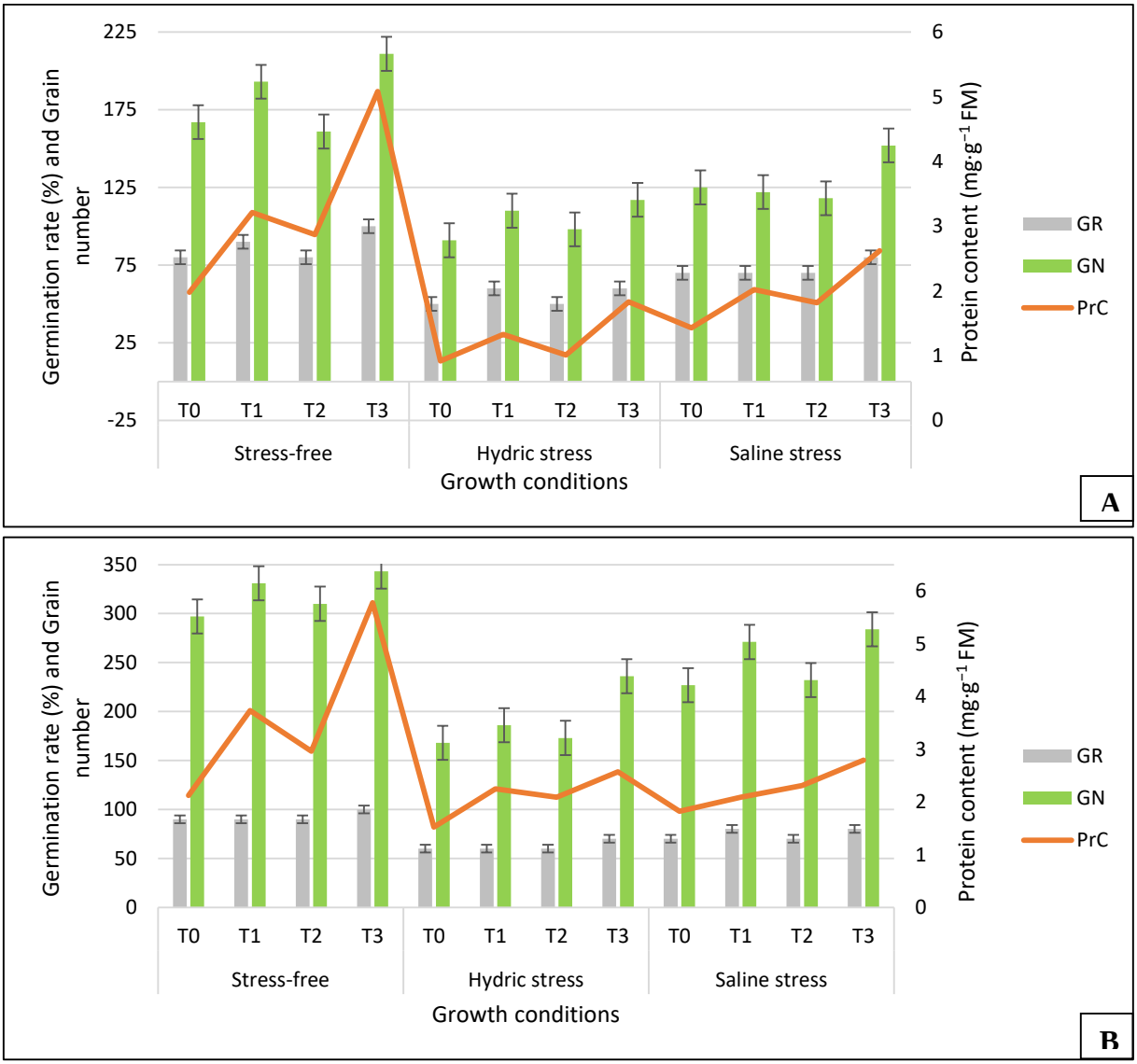


Figure 2. Grain protein content, germination rate, and grain number of studied legumes A) Broad bean, B) Pea after consortia inoculation under stress conditions.

In terms of nodulation, all consortia treatments showed a significant effect when compared to the control except T1 in pea with hydric stress; furthermore, we noted a significant variation between consortia treatments, with the T3 and T2 showing the best effect, respectively. Besides that, all consortia treatments show a significantly positive impact by promoting Pea and Broad Bean seedling growth. The present results showed a significant response regarding germination rate and protein content of Pea inoculated seeds better than Broad Bean inoculated seeds (Figure 2). Our results showed a significant relationship between protein content and the germination rate of inoculated seeds. Protein content increased considerably with increasing germination percentage, which explains a strong positive correlation (R^2= 0.9317). The protein content on T3 treatment compared to the non-inoculated control indicates an increase in each growth condition ranging from 45.42 to 61.02% for Broad Bean seeds and from 35.13 to

63.32% for Pea seeds. In addition, the maximum grain number results were observed in seeds inoculated with the T3 followed by the T1 treatment for both tested crops. The T3 treatment increased plant yields by 20.85% and 13.41%, respectively. We note that T3 treatment also gave the best germination rate for both tested plants, with an increase of 10, 14.29, and 12.5% for broad beans and 20, 16.66, and 12.5% for peas compared to the control of stress-free, hydric stress, and saline stress, respectively. However, we note that the treatments used (consortia) have an affinity with salt stress more than water stress where the growth parameters results of the latter are much weaker. Otherwise, based on all these results, we notice that PSB strains in consortium contribute to the development of the pea seeds more than the Broad Bean seeds.

3.2.2. Soil physicochemical properties after inoculation

The following table depicts the effects of various PSB consortia inoculations on the physicochemical properties of rhizosphere soils. In the P-available parameter, the T3 treatment increased P-available by 72.35% for Broad Bean and 71.89% for Pea compared to the control of the stress-free condition. On the other hand, The T3 inoculation had a significant impact on the rhizosphere's soil N content, which was significantly different from the values of other treatments, with an increase of 42.35% for Broad Bean and 45.41% for Pea compared to the control of stress-free condition (Table 3). These results confirm that the application of PSB strains was able to increase N- and P-available in the soil even in stress conditions. In addition, we note that the consortia treatment application can decrease soil pH. The minimum pH value was found with the T3 treatment in both Broad Bean and Pea plants in stress-free conditions, which was 5.31 and 5.56, respectively. On the other hand, the water deficit and salt stress contributed to an increase in soil pH and conductivity due to a reduction in microbiological activity, the release of hydrogen ions and an increase in the concentration of alkaline salts. This is accompanied by variation between treatments, where T3 remains the best able to withstand these extreme conditions. In terms of soil organic matter, the consortia treatments used show significantly lower values than normal conditions, which explains why the PSBs have accelerated the decomposition of organic matter to obtain the nutrients and water they need. In addition, Basal soil respiration varies have been strongly affected and were typically lower than those in stress-free conditions, which refer to the production of carbon dioxide when soil organisms respire. The increase in basal respiration under stress in consortia treatments is remarkable compared to their control, which is explained by the increase in their metabolic activity to maintain their survival. However, stress conditions significantly affected the alkaline phosphatase activity (APA) and β-glucosidase (GA) activity in the rhizosphere soil, despite

this, T3 treatment exceeds the control of normal conditions. The highest APA and GA were determined in T3 treatment in stress-free conditions with 22.97 µg pNP $g^{-1}$and 5.81 mg pNP g^{-1}, respectively for broad bean, and 22.62 µg pNP g^{-1} and 5.83 mg pNP g^{-1}, respectively for pea.

Table 3. Mean values for soil parameters as influenced by the applied PSBs and RP in legume experiments.

Legumes	Growth conditions	Treatments	N Total	P Available	OM	pH	EC	BSR	APA	GA
Broad Bean	Stress-free	T0	24.6^{g}	9.81^{e}	3.8^{a}	7.61^{b}	0.34^{g}	0.83^{e}	8.91^{g}	3.04^{g}
		T1	56.67^{a}	32.51^{b}	2.41^{c}	5.18^{f}	0.65^{f}	1.42^{b}	23.8^{a}	5.73^{a}
		T2	46.2^{b}	27.93^{c}	2.78^{b}	5.26^{f}	0.47^{g}	1.24^{c}	22.52^{b}	5.25^{b}
		T3	57.62^{a}	35.62^{a}	2.29^{c}	5.31^{f}	0.62^{f}	1.71^{a}	22.97^{b}	5.81^{a}
	Hydric stress	T0	11.3^{h}	4.32^{g}	1.7^{d}	7.94^{b}	2.87^{b}	0.23^{g}	3.39^{h}	1.01^{i}
		T1	32.73^{e}	26.21^{c}	1.52^{e}	6.22^{e}	2.03^{e}	0.86^{e}	15.28^{e}	3.62^{f}
		T2	29.81^{f}	20.03^{d}	1.67^{d}	6.56^{d}	2.21^{d}	0.73^{e}	13.57^{f}	3.45^{f}
		T3	33.56^{e}	28.32^{c}	1.73^{d}	6.13^{e}	1.98^{e}	0.98^{d}	17.87^{d}	3.94^{e}
	Saline stress	T0	13.43^{h}	6.34^{f}	0.91^{g}	9.19^{a}	3.11^{a}	0.42^{f}	4.22^{h}	1.74^{h}
		T1	37.57^{d}	32.12^{b}	1.29^{f}	6.56^{d}	2.53^{c}	1.01^{d}	19.49^{c}	4.32d
		T2	34.11^{e}	27.46^{c}	1.23^{f}	6.78^{c}	2.64^{c}	0.87^{e}	18.31^{d}	4.02^{e}
		T3	42.67^{c}	35.48^{a}	1.48^{e}	6.61^{c}	2.48^{c}	1.19^{c}	20.01^{c}	4.68^{c}
Pea	Stress-free	T0	23.33^{G}	9.31^{E}	3.87^{A}	7.43^{C}	0.31^{F}	0.92^{D}	8.72^{H}	2.98^{E}
		T1	51.42^{A}	29.11^{B}	2.59^{BC}	5.75^{EF}	0.54^{E}	1.69^{A}	22.32^{A}	5.57^{A}
		T2	48.61^{B}	27.51^{C}	2.83^{B}	5.32^{F}	0.51^{E}	1.42^{B}	21.83^{B}	5.67^{A}
		T3	52.23^{A}	30.19^{B}	2.72^{B}	5.56^{EF}	0.63^{E}	1.64^{A}	22.62^{A}	5.83^{A}
	Hydric stress	T0	10.64^{I}	5.28^{G}	1.21^{D}	7.82^{B}	2.79^{B}	0.37^{F}	3.61^{I}	1.01^{F}
		T1	30.81^{F}	23.57^{D}	2.07^{C}	6.75^{E}	1.96^{C}	0.92^{D}	15.73^{F}	3.83^{C}
		T2	31.21^{F}	19.46^{c}	2.04^{C}	6.96^{D}	2.05^{C}	0.85^{E}	13.97^{G}	3.58^{D}
		T3	32.64^{E}	29.84^{B}	2.02^{C}	6.71^{E}	1.8^{D}	1.18^{C}	18.1^{E}	4.14^{B}
	Saline stress	T0	14.5^{H}	7.56^{F}	0.94^{E}	9.14^{A}	3.03^{A}	0.39^{F}	3.92^{I}	1.29^{F}
		T1	38.22^{D}	32.84^{A}	1.27^{D}	6.92^{D}	2.32^{BC}	1.13^{C}	18.46^{E}	4.05^{B}
		T2	33.81^{E}	29.24^{B}	1.19^{DE}	6.57^{E}	2.51^{B}	0.79^{E}	19.1^{D}	3.86^{C}
		T3	42.74^{C}	33.13^{A}	1.36^{D}	6.68^{E}	2.15^{C}	1.21^{C}	20.61^{C}	4.13^{B}

Data represent the mean ± of two independent replicates with five repetitions each. The data are the average of two independent replicates with five iterations each. At $p < 0.05$, values with the same superscript letter did not differ significantly. N total (mg.Kg^{-1}), P available (mg.Kg^{-1}), EC, electrical conductivity (dS m^{-1}); OM, organic matter (%); BSR, basal soil respiration (mg CO_2 g^{-1} 24 h^{-1}); APA, alkaline phosphatase activity (µg pNP g^{-1}); GA, β-Glucosidase activity (mg pNP g^{-1}).

3.2.3. Plant mineral content acquisition

The PSB consortia inoculations were apparent to contribute to the response to P fertilization in both crops used. Seed-inoculated plant parameters were higher than those from the fertilized plant alone (Table 4). A high positive correlation between plant P content and root acid phosphatase activity in response to PSB inoculation was observed. In our study, higher acid phosphatase activities were found in the roots of seeds inoculated with PSB consortia, which could accelerate the decomposition of organic and inorganic P and promote plant P uptake. We noted an increase in P availability in the grain and an improvement in the plant aerial surface

in response to PSB inoculation; specifically in T3 treatment compared to the non-inoculated control indicating an increase in each growth condition ranging from 62.3 to 85.08% for Broad Bean seeds and from 48.64 to 73.36% for Pea seeds, respectively. With an average increase that exceeds 42.5 and 39.7% for Broad Bean and Pea, respectively, compared to non-inoculated plants, shoot N content increased in response to PSB consortia inoculation even in stress conditions. Likewise, grain N content marks an increase greater than 54 and 67.41% respectively, compared to non-inoculated plants.

Table 4. Variations in plant mineral contents in both shoot and grains with RP fertilization and PSBs inoculation.

Legumes	Growth conditions	Treatments	P contents ($mg.g^{-1}$)		Total N ($mg.g^{-1}$)		K contents ($mg.g^{-1}$)		RPAE ($mg.g^{-1}$)	RAPA ($nmol{\cdot}g^{-1}{\cdot}min^{-1}$)
			Shoot	Grains	Shoot	Grains	Shoot	Grains		
Broad Bean	Stress-free	T0	2.46^{c}	1.15^{d}	21.22^{g}	4.48^{e}	3.77^{f}	1.54^{f}	4.26^{f}	28.92^{h}
		T1	4.94^{a}	2.93^{a}	35.72^{b}	7.59^{b}	7.46^{d}	2.08^{b}	10.82^{b}	76.68^{d}
		T2	4.36^{b}	2.58^{b}	35.48^{b}	9.18^{a}	8.16^{c}	2.17^{b}	10.43^{b}	67.68^{e}
		T3	4.79^{a}	3.05^{a}	36.91^{a}	9.74^{a}	9.41^{b}	2.92^{a}	11.17^{a}	85.39^{bc}
	Hydric stress	T0	0.61^{g}	0.17^{h}	3.97^{h}	0.13^{h}	5.46^{g}	0.62^{g}	2.18^{h}	53.56^{f}
		T1	1.57^{e}	0.86^{e}	23.1^{f}	3.62^{f}	10.89^{a}	1.61^{e}	6.96^{e}	91.02^{b}
		T2	1.33^{d}	0.64^{f}	25.65^{e}	5.03^{d}	10.12^{b}	1.39^{f}	7.15^{e}	82.21^{c}
		T3	2.29^{c}	1.14^{d}	26.39^{e}	5.09^{d}	11.01^{a}	1.87^{d}	8.25^{d}	101.03^{a}
	Saline stress	T0	0.88^{g}	0.33^{g}	8.46^{g}	2.13^{g}	1.97^{h}	0.63^{g}	3.81^{g}	18.26^{i}
		T1	1.83^{d}	1.26^{d}	27.54^{d}	5.35^{d}	3.76^{f}	1.69^{e}	8.68^{d}	29.66^{h}
		T2	1.97^{d}	0.93^{e}	26.42^{e}	6.02^{c}	4.11^{e}	1.61^{e}	9.01^{d}	32.23^{g}
		T3	2.39^{c}	1.48^{c}	29.71^{c}	6.12^{c}	4.23^{e}	1.91^{c}	9.52^{c}	35.77^{g}
Pea	Stress-free	T0	2.45^{C}	1.36^{C}	20.31^{G}	3.36^{F}	4.55^{F}	1.21^{D}	3.88^{F}	22.59^{I}
		T1	4.63^{B}	2.83^{A}	33.8^{A}	10.29^{A}	8.22^{D}	2.42^{A}	9.98^{A}	74.42^{D}
		T2	4.59^{B}	2.61^{B}	33.53^{B}	10.13^{A}	8.76^{D}	2.73^{A}	9.32^{B}	65.51^{E}
		T3	4.92^{A}	2.81^{A}	33.68^{B}	10.31^{A}	9.81^{C}	2.64^{A}	9.72^{A}	81.87^{C}
	Hydric stress	T0	0.53^{G}	0.11^{H}	3.15^{I}	0.21^{H}	6.6^{E}	0.46^{E}	1.34^{G}	42.82^{F}
		T1	0.97^{F}	0.49^{G}	24.55^{F}	5.06^{E}	10.42^{B}	1.49^{C}	5.63^{E}	84.89^{B}
		T2	1.09^{F}	0.67^{F}	24.31^{F}	6.21^{D}	10.05^{B}	1.17^{D}	6.97^{D}	86.33^{B}
		T3	1.64^{E}	0.89^{E}	25.19^{E}	5.97^{E}	11.61^{A}	1.79^{B}	7.21^{D}	95.97^{A}
	Saline stress	T0	0.94^{F}	0.63^{F}	9.66^{H}	1.63^{G}	2.07^{H}	0.39^{E}	3.73^{F}	19.34^{J}
		T1	2.07^{D}	1.01^{D}	27.88^{D}	7.51^{B}	3.56^{G}	1.69^{C}	8.86^{C}	31.02^{H}
		T2	1.73^{E}	0.79^{E}	27.28^{D}	7.2^{C}	4.44^{F}	1.58^{C}	9.21^{B}	33.67^{H}
		T3	2.12^{D}	1.23^{C}	29.32^{C}	7.82^{B}	4.86^{F}	1.83^{B}	8.97^{C}	37.49^{G}

The data are the average of two independent replicates with five iterations each. At $p < 0.05$, values with the same superscript letter did not differ significantly. RPAE, Root P acquisition efficiency; RAPA, Root acid phosphatase activity.

The same results were noted for the shoot and grain K content with significant improvement in T3 treatment. Except that in hydric stress, we observe an increase in the K in plant tissues, rather than an actual accumulation of potassium in the seed in both crops used. This explains that a lack of water can disrupt plants' ability to absorb nutrients, including potassium, effectively, due to reduced metabolic activity and root absorption processes. This was also observed for the other two nutrients under both hydric stress and saline stress, which reduce the

nutrient content in the seeds due to their effects on ionic competition, osmotic imbalance and other metabolic processes. On the other hand, a notable increase in RPAE significantly higher than the control was seen, indicating that PSB strains probably help to improve internal P use efficiency.

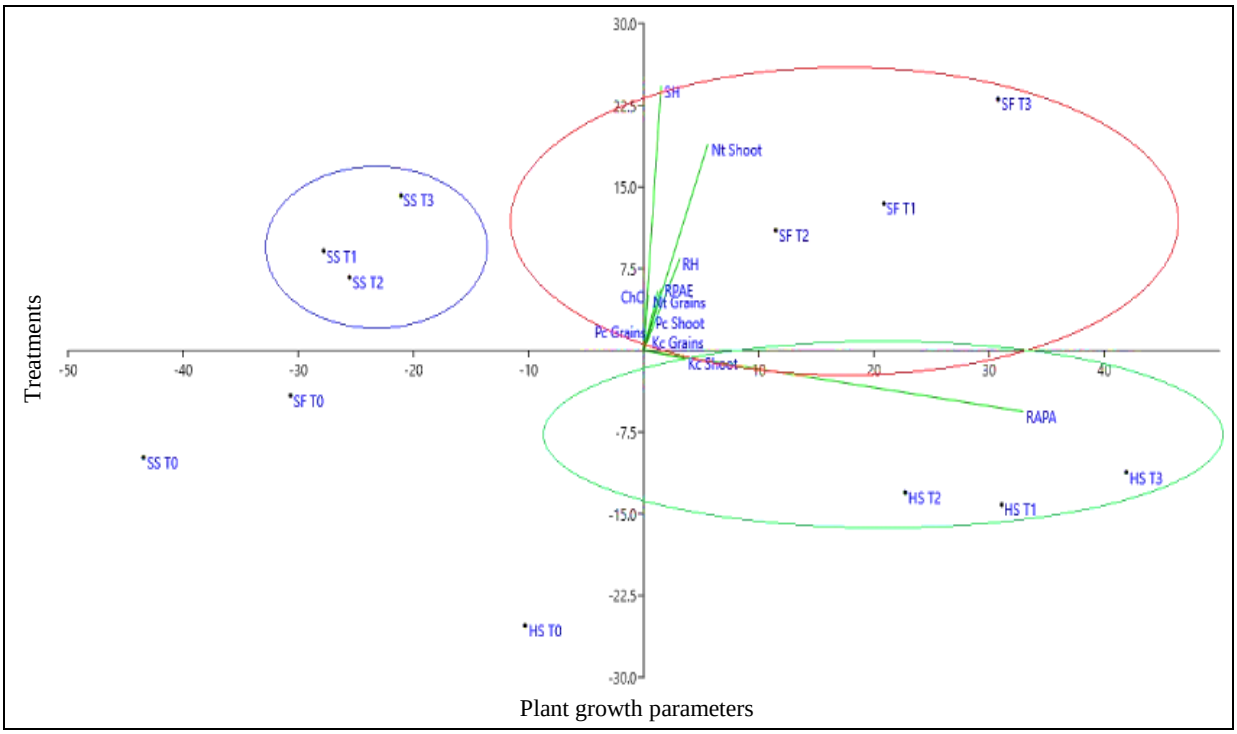


Figure 3. Principal component analysis of plant growth parameters in the function of different treatments.

The plant growth parameters in terms of different treatments used were studied by a dimensional analysis (Principal component analysis (PCA)) (Fig 3) to identify how these variables are correlated with the consortia inoculation. According to the PCA analysis, the first synthetic component (axis x) corresponds to the plant growth parameters, while the second synthetic component (axis y) corresponds to the different treatments. The studied parameters (mineral contents, growth response) explained 78.011 % of the total variation, while the different treatments explained 19.699 %. According to Figure 3, we note that T0 of stress-free, saline stress, and hydric stress are in the opposite direction of all studied parameters, where a negative correlation is found. While T3, T1, and T2, respectively, are heavily clustered with all studied parameters in stress-free conditions, except RAPA and potassium content in the shoot. On the other hand, T3, T1, and T2, respectively, are positively correlated with RAPA and potassium content in shoot in hydric stress with a negative correlation with T3, T1, and T2, respectively, in saline stress. From our results, we can note that the consortia treatments have a positive effect on plant development with the superiority of T3, T1, and T2, respectively.

3.3. Molecular identification of isolates

Genetic sequencing of the amplified 16S rRNA gene of the randomly selected isolates was analyzed using the web-based Basic Local Alignment Search Tool (BLAST) program of the National Centre for Biotechnology Information (NCBI). Based on the 16S rRNA gene sequence analyses, a total of five species of bacteria were identified that exhibited 98.67 to 100% homology with type or reference strains (Table 5). These were: *Pseudomonas lutea* (WJEF15), *Pseudomonas hunanensis* (WJEF26), *Pseudomonas pergaminensis* (WJEF38), *Bacillus amyloliquefaciens* (WJEF46), and *Bacillus rugosus* (WJEF63). The sequence data of the isolates were submitted to NCBI and assigned the accession number represented in Table 5.

Table 5. Isolates from rhizospheric soils of legumes by 16S rRNA gene sequence homology and their GenBank accession numbers.

Isolates	Sequence homology			Phylum
	Closest species	Percent homology	Accession no.	
WJEF15	*Pseudomonas lutea*	100	OR591463	γ-proteobacteria
WJEF26	*Pseudomonas hunanensis*	100	OR592440	γ-proteobacteria
WJEF38	*Pseudomonas pergaminensis*	99.35	OR592458	γ-proteobacteria
WJEF46	*Bacillus amyloliquefaciens*	98.67	OR592459	Firmicutes
WJEF63	*Bacillus rugosus*	99.59	OR592467	Firmicutes

A phylogenetic tree was constructed comprising all of the sequenced isolates using the maximum likelihood method in MEGA 11 with 5000 bootstrap replicates. The 16S rRNA gene sequences of bacterial isolates used in this study and the closest similar sequences of type or reference strains found in the NCBI nucleotide database were aligned in MEGA 11 using the muscle-clustering program. The scale bar indicated the number of differences in nucleotide substitutions per sequence.

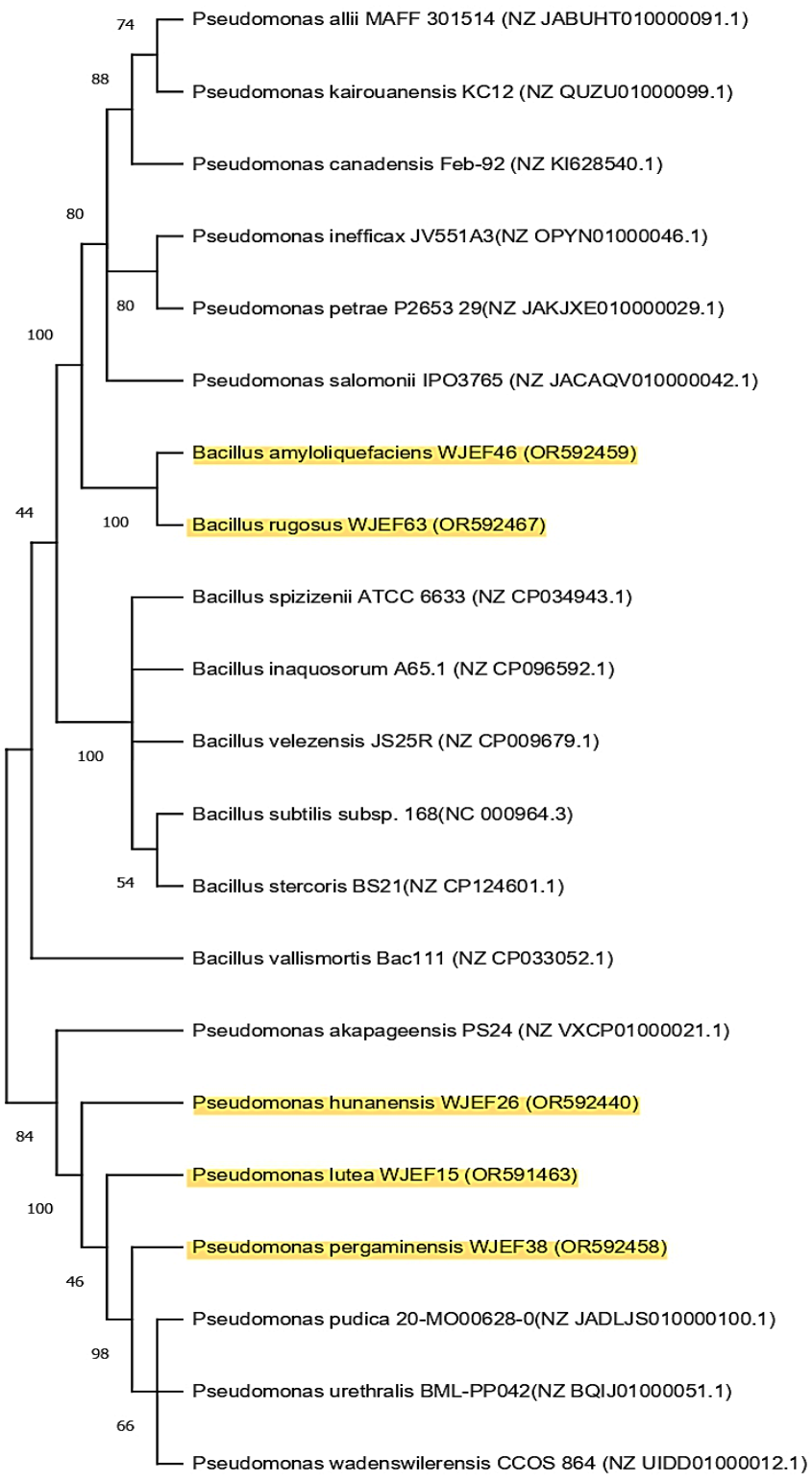


Figure 4. Phylogenetic tree based on 16S rRNA gene sequences of isolated strains and closely related bacteria using maximum likelihood method.

4. Discussion

More food is required to feed the world's growing population, but agricultural yields are declining due to abiotic stressors such as nutrient scarcity, drought, salinity, and temperature,

which are the primary barriers to sustainable agriculture worldwide (Kapadia et al., 2021). In this study, we look at how PSB consortia affect the growth and yield parameters of broad beans and pea legumes under stress conditions. We adopted a more appropriate approach for the selection of competent strains of PSB to increase the chances of successful inoculation in the field and to improve significantly the nutritional status of the plants. We started our research by evaluating PSB-PGP traits and *in vivo* plant-bacterial interaction with bacteria isolated from a typical rhizospheric environment (Janati et al., 2023) and two crops intolerable to abiotic stress. This was done in light of the knowledge that PSB strains with plant growth promotion (PGP) abilities can benefit plant development under saline and drought stress (Latif et al., 2023; Slimani et al., 2023). Although all the activities of PSBs are crucial when choosing bacterial strains as bio-inoculants, the ability to tolerate a high degree of dryness and increasing NaCl concentration is of particular interest; in this respect, it was chosen to begin the stepwise screening of PSBs.

However, although PSBs are present in the majority of soils, their performance is strongly influenced by environmental factors such as soil type, temperature, salinity, cation exchange capacity, pH, organic matter, and availability of substances in soils (Rfaki et al., 2018; Masdariah et al., 2019). Our results indicate that PSBs have limited resistance to high concentrations of NaCl and drought. However, our in vitro assay confirmed that all strains selected showed a good resistance to the increasing concentration of NaCl which could be explained by the environmental conditions required by the strains. However, a remarkable growth decrease is observed from 1.6M of NaCl, except for WJEF15 and WJEF38 strains where their sensibility ranges between 0.8 and 1.2M. These salinity values are much higher than the crop's toleration and higher than the maximum salinity levels used in the broad bean and pea trials. As a result, we were able to choose the concentration of NaCl (2M) that we used during the *in vivo* salt stress. For their part, Yañez-Yazlle et al. (2021) noted that Halophilic rhizobacteria decreases the salt level in soil, protects the plant from osmotic effects, and thus, helps the growth of the plant under salinity stress while ameliorating soil salinity. Likewise, Latif et al. (2023) indicate that novel rhizospheric bacterial strains *P. oxydans* and *S. pasteuri* can be considered important halotolerant PGPR to reduce harmful effects of salt stress and may serve as useful tools for alleviating salinity in plants. Similarly, Ait-Ouakrim et al. (2023) mentioned that PSBs such as *Brevibacterium frigoritolerans*, *B. valismortis*, and *Streptomyces venezuelae*, were resistant to extreme abiotic stresses (salinity, high temperature, and pH change) and tolerant to heavy metals (Fe, Zn, Cu, and Pb). In addition, Namli et al. (2017) stated that resistance to extreme environmental conditions is an important

characteristic that allows inoculant bacteria to survive and maintain optimal population numbers throughout a specific crop life cycle. In contrast, The PSB strains were screened for their drought tolerance ability. We noted that all PSBs have the same behaviour facing the PEG 6000 concentration and tolerate concentrations ranging between 10 and 20% and their resistance decreased under high osmotic stress created by PEG 6000 (30%) with remarkable superiority of strain WJEF46. Our results are in concordance with Slimani et al. (2023) who tested the tolerance of PSBs to drought, where they mentioned that the PSBs used were able to tolerate up to 30% PEG 6000. Likewise, it was reported that the PSB also increase resistance to drought and diseases, acidifies soils, enhances nutrient availability, and enhances root growth, water and nutrient uptake (Jabborova et al., 2021). However, it was reported that salinity and hydric stress can induce an accumulation of free radicals and reactive oxygen species (ROS) in plant cells, causing damage to cell membranes, proteins, and DNA (Mansoor et al., 2022). This led us to test the antioxidant activity of the PSBs used, given that they can help plants tolerate salt and drought stress by several mechanisms. All tested strains showed significant antioxidant activity, both in terms of TAC and DPPH scavenging, where strains WJEF26 and WJEF15 showed superior results. According to Ilyas et al. (2020), inoculated plants showed a high concentration of antioxidants (SOD, CAT, and POD) as compared to un-inoculated plants under stress. In addition, (Gowtham et al., 2020) also reported a significant increase in antioxidant enzymes by the inoculation of bacterial strains under drought-stress conditions. These antioxidants have the potential to minimize oxidative damage and make plants drought-tolerant, they enhance water retention by maintaining the diffusion of organic carbon sources (F. Yasmin et al., 2020). In addition, resistance to intrinsic and extrinsic stresses is an important factor in the proliferation, development and survival of microorganisms in rhizospheric soils. For this reason, the formation of biofilms helps to alleviate stresses such as saline stress by reducing the Na^+ content available for uptake by plants, thereby enhancing plant growth, increasing biomass and crop productivity, and improving yields in saline soils (Banerjee et al., 2019). Our results showed that all the PSBs tested were capable of forming biofilms except WJEF46, while WJEF38 and WJEF26 exhibited significant biofilm production. In addition, Balasundararajan & Dananjeyan, (2019) studied AHL-mediated biofilm formation by bacteria from rice rhizoplane and found that AHL-positive bacteria profusely colonized the rice root upon inoculation and formed biofilm on the surface of the root under gnotobiotic conditions. They suggested that biofilm ensures competitive colonization on the rhizoplane and thereby improves plant growth and health. Therefore, the inoculation with biofilm-producing

bacteria, like the ones tested in this study, could serve as a useful tool for alleviating salinity stress in salt-sensitive plants due to a reduced passive flow of Na^+.

Soil salinity is a global menace to plant growth and development causing significant economic losses to legume crops (Haj-Amor et al., 2022). Increasing soil salinity and saline irrigation are the most limiting factors for cultivating legume crops, which creates water deficit conditions and prevents water uptake from soil ultimately disturbing the osmotic balance in the affected plants (M. I. Hussain et al., 2020). Likewise, it was mentioned that drought stress has adverse effects on seed germination and growth in various plants by oxidative stress and ionic imbalance in addition to reducing the Chl content resulting in a decreased rate of photosynthesis (Sohag et al., 2020). For this purpose, a growth experiment was performed to check the ameliorative effect of selected bacterial strains in stressed conditions. The response of crops to saline and drought stress was different, as expected. Broad bean is a crop with a high tolerance to the different types of abiotic stress compared to pea crops. Our *in vivo* results confirmed these reports as broad bean germination rate and yield percentage surpassed that of pea. However, the studied traits showed the highest values by applying the treatments T3 and, T1 followed by T2 (Tables 2, 3, and 4). The inoculation by PSB consortia significantly enhanced agronomic and yield-attributed traits than the control (without bio-fertilizer) treatment. The negative impacts of drought and salinity on seed germination, plant growth, nodulation, and crop yield have been reported. The growth parameters such as germination percentage root and shoots length and biomass decreased under high osmotic stress created by drought conditions or under high salinity stress. These problems can be explained by the increased osmotic potential resulting in the inhibition of water absorption by the seeds and impairment of other metabolic processes (Masrahi et al., 2023). Additionally, repercussion in the value of nutrients, amount of organic matter, and cultivation is observed with the change in salinity value. Our findings are consistent with those in different crops, and the activation of antioxidant enzymes is a great tactic for coping with salinity-induced oxidative stress (Osman et al., 2022). In this study, bacterial combination increased germination and germination-related parameters significantly as compared to control. However, using a combination of PSB consortia and P fertilizer rates increased yield and its components, therefore growth of the plant even in stress conditions. On the other hand, we noted that root/shoot length and biomass were increased significantly by the inoculation of bacterial strains as compared to the control. The T3 treatment was the most promising for both crops showing the best values in measured growth attributes under stress conditions. In addition, the results of the present study revealed that consortia used increased osmotic potential, water potential, growth development, and chlorophyll content in plants,

which facilitate plant growth under drought and saline stress conditions. The microbial amendment in the soil at the rhizosphere provides hormones, and the siderophores converts complex organic matter to readily available forms and assists in the transport and uptake of molecules (Billah et al., 2019; Gusain & Bhandari, 2019). In this respect, when combined with P, PSB has a significant function in cell division, and root elongation, and is an essential component of ATP and ADP, which are responsible for higher yields (Elhaissoufi et al., 2020). The promotion effect of PSB as bio-fertilizers may be due to the effect of non-symbiotic P solubilizing microorganisms in exerting a positive influence on plant growth by the synthesis of phytohormones and enzymes such as ACC deaminase that modulate the plant hormonal level (Yañez-Yazlle et al., 2021). These beneficial roles of microbes help the plants to tolerate various abiotic stress conditions.

Furthermore, different studies have shown the effects of salinity and drought stress on seed germination, vegetative growth, reduced growth and development, reproductive development, and fertility (L. Xu et al., 2020). The results were from a previous study carried out by Molazem et al., in which there was a statistically significant decrease in both chlorophyll a and chlorophyll b during higher concentrations of salts compared to the PSBs inoculation (A. Kumar, Singh, Mukherjee, et al., 2021). Accumulation of ions and functional disorder in stomatal opening and closing can be a possible reason for the depletion of the total amount of chlorophyll content under salt stress (Kapadia et al., 2021). On the other hand, Kusale et al. (2021) reported a significant reduction in root length by 32.1%, shoot height by 19.7% and a 31.1% reduction in chlorophyll content in wheat seedlings after 45 days of growth under saline soil. They found that the inoculation of halotolerant *Klebsiella variicola* not only reverted this negative impact but also significantly improved these plant growth parameters. These results were also in harmony with Danish et al. (2020) also reported that under drought stress PGPR inoculated plants showed higher dry matter accumulation and a sustainable amount of chlorophyll levels as compared to un-inoculated drought-exposed plants. On the other hand, high salt levels have a significant role in accelerating oxidative damage to cell structures and membrane components, which could explain the greater value of EC at the highest salinity levels (Masrahi et al., 2023). However, the inoculation of PSB consortia could alleviate the stress factor by providing the necessary substances for their growth, opening a new possibility for extending broad bean and pea cultivation to saline and drought soils. These features represent an advantage for agricultural and biotechnological applications and the possibility to apply and evaluate the strains in multiple conditions. Furthermore, the reduction in the primary and secondary root length will lead to lesser absorption of nutrients, which could be attributed

to a lower shoot, leaf dryness, and fresh biomass (Tahir et al., 2023). In addition, PSBs alone or in combination with other rhizobacteria have been reported to improve growth, nutrient uptake and root architecture in legumes as well as to improve crop resistance (Seenivasagan & Babalola, 2021). In our funding, we note changes in fresh and dry weight, length, growth, and dimension of plants due to the application of microbial consortia. Our results are in line with Song et al. (2022) who reported an improvement in root, shoot dry weight and yield of maize with PSB inoculation. The present results are supported by the observation that inoculation of *Bacillus sp*., *Delftia sp*., *Enterobacter sp*., *Achromobacter sp*., consortia could alleviate the deleterious effects of salinity stress and improve the growth of tomato plants under salinity stress. In addition, Van Oosten et al. (2018) found that *A. chroococcum* 76A inoculation under both moderate (50 mM NaCl) and severe (100 mM NaCl) salt stresses improved plant growth parameters, such as shoot dry weight, fresh fruit weight, and fruit numbers per plant compared to the un-inoculated plant. According to Kapadia et al. (2021), they explained that the length, growth, and dimension of plants are dependent on the processes of assimilation and partitioning of various carbohydrates, this ratio is observed to change only when the plant is encountered with any stressful condition.

On the other hand, under stressed conditions, grain yield decreased in both crops used compared to un-stressed plants. The reason for this decrease in yield may have resulted from the reduction in the growth of stressed plants and the limitation of photosynthetic pigments in leaves. The results of this study showed that inoculation with bio-fertilizer application under drought and salinity stress led to a significant increase in broad bean and pea grain yield as compared to control, in addition to significant uptake of nitrogen, phosphorus, and potassium in grain and shoot. On their part, Kusale et al. (2021) reported that inoculation of halotolerant *K. variicola* SURYA6 significantly improved N, P, K, Na, and Mg contents in wheat and maize under salinity. This isolate resulted in a 35.1 to 89.9% increase in the mineral content compared to the un-inoculated plants. Moreover, it is known that salinization affects the normal transformations of N in soil by retarding or inhibiting several biological/microbial processes as nitrification, causing an accumulation of nitrite or increasing NH_4 volatilization rates (Haj-Amor et al., 2022). The overall growth, development, and health of crop plants depend on the availability of sufficient amounts of nutrients and it is regulated by the ability of its microbiome to produce a variety of plant growth-promoting traits to improve plant growth (Khumairah et al., 2022). The positive effect of PSB consortia on the uptake of N, P, and K in grain and straw yields can be due to the production of organic acids, which led to an increase in plant uptake of nutrients by reducing soil pH. The data in Table 4 showed that the maximum values of NPK

uptake in grain and shoot were obtained by the T3 treatment followed by T2 and T1 respectively. Inoculation of multifarious rhizobacteria provides multiple nutrients to the plants and thus helps grow plants (Alishahi et al., 2020). The application of PSB consortia plays a significant role in the optimization of P solubilization, increasing nutrient levels, and mineralization of organic P, therefore, increasing the boosting plant tolerance to environmental challenges (Masrahi et al., 2023). An integrated crop and soil nutrient management through the application of multifarious PSBs with drought and salinity ameliorant potential can help in managing the biodiversity of microbial fertilizers, improve soil nutrients, alleviate salinity stress, and sustain crop growth under stress conditions. Drought stress also adversely affects legumes nutrient uptake such as N, P, K, Ca, and Mg. Several studies reported that drought stress reduces the concentration of N, K, and P in plant tissue and declines nutrient uptake from soil (Jabborova et al., 2021). Likewise, Hinsinger et al. (2009) reported that drought-stressed conditions significantly decrease soil nutrients such as N, P, and K, and microelements such as B, Fe, Mn, and Zn. Our results showed that both P concentration and uptake were improved when PSBs were applied (Table 4). Results of the present study show that co-inoculation with T3 treatment significantly increased the N content, P content, and K content compared to the control under drought conditions. However, PSBs are known to colonize the plant's rhizosphere, adhere to the root surface, and maintain moisture content, this makes stable aggregates that help in nutrient absorption in plants (Gamalero et al., 2020). On the other hand, drought stress in the soil is known also to decrease enzyme activities. The decrease in soil enzyme activities observed in this study is in agreement with the decrease in P content in drought-stressed conditions. Similarly, Jabborova et al. (2021) have also observed the enhancement in soil enzymes such as phosphatase acid due to rhizobial inoculation. Our results are in line with Han & Lee, (2006) who reported an improvement in N, P and K uptake in pepper and cucumber has been observed with PSB inoculation (Adnan et al., 2020). These authors stated that, at the germination stage, seeds obtain most of their nutrients from internal reserves, but growth hormones like auxins or gibberellins produced by PSB stimulate the process of germination. One of the possible reasons for such improvements is the PGPR behaviour of PSBs, as reported in our previous studies (Janati et al., 2023). Our results confirm that PSB enhances plant growth by various mechanisms, of which the most prominent are the release of valuable metabolites, such as phytohormones, antioxidants, siderophores, root colonization, P solubilization, siderophores, auxins and ACC deaminase syntheses by PSB. In addition, PSB releases substances like phosphatases and different organic and mineral acids, which amplify P solubility and progress, crop growth. Based on the result it is concluded that

seed inoculation with PSBs is beneficial when P is supplemented from mineral sources, especially as RP. Phosphorus solubility from RP can be increased with PSB inoculation and it can be used as an environmentally friendly and economically beneficial alternative.

Many such investigations and experiments conclude that PSBs are a potential alternative to withstand abiotic stress in plants and enhance plant growth promoters (Kapadia et al., 2022). These studies indicate the role of various mechanisms at the molecular level during the plant-PSB interactions. On the other hand, as discussed by Crowther et al. (2019), although there is a clear potential for the development of inoculants with PSBs, their widespread application remains limited by a poor understanding of microbial ecology, dynamics population in the soil, and inconsistent performance in a range of environmental factors. The result obtained in the current paper revealed that the investigated bacterial consortia have PGP traits that can alleviate drought and salt stress. Thus, the inoculated PSB consortia mentioned in the results enhanced the tolerance limit of both used crops. This consortium enabled the plants to survive properly at higher stress than their normal tolerance level. Thus, this study underlines the commercial potential of these selected PSB isolates for sustainable broad bean and pea cultivation under salt and drought stress.

5. Conclusion

Pots experiments demonstrate that PSB strains can significantly encourage early plant growth while raising the germination rate of the two plants under stress conditions. Seeds treated with PSB consortia had a higher germination rate and longer rooting length than the untreated controls. The result obtained in the current paper revealed that the investigated bacterial consortia have PGP traits that can alleviate stress conditions The pot experiment further demonstrated that PSB consortia inoculation could enhance soil acid phosphatase activity and nutrient available plant nutrients uptake. Higher dry matter accumulation and a sustainable amount of chlorophyll levels were observed in inoculated plants compared to control. It is possible to conclude that these species have a high potential for reducing the use of chemical P fertilizers and promoting long-term agricultural development. Based on these findings, we conclude that PSB-tested strains are potential P-solubilizing and plant-growth-promoting strains that can supplement Broad Bean and Pea P requirements under saline and drought stress. To understand the existence of microbial communities in different conditions and their effect in field conditions, a consortium of PGPs and their interactive effect must be studied at the molecular level. Further research into the mechanisms and experiments with these organisms in different plants would lead to the effective use of these PGPRs as bioinoculants for various

soil properties. Collectively, the application of abiotic-tolerant PSB consortia may provide an eco-friendly strategy to mitigate drought and saline stress. To facilitate the more widespread and efficacious use of PSBs, scientists need to make the leap from greenhouse experiments to field studies, farmers and consumers must be educated about the role that PSBs play in plant growth and soil health. The studies highlighted give us a suggestion that these strains could be used to formulate a consortium of biofertilizers for sustainable production of Broad beans and peas under drought or saline-stressed field conditions.

Conclusion générale et perspectives

Ce projet de thèse a été mené dans le but d'isoler des bactéries qui solubilisent les phosphates à partir de la rhizosphère et créer une banque de souches compétentes suites à une identification biochimique et moléculaire. Il vise également l'étude des facteurs abiotiques qui influencent l'activité de ces bactéries. Les interactions entre les légumineuses et les PSB sont en fin explorés afin de mettre en évidence les mécanismes sous-jacents de cette symbiose, et expliquer la contribution des PSB à réduire la dépendance aux engrais phosphatés et à augmenter les caractères de croissance des plantes.

La première partie a mis en évidence les différents effets climatiques et pédologiques sur l'abondance, la diversité et le potentiel des PSB dans la rhizosphère des légumineuses dans quatre stations bioclimatiques dissemblables de la région Fès - Meknès, au Maroc. Au total, 51 PSB ont été isolées et caractérisées dans ce travail. La moyenne de solubilisation du P rocheux (RP) et du P tricalcique (TCP) de toutes les souches isolées dans chacune des quatre stations varie de 18,69 $mg.L^{-1}$ à 40,43 $mg.L^{-1}$ et de 71,71 $mg.L^{-1}$ à 94,54 $mg.L^{-1}$, respectivement. Les caractéristiques PGP des souches isolées sont positivement corrélées avec l'abondance des PSB et les caractéristiques de l'échantillon (sol et climat). La densité de la population de PSB était significativement différente entre les quatre stations en raison des conditions environnementales variées : température, humidité et précipitations. Le site d'Ait Saleh, le plus chargé en PSB, montre 70% et 69% de la production d'auxines et de siderophores, respectivement. La solubilisation de zinc et l'activité de l'ACC désaminase sont corrélées avec la distribution des isolats. Les résultats montrent que la diversité des communautés bactériennes, leur densité et leur potentiel sont étroitement liés à diverses conditions édapho-climatiques telles que la température, les précipitations, le statut nutritif du sol et la texture du sol. Les caractéristiques morphologiques et biochimiques des souches nous ont permis d'identifier environ neuf genres bactériens différents, dont *Bacillus*, *Pseudomonas* et *Rhizobium*. Le climat exerce une forte pression sélective sur la structure des communautés de PSB et leurs activités tels que la compétence de solubilisation du P. En général, les résultats de l'étude pourraient fournir une approche efficace pour l'amélioration des inoculant microbiens au but agronomique afin d'augmenter la mobilisation du P du sol, que ça soit en production intensive ou dans des applications agro-écologiques.

La deuxième partie a mis le point sur les souches de PSB isolées des sols situés à différents stades bioclimatiques au Maroc et présentes dans la rhizosphère de diverses légumineuses afin

d'améliorer l'efficacité des engrais microbiens agronomiques. Il s'agissait de tester la capacité de ces souches à améliorer l'activité de solubilisation du P dans un milieu NBRIP avec du P tricalcique (Ca_3 $(PO_4)_2$) (TCP) et du P naturel (RP) séparément, ainsi que leur combinaison (RP/TCP) selon un plan d'expérience complet à deux niveaux ($2^2 = 4$ expériences). Parmi toutes les souches isolées, 12 présentent un intérêt biotechnologique prometteur en raison de leur solubilisation du P et de leur bonne résistance à différentes conditions physicochimiques extrêmes. En outre, la souche WJEF15 a montré la plus grande efficacité de solubilisation du P en milieu solide NBRIP avec un PSI de 4,1 ; tandis que la souche WJEF61 a été localisée comme la souche la plus efficace sur milieu liquide NBRIP-TCP en libérant 147,62 $mg.l^{-1}$ de P soluble. En revanche, dans le milieu liquide NBRIP-RP, la souche WJEF15 a présenté une solubilisation maximale avec 25,16 $mg.l^{-1}$. Le plan d'expérience élaboré a montré qu'une combinaison de RP et de TCP avec le niveau maximum augmente progressivement la solubilisation du P de 20,58%, tandis que la souche WJEF63 a la concentration la plus efficace de 102,69 $mg.l^{-1}$. Les PSB isolées révèlent une tolérance à la salinité (2M), le pH (3-13), la sécheresse (30% PEG) et la température (55 °C). Les souches PSB isolées ont un effet antifongique significatif allant jusqu'à 49,15% d'inhibition de la croissance mycélienne. Les résultats révèlent donc un effet potentiel des PSB pouvant ainsi soutenir les cultures en tant que rhizobactéries favorisant la croissance des plantes (PGPR) comme engrais microbiens dans le domaine agronomique.

La troisième partie du travail a évalué l'impact de cinq souches de PSB compétentes, sur la croissance de deux légumineuses (*Vicia faba* et *Pisum sativum*) et sur certaines propriétés du sol. Les souches de PSB utilisées se caractérisent par la solubilisation du P, l'activité ACC désaminase, la fixation de N, la production d'auxines, de l'acide cyanidrique, et des siderophores. Les expériences d'inoculations des graines montrent que les souches de PSB induisent de manière significative la croissance précoce des plantes. Les graines traitées avec des PSB ont un taux de germination plus élevé et un enracinement très développé que les témoins non traités. Toutes les souches de PSB ont surpassé le témoin en termes d'amélioration de la croissance des deux légumineuses testées avec un pourcentage allant de 77,78% à 88,88%, respectivement. Les résultats ont montré également que tous les traitements ont amélioré de manière significative les paramètres nutritionnels des plantes comme la teneur en N et en P 67,50 %, 23,11 %, respectivement. Nous avons observé parallèlement une augmentation du poids frais et secs des biomasses aériennes (41,17%, 38,57%) et souterraines (56,6%, 42,28%), respectivement. Par rapport au témoin, l'inoculation des PSB montre une augmentation de 72%

d’enracinement, de 40,91% du poids sec de la plante et de 40,07% du poids frais. Le sol rhizosphérique des traitements PSB présente des niveaux élevés de N, P et de matière organique. Tous les traitements ont montré des niveaux significativement plus élevés de phosphatase alcaline, de respiration basale du sol (CO_2) et d'activité β-glucosidase que le contrôle. L'expérience en pot a également démontré que l'inoculation de PSB améliore l'activité de la phosphatase acide du sol et le P disponible, ainsi que l'absorption du P par les plantes étudiées. Sur la base de ces résultats, nous concluons que les souches testées sont effectivement solubilisantes du P et favorisant ainsi la croissance des plantes complétant ainsi les besoins nutritionnels de la fève et du petit pois. Les résultats de l'étude offre une méthode pratique pour améliorer d’une manière agronomique les inoculant microbiens dans le but d'augmenter la mobilisation du P dans le sol nécessaire pour la croissance des plantes hôtes dans les applications agronomiques.

La quatrième partie a mis en évidence le rôle des PSB tolérantes aux stress abiotiques dans l'amélioration de la tolérance au stress hydrique et salin chez la fève et le petit pois en forme de consortium et en tenant compte des processus morphologiques, biochimiques et physiologiques des plantes testées. Cinq souches de PSB présentent une capacité à tolérer le sel et la sécheresse, elles présentent une capacité de former un biofilm et une activité antioxydant. Les isolats WJEF15, WJEF26 et WJEF38 ont été identifiés avec succès à l'aide de la technique de l'ARNr 16S comme étant *Pseudomonas lutea* (WJEF15), *Pseudomonas hunanensis* (WJEF26), *Pseudomonas pergaminensis* (WJEF38), *Bacillus amyloliquefaciens* (WJEF46), and *Bacillus rugosus* (WJEF63). Les expériences en pots démontrent que les souches de PSB encouragent de manière significative la croissance précoce des plantes tout en augmentant le taux de germination des deux plantes dans des conditions de stress. Les graines traitées avec des consortiums de PSB présentent un taux de germination plus élevé et une longueur des racines importante que les témoins non traités. L'inoculation des consortiums PSB peut améliorer l'activité de la phosphatase acide du sol et l'absorption des éléments nutritifs disponibles pour les plantes. Une plus grande accumulation de matière sèche et une teneur en chlorophylle significativement plus importante dans les plantes inoculées par rapport au témoin. Les résultats se sont avérés significatifs avec les trois traitements de consortium utilisées (T1, T2, T3) avec une efficacité remarquable du T3 qui comporte les cinq souches utilisées a atténué l'effet du stress salin et hydrique et a amélioré le développement de la plante. Le traitement T3 favorise la croissance des plantes avec une augmentation de 34,5 % et 32,45 % de la longueur des pousses, 39,58 % et 36,3 % de la longueur des racines, 46,9 % et 42,3 % de la biomasse, pour

la fève et le petit pois, respectivement. Les attributs physiologiques des plantes ont également été améliorés par l'inoculation bactérienne, montrant une augmentation de 39,8 % et 39,8 % de la chlorophylle (a et b) pour la fève et le petit pois, respectivement. En outre, le poids frais des pousses (49,72 % et 65,13 %) et le poids frais des racines (48,32 % et 56,98 %) ont été améliorés pour la fève et le petit pois, respectivement. De plus, les minéraux du sol et l'accumulation de nutriments minéraux dans les pousses et les grains, tels que l'azote, le phosphate, et le potassium après l'inoculation, se sont avérés significativement augmentés. Sur la base de ces résultats, l'application de ces consortiums de PSB tolérants les stress abiotiques pourrait constituer une stratégie écologique pour compléter les besoins en P de fèves et de petit pois dans des conditions de stress salin et hydrique, contribuant ainsi à la stratégie nationale du Maroc (GREEN GENERATION 2020-2030), qui vise à atteindre un développement durable en améliorant la qualité des sols et en augmentant les rendements grâce à l'utilisation de méthodes biologiques.

En guise de conclusion, l'utilisation de PSB compétentes avec un potentiel significatif et une tolérance au stress abiotique sous forme d'inoculum bactérien ouvre la voie vers la promotion d'une pratique agricole durable tout en préservant la fertilité des sols et en réduisant l'empreinte environnementale de l'agriculture moderne. Cela ajoutera une grande valeur écologique et économique au secteur agricole, le rendant ainsi plus concurrentiel sur les marchés nationaux et internationaux. Les résultats obtenus dans cette thèse ouvrent la voie à de nouvelles perspectives de recherche et développement intéressantes afin d'exploiter le plein potentiel de ces bactéries telluriques :

- Utiliser d'avantage les tests de confrontation en se basant beaucoup plus sur le type d'interaction et aussi les fonctions de chaque isolat afin de mettre en place une communauté synthétique ayant plusieurs caractéristiques et des performances multiples.

- Vérifier l'efficacité de ces biofertilisants sur les terres agricoles, qui pourrait éventuellement être variable chez d'autres espèces, et comprendre les différents mécanismes impliqués dans la disponibilité du P dans la rhizosphère ainsi que l'interaction entre les PSB, le phosphate et les légumineuses.

- Développer de nouvelles techniques innovantes sur les mécanismes génétiques déterminant le comportement des inoculant microbiens et se concentrer sur les concepts d'application de ces inoculant en agriculture afin d'identifier des bactéries plus compétentes dans des systèmes de production de cultures durables dans différentes conditions.

- Identifier des gènes impliqués dans la solubilisation du phosphate par les PSB et étudier les points de contrôles et d'expression des différents gènes identifiés.

- Appliquer des outils biotechnologiques et nano-technologiques pour créer des bio- et nano-fertilisants afin de garantir une grande surface d'échange, une réactivité élevée, une bonne absorption des nutriments et des rendements élevés.

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Appendixes

Appendix 1

Composition of NBRIP medium

For 1 litre of medium

$MgCl_2 \cdot 6H_2O$ (5g); $MgSO_4 \cdot 7H_2O$ (0.25g); KCl (0,2g); $(NH_4)2SO_4$ (0.1g); glucose (10g); Agar (15g), $Ca_3 (PO4)_2$ (5g) 1l of distilled water; pH = 7.

For 250ml of concentred solution *10

$MgCl_2 \cdot 6H_2O$ (12.5g); $MgSO_4 \cdot 7H_2O$ (0.625g); KCl (0,5g); $(NH_4)_2SO_4$ (0.25g), 250ml of distilled water

In addition, we take 100ul of solution *10 + 900ul of distilled water. Then we add glucose (10g); Agar (15.0g), $Ca3 (PO_4)_2$ (5g); pH = 7.

Composition of LB medium

For 1 litre of medium

Tryptone (10g); NaCl (5g); Yeast extract (15g)

Composition of YEM medium

For 1 litre of medium

CH2PO4 (5g), NaCL (0.1g), mannitol (10g), $MgSO_4 \cdot 7H_2O$ (0.2g), yeast extract 1g

Composition of tryptic Soy Broth (TSB) medium

For 1 litre of medium

Bovine casein pepton (17g) ; Soya pepton (3g) ; sodium chloride (5g) ; bio-potassium phosphate (2.5g) ; Glucose (2.5g) ; the (30g) of powder is poured into a litre of purified water and then 15min in an autoclave at 121°C; pH 7.3 ± 0.2 at 25°C

Composition of TSA (BioKar Diagnostics) medium

For 1 litre of medium

Tryptone (15g); Papain peptone of soya (5g); Sodium chloride (5g); Bacteriological agar (15g); pH of the ready-to-use medium at 25°C: 7.3 ± 0.2.

Peptone Water

For one litre, we have Peptone 10g; NaCl 5g

Appendix 2

PNP 10mM 69.55mg in 50ml distilled water, and then put in darkness at 4°C

pNPP 10mM 0,0742g in 20ml distilled water, put in darkness at 4°C (expired in 2d)

M (pNPP) = 371.12g/mol

NaOH (0.5M (mol/l)) 9.99g of NaOH in 0.5L of Distilled water

$CaCl_2$ (0.5M) 27.746g in 0.5L of Distilled water

Acid acetic (A solution 0.2M) 11.42 ml de CH_3COOH pure, in a litre of Distilled water

Sodium acetate (B solution 0.2M) 16.4 g de CH_3COONa or 27.2 g de $CH_3COONa•3H_2O$ in a litre of Distilled water.

Buffer Acetate solution

In a beaker put 164.8ml of (B) solution, then adjust the pH to 6.5 with (A) solution, after that, transfer the whole to a 200ml flask and fill in to the mark with distilled water, shake and store in the refrigerator.

Reagent 1: 1 litre of acidic ammonium heptamolybdate $(NH4)6Mo7O_{24}•4H_2O$ of 17.55g/L containing 168mL of pure H_2SO_4

Reagent 2: 1 litre of 3.5g/L solution of polyvinyl alcohol or PVA (obtained after dissolution of the reagent; and in water at 80°C) containing 0.35g of malachite green oxalate;

Grinding buffer Acetate solution

For 40ml of medium:

0.88ml of Solution A,

4.12ml of Solution B,

35ml of distilled water, the pH must be adjusted to 5.36 with NaOH 0.5M

Reaction solution for the ascorbic acid method (expired in 4 hours)

For 100ml, we combine:

50ml of sulphuric acid 5N (dilute 70ml of concentrated sulphuric acid in 500ml of H_2O),

5ml of Potassium antimonyl tartarate (Dissolve 1.3715g in 400ml H_2O and diluted in 500ml),

15ml of Ammonium Molybdate 4% (Dissolve 20g in 500ml of H_2O),

30ml of ascorbic acid (dissolve 1.76g in 100ml of H_2O).

Reaction solution for the Salkowsky method

<u>For 100ml, we combine:</u>

2ml of $FeCl_3$ 0.5M (Dissolve 1.35g in 10ml of H_2O),

49ml of H_2O and 49ml of perchloric acid 70%.

Preparation of phosphorus assay standard range

Stock solution; dissolve 219.5mg of Potassium dihydrogen phosphate KH_2PO_4 in 1000ml.

Standard solution; dilute 25ml of the stock solution in 500ml of distilled water.

DO measure in 880nm

Plot the curve on excel DO = f (KH_2PO_4 concentration).

Appendix 3

Production of siderophores

First step: Blue colourant

- Solution 1: Dissolve 0.06 g CAS (Fluka Chemicals) in 50 ml H_2O.
- Solution 2: Dissolve 0.0027 g of $FeCl_3$-6 H_2O in 10 ml of 10 mM HCl.
- Solution 3: Dissolve 0.073 g of HDTMA in 40 ml of H2O.

Mix solution 1 with 9 ml of solution 2, then mix with solution 3.

The solution should be blue in colour. Autoclave and store in a plastic container/bottle.

Second step: Mixing solution

- Salt solution Minimum medium 9 (MM9):

Dissolve 15g KH_2PO_4, 25g NaCl and 50g NH_4Cl in 500 ml H2O.

- 20% glucose stock:

Dissolve 20g glucose in 100ml H2O.

- NaOH Stock:

Dissolve 25g NaOH in 150 ml H2O; the pH should be around 12.

- Casamino acid solution:

1. Dissolve 3g of Casamino acid in 27 ml of H_2O.
2. Extract with 3% 8-hydroxyquinoline in chloroform to remove all traces of iron.

- Preparation of CAS agar

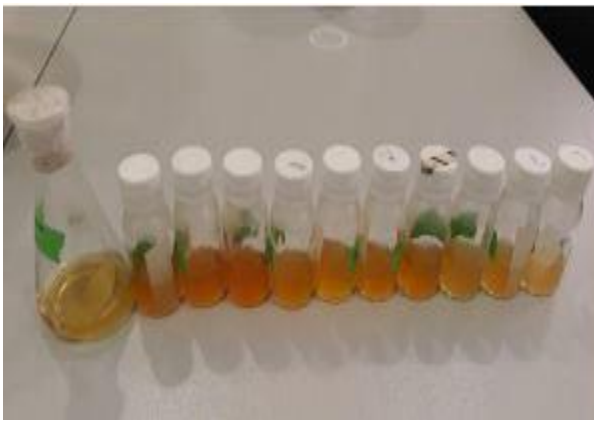
1. Add 100 ml MM9 saline solution to 750 ml H_2O.
2. Dissolve 32.24 g of piperazine-N, N-bis (2-ethanesulfonic acid) PIPES.

The PIPES do not dissolve below pH 5. Bring the pH to 6 and slowly add the PIPES while stirring.

PIPES while stirring. The pH will decrease as the PIPES dissolve. While stirring slowly bring the pH to 6.8. Do not exceed 6.8 as this will turn the solution green.

3. Add 15g of Bacteriological Agar.
4. Autoclave and cool to 50°C.
5. Add 30ml of sterile Casamino acid solution and 10ml of sterile 20% glucose solution to the MM9 / PIPES mixture.
6. Slowly add 100ml of blue dye solution along the glass wall with sufficient agitation to mix well.
7. Aseptically pour the dishes.
8. Inoculate the bacterial isolates onto the medium and incubate at 28°C for one week.

Appendix 4



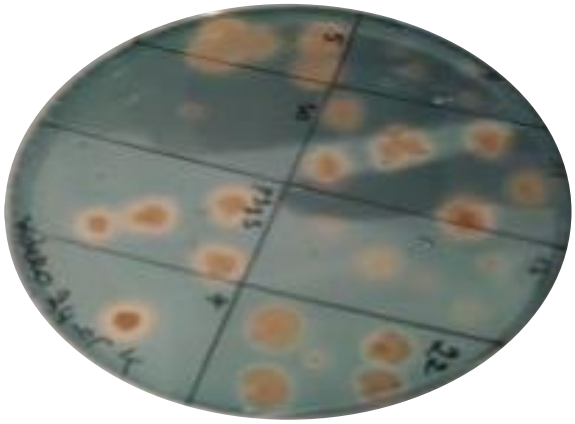
HCN production



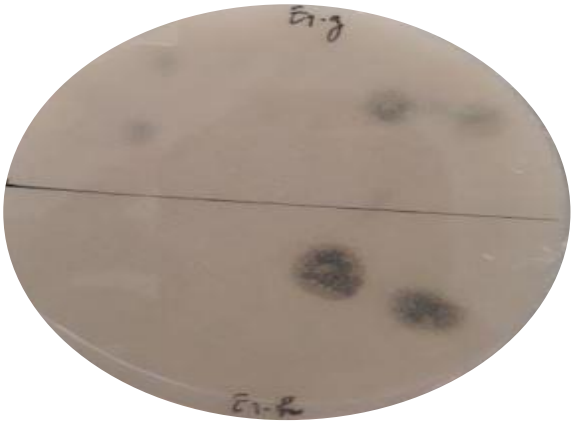
AIA production



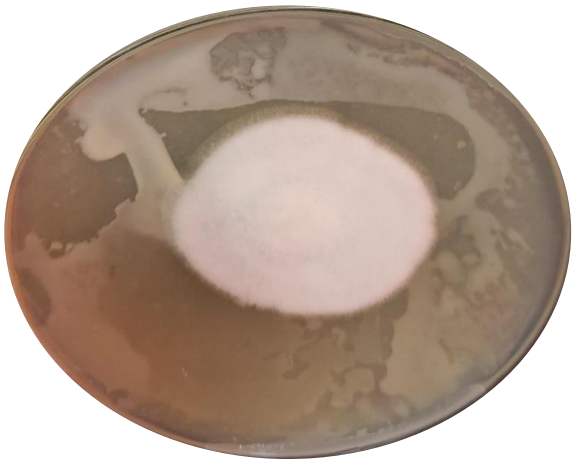
Nitrogen fixation



Siderophores production



Zinc solubilisation



Antifungal activity



P solubilization



Biochemical identification



Laboratory practices



Grain inoculation experiment

Résumé

Le phosphore est majoritairement présent dans le sol sous des formes insolubles et non disponible pour la plante. Le développement d'inoculant microbiens contenant des bactéries solubilisant le phosphate (PSB) est une solution biologique pour améliorer la disponibilité du phosphate (P) dans la rhizosphère. L'objectif de cette thèse est d'isoler, d'identifier et de caractériser les PSB de la rhizosphère de légumineuses au niveau de quatre sites de la région Fès-Meknès, en mettant en évidence la symbiose plante-PSB et en expliquant la contribution de ces rhizobactéries à réduire la dépendance aux engrais phosphatés et à stimuler la croissance des plantes.

Les conditions environnementales des sites étudiés exercent une forte pression sélective sur les communautés de PSB et sur leurs propriétés, tels que la capacité de solubiliser le P. L'identification classique des souches isolées a révélé neuf genres bactériens, dont *Bacillus spp*., *Pseudomonas spp*. et *Rhizobium spp*. La solubilisation moyenne du P rocheux et du P tricalcique par ces PSB atteint 40,43 $mg.L^{-1}$ et 94,54 $mg.L^{-1}$, respectivement.

Les souches isolées améliorent l'activité de solubilisation du P dans un milieu NBRIP avec une combinaison (RP/TCP) et augmentent ça solubilisation de 20,58% par rapport au témoin, où la souche WJEF63 montre la concentration de P soluble la plus efficace (102,69 $mg.L^{-1}$). Parmi toutes les souches isolées, douze présentent une résistance significative aux conditions stressantes (Température, pH, sécheresse et salinité) associés à une activité antifongique allant jusqu'à 49,15% d'inhibition de la croissance mycélienne d'*Aspergillus niger*, *Fusarium culmorum* et *Rhizopus stolonifer*.

L'inoculation des graines de *Vicia faba* et de *Pisum sativum* par ces PSB dans des conditions contrôlées et supplémentées du RP augmentent la teneur en N, en P, le poids frais et le poids secs des plantes testées de 67,50 %, 23,11 %, 56,6% et 42,28%, respectivement. La phosphatase alcaline, la respiration basale du sol et l'activité β-glucosidase ont augmenté de 45.46%, 67.5% et 66.12%, respectivement. En outre, les souches de PSB utilisées présentent plusieurs activités dont la fixation de N, la production d'auxines et des sidérophores.

L'application de ces PSB sous forme de consortium stimule le développement de *Vicia faba* et de *Pisum sativum*, améliore la qualité de sol et augment le rendement de plantes étudiées de 20.85% et 13.41%, respectivement, dans des conditions de stress hydrique et salin. De plus, ces souches sont capables de former un biofilm, présentent une activité antioxydant et tolèrent la salinité et la sécheresse. L'identification moléculaire (ARNr 16S) a permis d'identifier ces souches comme étant des *Pseudomonas spp*. et des *Bacillus spp*.

Les PSB étudiées montrent un potentiel bénéfique en tant que rhizobactéries promotrices de la croissance. Les retombés de ce projet de thèse fournissent une méthode pratique pour augmenter la mobilisation du P dans le sol et la croissance des plantes hôtes.

Mots clés : Maroc ; Phosphate ; PSB ; Légumineuses ; Agronomie ; Biotechnologie ; Développement durable.