ROYAUME DU MAROC
Université Mohammed V
- RABAT -
جامعة محمد الخامس
– الرباط –
Faculté des sciences
كلية العلوم

CENTRE D'ETUDES DOCTORALES - SCIENCES ET TECHNOLOGIES

N° d'ordre 3493

THESE

En vue de l'obtention du : ***DOCTORAT***

Structure de Recherche : *Biotechnologies Végétale et Microbienne, Biodiversité et Environnement*
Discipline *: Biologie*
Spécialité : *Biotechnologie végétale*

Présentée et soutenue le 09/07/2021 par :

Mutale Joan CHANDA

Microalgues et cyanobactéries comme sources de biostimulants de la croissance et de la tolérance au stress chez la tomate (Solanum lycopersicum L.) : approches moléculaires, métaboliques et biochimiques

JURY

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Année Universitaire : 2020-2021

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DEDICACE

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RESUME

Les microalgues et les cyanobactéries sont une source prometteuse de nouveaux biostimulants due à leur capacité de produire une variété des molécules biologiquement actives. Cette étude met en évidence l'effet des extraits des microalgues et des cyanobactéries sur les voies moléculaires, métaboliques et biochimiques liées à la croissance et la tolérance de la tomate (*Solanum lycopersicum* L.). Le traitement de la tomate avec les extraits des microalgues et des cyanobactéries a significativement amélioré la teneur en chlorophylle dans les feuilles, le poids sec (aérienne et racinaire), la croissance racinaire et l'absorption racinaire des nutriments (azote, phosphore et potassium), par rapport aux plantes non traitées. Les plantes traitées ont démontré une accumulation plus élevée des acides gras C16 et C18 (indiquant une stimulation de la synthèse lipidique de novo), et d'autres métabolites lipophiles essentiels dans la biosynthèse des vitamines (la pyridine-3-carboxamide), et des hormones (l'acide linolénique, le précurseur clé dans la voie de la biosynthèse des jasmonates). Les extraits des microalgues et des cyanobactéries ont stimulé la croissance et la tolérance de la tomate dans les conditions du stress salin modéré par l'amélioration de l'activité des enzymes antioxydantes et l'absorption des nutriments, notamment le K^+, dont l'accumulation foliaire a induit l'augmentation du rapport K^+/Na^+ cytosolique. Les extraits ont également augmenté l'efficacité de l'absorption du P par la stimulation de la croissance racinaire. Une faible expression des gènes codant les protéines transporteurs du phosphate a été détectée dans les racines des plantes traitées soumises à l'engrais TSP (0,3 mM et 0,6 mM de P).

Mots clés : Microalgues, cyanobactéries, biostimulants, *Solanum lycopersicum* L, croissance, stress abiotique.

ABSTRACT

Microalgae and cyanobacteria are a promising source of new biostimulants, due to their ability to produce a variety of biologically active molecules. This study investigates the biostimulant properties of microalgae and cyanobacteria extracts as enhancers of molecular, metabolic and biochemical mechanisms related to the growth and stress tolerance of tomato plants (*Solanum lycopersicum* L.). Treatment of tomato plants with microalgae and cyanobacteria extracts significantly improved chlorophyll concentrations in leaves, as well as the root length, nutrient uptake (nitrogen, phosphorus and potassium) and shoot and root dry weights of treated plants compared with the control. Treated plants accumulated higher levels of C16 and C18 fatty acids (indicating improved *de novo* lipid synthesis), and of other lipophilic metabolites essential in the biosynthesis of vitamins (pyridine-3-carboxamide), and hormones (linolenic acid, a key precursor in the biosynthetic pathway of jasmonates). Microalgae and cyanobacteria extracts also enhanced antioxidant enzyme activities and NPK absorption in plants subjected to low and moderate salinity. Enhanced K^+ uptake and leaf accumulation in treated plants improved their cytosolic K^+/Na^+ ratio and tolerance to low and moderate salinity. Microalgae and cyanobacteria extracts also enhanced P-absorption efficiency by stimulating root growth, resulting in increased root surface area for P absorption. Low level expression of genes encoding phosphate transporter proteins in roots was also detected in treated plants subjected to TSP fertilizer (0.3 mM and 0.6 mM P).

Key words: Microalgae, cyanobacteria, biostimulants, *Solanum lycopersicum* L, plant growth, abiotic stress.

ABREVIATIONS

ABA - Acide abscissique

ADN - Acide DésoxyriboNucléique

ARN - Acide RiboNucléique

BRs - Brassinostéroïdes

Ca - Calcium

$CaCO_3$ - Calcium carbonate

Car - Carotenoids

CAT - Catalase

$CHCl_3$ - Chloroform

Chl a - Chlorophyll a

Chl b - Chlorophyll b

CK - Cytokinines

$CuCl2\_2H_2O$ - Copper (II) chloride dihydrate

EPS - Exopolysaccharides

ER - Endoplasmic reticulum

ET - Ethylène

GA - Gibbérellines

GC-MS - Gas Chromatography-mass spectrometry

H_2O_2 - Hydrogen Peroxide

H_2SO_4 - Sulfuric acid

H_3BO_3 - Boric acid

HKT - High-affinity Potassium transporter

IAA - Acide indole acétique

JA - Acide jasmonique

K - Potassium

K_2HPO_4 - Potassium Phosphate dibasic

K_2SO_4 - Potassium sulfate

KH_2PO_4 - Potassium phosphate monobasic

KNO_3 - Potassium nitrate

LOX - Lipoxygénase

MAMPs - Microbial-associated molecular patterns

MAPK - Mitogen-activated protein kinase

MDA - Malondialdehyde

$Mg\ (NO_3)_{26}H_2O$ - Magnesium nitrate hexahydrate

$MnCl_2\_4H_2O$ - Manganese (II) chloride tetrahydrate

N - Azote

Na - Sodium

Na_2SO_4 - Sodium sulfate

NaCl - Sodium Chloride

NAD+ - Nicotinamide adenine dinucleotide

$(NH_4)_6Mo_7O_{24}\ 4H_2O$ - Ammonium molybdate tetrahydrate

NH_4NO_3 - Ammonium nitrate

NO_3 - Nitrate

NPK - Azote, Phosphore et Potassium

P - Phosphore

PAL - Phenylalanine ammonia lyase

PBR - photobioréacteurs

PCA - Principal Component Analysis

PGPB - Plant Growth Promoting Bacteria

PGPR - Plant Growth-Promoting Rhizobacteria

PHT - Phosphate transporter

POD - Peroxydase

PR - Pathogenesis-related proteins

PUFAs - Polyunsaturated fatty acids

ROS - Espèces réactives de l'oxygène

SA - Acide salicylique

SFA - Saturated Fatty Acids

SLs - Strigolactones

SOD - Superoxyde dismutase

SOS - Salt overly sensitive pathway

UFA - Unsaturated Fatty Acids

VLCFA - Very Long Chain Fatty Acids

$ZnCl_2$ - Zinc chloride

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AVANT PROPOS

Cette étude a été réalisée dans le cadre d'une collaboration entre le Laboratoire de Microbiologie et Biologie Moléculaire de la Faculté des Sciences de Rabat (Université Mohamed V) et le Centre de Biotechnologie Verte de la Fondation MAScIR. Le travail a été réalisé sous l'encadrement scientifique conjoint de Pr. Laila SBABOU, Professeur habilité à la Faculté des Sciences, Université Mohammed V- Rabat, et de Dr. Hicham EL ARROUSSI du centre de biotechnologie végétale de la fondation MAScIR-Rabat.

Ce travail a été réalisé dans le cadre d'un programme de recherche sur les biostimulants à base de microalgues coordonné par MAScIR en collaboration avec des partenaires industriels et académiques nationaux et internationaux. Ce programme vise le développement de biostimulants à base de microalgues pour l'amélioration de la croissance, de la productivité, de l'assimilation des engrais et de la santé des plantes.

PUBLICATIONS

- **Chanda, Mutale-joan**, Nawal Merghoub, and Hicham EL Arroussi. "Microalgae polysaccharides: the new sustainable bioactive products for the development of plant bio-stimulants?". *World Journal of Microbiology and Biotechnology* 35, no. 11 (2019): 1-10.
- **Chanda, Mutale-Joan**, Benhima Redouane, Elmernissi Najib, Kasmi Yassine, Karim Lyamlouli, Sbabou Laila, Youssef Zeroual and Hicham EL Arroussi. "Screening of microalgae liquid extracts for their bio stimulant properties on plant growth, nutrient uptake and metabolite profile of Solanum lycopersicum L.": *Scientific reports* 10, no. 1 (2020): 1-12.
- **Chanda, Mutale-joan,** Farid Rachidi, Hachimi Alaoui Mohamed, Najib El Mernissi, Abderrahim Aasfar, Mustapha Barakate, Danouche Mohammed, Laila Sbabou, and Hicham El Arroussi. "Microalgae-cyanobacteria–based biostimulant effect on salinity tolerance mechanisms, nutrient uptake, and tomato plant growth under salt stress." *Journal of Applied Phycology (2021): 1-17.*
- **Chanda, Mutale-joan**, Farid Rachidi, Najib El Mernissi, Abderrahim Aasfar, Hicham El Hadi, Laila Sbabou, Karim Lyamlouli, *Hicham El Arroussi. "Metabolic and transcriptomic study of *Aphanothece* sp. biostimulant effects on plant growth and phosphorus acquisition traits in Tomato plants (*Solanum lycopersicum* L.)".
- **Chanda, Mutale-joan**, Laila Sbabou and EL Arroussi Hicham. "Microalgae and cyanobacteria: How exploiting these microbial resources can address the underlying challenges related to food sources and sustainable agriculture. A review". **Soumis** : *Journal of plant growth regulation* SPRINGER

COMMUNICATIONS

- **Effect of microalgae polysaccharides on the metabolic and biochemical pathways involved in plant defense against biotic and abiotic stress.**
  MICROBIO congress, April 24-26, 2019, Agadir, Morocco.
- **Screening of microalgae liquid extracts for their bio stimulant properties on plant growth, nutrient uptake and metabolite profile of *Solanum lycopersicum* L.** POSTER
  Biostimulant World Congress, November 18- 21, 2019, Barcelona, Spain.
- **Microalgae polysaccharides as bio-elicitors of biochemical and metabolic pathways related to plant defense in *Solanum lycopersicum* L.**
  PLANT BIOPROTECH, November 19-22, 2019, Marrakech, Morocco.
- **Microalgae extracts and their biostimulantory effects on biochemical and metabolomic pathways related to plant defense in *Solanum lycopersicum* L.**
  Doctoriales BioBio, December 25-26, 2018, Faculty of Sciences Rabat-Agdal.

INTRODUCTION

La régulation de la croissance et le développement des plantes, et la mitigation des effets négatifs de stress environnementaux sont des facteurs importants déterminant la productivité des plantes. Les stress biotiques et abiotiques empêchent essentiellement tous les systèmes de culture d'atteindre leur potentiel de rendement. Les conditions de croissance des plantes peuvent être optimisées par l'application adéquat de l'eau, des nutriments et des régulateurs de croissance des plantes tels que les rhizobactéries promotrices de la croissance des plantes (PGPR), les phytohormones, les strigolactones etc. En plus de ces approches traditionnelles, les biostimulants sont de plus en plus intégrés dans les systèmes de production dans le but de modifier les processus physiologiques des plantes pour optimiser leur productivité. Les biostimulants sont des produits dont la fonction est d'améliorer une ou plusieurs des caractéristiques suivantes de la plante ou de la rhizosphère végétale : i) l'efficacité d'utilisation des nutriments, ii) la tolérance au stress abiotique, iii) les traits de qualité, ou iv) la disponibilité de nutriments confinés dans le sol ou la rhizosphère (Ricci 2020). Ils sont aussi définis comme des fertilisants dont la fonction est de stimuler les processus de nutrition des plantes, indépendamment de leur teneur en nutriments (Ricci 2020). Plusieurs produits ou substances biologiques classés comme biostimulants ont la capacité de déclencher des réactions métaboliques, favorisant la croissance et la réponse des plantes aux stress abiotiques (Du Jardin 2015; Yakhin et al., 2017). Les biostimulants sont classés en différentes catégories qui sont : les hydrolysats de protéines et d'autres composés contenant l'azote, les polysaccharides, les composés inorganiques comme le sélénium, les microorganismes, les acides humiques et fulviques et les extraits d'algues. Parmi les nombreuses catégories de biostimulants des plantes, les algues sont largement exploitées et représentent une catégorie importante de biostimulants organiques, comparé aux microalgues (Khan et al. 2011; Battacharyya et al. 2015; Colla et al. 2017).

Les microalgues et les cyanobactéries gagnent maintenant en popularité en tant que ressources bioactives renouvelables pouvant être exploitées en agriculture pour produire des biostimulants. Les microalgues (eucaryotes) et les cyanobactéries (procaryotes) sont des organismes photosynthétiques microscopiques unicellulaires qui poussent dans divers habitats aquatiques et même dans des sols humides. Ils ont suscité un intérêt considérable dans les communautés scientifiques et industrielles pour leur capacité à produire une diversité de molécules biologiquement actives telles que les polysaccharides sulfatés, les osmolytes, les phytohormones, les acides aminés et les composés phénoliques (Cuellar-Bermudez et al. 2015; de Morais et al. 2015; Renuka et al. 2018). Au cours des dernières années, de nombreuses études ont été menées pour mettre en évidence les propriétés biostimulantes des extraits de différentes espèces de microalgues et de cyanobactéries sur un large éventail d'activités biologiques chez les plantes supérieures, y compris l'absorption des nutriments et la tolérance au stress abiotique (de Morais et al. 2015; EL Arroussi et al. 2016, 2018; Garcia-Gonzalez and Sommerfeld 2016; Barone et al. 2018; Farid et al. 2019).

Pourquoi la tomate ?

La tomate (*Solanum lycopersicum* L.), un membre des Solanacées, est l'une des plantes maraîchères les plus importantes au monde avec une grande valeur économique mondiale (Kimura and Sinha 2008). La tomate est cultivée dans plus de 140 pays. Le Maroc est l'un des plus grands exportateurs mondiaux de la tomate, générant environ 5 milliards de dollars d'exportations en 2016 (Carbonell et al. 2019). La tomate produit des fruits charnus importants pour l'alimentation humaine en tant que source de vitamines et de carotènes tels que le bêta-carotène et le lycopène (Canene-Adams et al. 2005). La tomate n'est pas seulement une plante agricole majeure à l'échelle mondiale, mais aussi un organisme modèle pour les recherches physiologiques et moléculaires, basées sur son génome séquencé et bien étudié (Sato et al. 2012; Gupta et al. 2020). Elle est particulièrement étudiée comme un organisme modèle pour le développement des fruits charnus (Hyojin et al. 2017). La compréhension des réponses de la tomate face aux extraits de microalgues en tant que stimulants de croissance et l'identification des voies améliorant sa productivité est d'une importance primordiale pour l'agriculture de la tomate.

Objectif du travail :

Cette étude vise à mettre en évidence le potentiel des microalgues et des cyanobactéries en tant que ressources bioactives idéales pour le développement de produits agricoles innovants. Le traitement des plantes avec les extraits de microalgues ou de cyanobactéries vise à améliorer la défense, la tolérance, la croissance et le développement des plantes via la stimulation des voies moléculaires, métaboliques et biochimiques liées à la croissance et la tolérance de la plante.

L'étude est faite sur la tomate (*Solanum lycopersicum* L) ; en tant que plante modèle optimale pour étudier différents aspects de la physiologie végétale.

Volets d'étude :

i. Les polysaccharides de microalgues en tant que nouveaux produits bioactifs durables pour le développement de biostimulants de plantes.
ii. Le criblage d'extraits liquides de microalgues pour leurs propriétés biostimulantes de la croissance des plantes, l'absorption des nutriments et le profil des métabolites de *Solanum lycopersicum* L.
iii. L'effet du biostimulant à base des microalgues et cyanobactéries sur les mécanismes de tolérance de la tomate au stress salin.
iv. L'étude métabolique et transcriptomique des effets d'extraits d'*Aphanothece* sp. sur la croissance et les traits d'acquisition du phosphore chez la tomate (*Solanum lycopersicum* L.)
v. Les microalgues et les cyanobactéries : comment l'exploitation de ces ressources microbiennes peut permettre de faire face aux défis sous-jacents liés aux sources alimentaires et à l'agriculture durable

CHAPITRE I

REVUE BIBLIOGRAPHIQUE

1. Les Biostimulants des plantes

1.1 Définition

Les Biostimulants sont des produits naturels ou synthétiques qui peuvent être appliqués au niveau de la graine, la plante ou le sol. Ces produits provoquent des changements dans les processus vitaux et structurels afin d'influencer la croissance et le rendement des plantes, et/ou leur tolérance aux stress abiotiques. De plus, les biostimulants réduisent le besoin en produits agricoles conventionnels (Calvo et al. 2014; Du Jardin 2015). Les biostimulants, qui diffèrent des fertilisants traditionnels, peuvent contenir dans leur formule une variété de composés organiques, tels que les vitamines, les acides aminés, les phytohormones, de l'acide ascorbique et d'autres produits, qui peuvent varier selon leur fabricant ou marque.

Selon Yakhin et al. (2017), un biostimulant pourrait être un produit formulé d'origine biologique qui améliore la productivité des plantes grâce à l'action d'un ensemble de constituants complexes et bioactifs du produit, et non pas une seule conséquence de la présence de nutriments essentiels des plantes, de régulateurs de croissance des plantes ou de composés protecteurs des plantes. La fonction biologique peut être modulée positivement par l'application de molécules ou de mélanges de molécules pour lesquelles un mode d'action explicite n'a pas été défini.

1.2 Effets des Biostimulants

Les effets des biostimulants sont divers. Ils peuvent agir sur la production des plantes comme une réponse directe des plantes à l'application du biostimulant. Un biostimulant appliqué au sol peut aussi améliorer le microbiome du sol avec des effets ultérieurs sur la productivité des plantes. Plusieurs recherches ont été développées afin d'évaluer les effets des biostimulants dans l'amélioration de la croissance des plantes soumises à des stress abiotiques (El Arroussi et al. 2018; Barone et al. 2018; de Morais et al. 2015; EL Arroussi et al. 2016; Garcia-Gonzalez and Sommerfeld 2016). Plusieurs effets ont été décrits à savoir l'induction de la croissance racinaire et l'amélioration de l'absorption des nutriments. De plus, les biostimulants influencent positivement l'activité et l'expression de gènes codant les enzymes agissant dans le métabolisme primaire et secondaire de la plante (Nardi et al. 2016).

2. Classification des Biostimulants

De nombreux biostimulants ont été classés en groupes et catégories complètement divergents en fonction de leur utilisation et de leur type d'activité. Par exemple, les produits à base d'acide humique sont souvent décrits comme des amendements à la santé du sol, tandis que les rhizobactéries promotrices de la croissance des plantes (PGPRs) pourraient être classées comme biofertilisants, phytostimulateurs et biopesticides (Martínez-Viveros et al. 2010; Bhattacharyya and Jha 2012). Les biofertilisants peuvent aussi être une sous-

catégorie de biostimulants (Du Jardin, 2015). Les extraits d'algues et des microorganismes ont également été décrits comme des biofertilisants (Zodape 2001; Malusá et al. 2012; Bhardwaj et al. 2014; Malusá and Vassilev 2014). De plus, certains éléments inorganiques qui ne sont pas connus pour être essentiels aux plantes peuvent également être classés comme biostimulants s'ils sont capables d'améliorer ou promouvoir la croissance des plantes (Kleiber and Markiewicz 2013; Radkowski and Radkowska 2013). Par exemple, Thao and Yamakawa, (2009), considèrent les phosphites comme des biostimulants, car la réponse des plantes aux phosphites ne peut souvent pas être expliquée par la fonction antifongique connue de ces molécules.

Les biostimulants pourraient être regroupés sur la base de formulations à un ou plusieurs composants et peuvent être classés selon l'origine et le mode d'action de l'ingrédient actif (Basak, 2008). Du Jardin.(2015), a élaboré une justification scientifique de la classification de biostimulants en 7 catégories. Plus tard, Bulgari et al. (2015), ont proposé une classification des biostimulants sur la base de leur mode d'action plutôt que sur leur composition.

Tableau 1 : *Les catégories des biostimulants et leurs descriptions* (Du Jardin 2015).

	Catégorie	Description
1	Les acides humique et fulvique	Constituants naturels de la matière organique du sol, résultant de la décomposition des résidus végétaux, animaux et microbiens, ou de l'activité métabolique des microorganismes du sol utilisant ces substrats.
2	L'hydrolysats de protéines et autres composés contenant de l'azote	Les mélanges d'acides aminés et de peptides obtenus par l'hydrolyse chimique et enzymatique de protéines à partir de sous-produits agro-industriels provenant de sources végétales et de déchets animaux. D'autres molécules azotées comprennent les bétaïnes, les polyamines et les acides aminés non protéiques.
3	Les extraits d'algues ou des plantes	Les extraits totaux ou des composés purifiés d'algues, notamment les polysaccharides comme la laminarine, les alginates et les carraghénanes.
4	Le chitosane et autres biopolymères	Plusieurs poly - et oligomères d'origine biologique ou des variantes (hémi-) synthétiques, notamment les polysaccharides d'algues (déjà mentionnés) tel que la laminarine ou un glucane de stockage d'algues brunes.
5	Les composés inorganiques	Les éléments chimiques appelés « éléments bénéfiques » qui

		favorisent la croissance des plantes et peuvent être essentiels à des taxons particuliers mais ne sont pas requis par toutes les plantes. Les cinq principaux éléments bénéfiques sont Al, Co, Na, Se et Si, présents dans les sols et dans les plantes sous forme de différents sels inorganiques et de formes insolubles comme la silice amorphe (SiO2.nH2O) chez les espèces de graminées.
6	Les champignons bénéfiques	Les champignons mycorhiziens : un groupe hétérogène de taxons qui établissent des symbioses avec plus de 90% de toutes les espèces végétales.
7	Les bactéries bénéfiques	Deux types principaux doivent être considérés selon la diversité taxonomique, fonctionnelle et écologique : (i) les endosymbiontes mutualistes du type Rhizobium et (ii) les PGPRs mutualistes et rhizosphériques. Les rhizobiums et les taxons apparentés sont aussi commercialisés comme des biofertilisants.

2.1 Les modes d'application

Il existe divers modes d'application des biostimulants, notamment le traitement foliaire par pulvérisation, le traitement du sol par irrigation et le traitement des graines. La méthode consistant à tremper les graines dans le biostimulant avant le semis a été utilisée pour accélérer la germination. Cette technique peut être réalisée en utilisant des extraits d'algues à de faibles concentrations. Le traitement des grains stimule la croissance, la teneur en chlorophylle et les enzymes de la défense des plantules (Kumar and Sahoo 2011). Le traitement foliaire est le plus connu parmi les modes d'application. Plusieurs études ont montré la performance de l'application foliaire des biostimulants d'origine algale, sur la croissance, la composition minérale et biochimique et sur la stimulation de la défense naturelle des plantes (El Modafar et al. 2012; Shah et al. 2013). En outre, l'application directe des biostimulants au niveau du sol, peut généralement améliorer la structure du sol et la flore microbienne, ainsi que stimuler le développement racinaire des plantes, favorisant l'absorption des nutriments.

2.2 Le réglementations des biostimulants

L'enregistrement des produits utilisés dans l'agriculture est crucial pour garantir leur application pratique, sûre et légitime. En l'absence d'une définition solide des biostimulants en tant que groupe distinct de produits (Basak 2008), la procédure d'enregistrement ainsi que le régime de classification sont intenables,

ce qui crée inévitablement un obstacle au commerce et au développement. Divers pays, États et régions administratives ont élaboré différentes catégories pour l'enregistrement des biostimulants potentiels, y compris des terminologies telles que : *plant conditioners, supplements, soil improvers, plant enhancers, phytofortifiers*, etc. (Basak 2008; Traon et al. 2014; Yakhin et al. 2017). Dans de nombreuses administrations, les pratiques réglementaires exigent une description détaillée et une identification des substances dans toutes les classifications de produits commerciaux, tandis que dans d'autres, l'enregistrement des substances non entièrement identifiées est autorisé si ces produits sont considérés comme un mélange complexe de substances chimiques (Traon et al. 2014). Si nous acceptons le concept selon lequel un biostimulant est un produit à bénéfice clair mais dont le mode d'action est inconnu, alors il ne peut être réglementé que par sa sécurité et sa preuve d'efficacité. En réalité, plusieurs biostimulants sont à nature complexe et multi composante. Cette nature complexe complique clairement la découverte de leurs modes/mécanismes d'action, de production, d'enregistrement et d'utilisation de nombreux biostimulants. Ce qui est clairement nécessaire cependant, c'est un mécanisme de réglementation pour s'assurer que les produits sont "généralement reconnus comme sûrs", ont "un avantage positif sur la productivité agricole" et sont distincts des catégories de produits existantes. La tâche d'identification de la fonction et de l'utilité agronomique peut alors être poursuivie indépendamment et sera motivée par l'impératif du marché pour la qualité et la cohérence des produits. La coordination de la législation nationale dans ce cadre deviendra essentielle pour l'optimisation des biostimulants et le commerce entre les différents pays (Yakhin et al. 2017).

3. Les microalgues et les cyanobactéries comme source des biostimulants

Les microalgues et les cyanobactéries sont des microorganismes qui habitent dans divers écosystèmes aquatiques et terrestres et sont capables de produire une grande variété de substances uniques et bioactives (Rajvanshi and Sharma 2012). Les microalgues et les cyanobactéries effectuent le processus d'oxydation de l'eau en oxygène moléculaire et la réduction du dioxyde de carbone en composés organiques (Tamagnini et al. 2002), un processus très exigeant en énergie connu sous le nom de photosynthèse oxygénique. La photosynthèse oxygénique est apparue pour la première fois chez les ancêtres des cyanobactéries actuelles il y a plus de 3,7 milliards d'années. Les cyanobactéries sont considérées parmi les formes de vie les plus anciennes sur terre et sont les producteurs originaux de l'atmosphère oxygénée de la Terre (Chauvat and Cassier-Chauvat 2012; Saad and Atia 2014). Une association étroite de cyanobactéries avec des algues vertes, des plantes vertes et d'autres organismes est née d'un événement précoce d'endosymbiose il y a plus de 1,2 milliard d'années, où une cyanobactérie a été absorbée par un organisme hétérotrophe. Cet événement explique l'origine des chloroplastes chez les végétaux.

Les scientifiques ont d'abord échoué à reconnaître adéquatement les cyanobactéries comme procaryotes

puisque l'identification traditionnelle se basait sur la morphologie cellulaire. Ainsi, ces micro-organismes étaient à l'origine classés comme algues bleu-vert (Cyanophyta) sous des codes botaniques (Oren 2014; Demoulin et al. 2019) jusqu'à ce que leurs caractéristiques procaryotes soient établies dans les années 1960. Une proposition a ensuite été faite en 1978 pour inclure les cyanobactéries dans le code bactériologique (Stanier et al. 1978). Ainsi, les cyanobactéries sont classées comme procaryotes, dans la division eubactéries. Les vues microscopiques de cyanobactéries et de microalgues sont présentées dans la **Figure 1**.

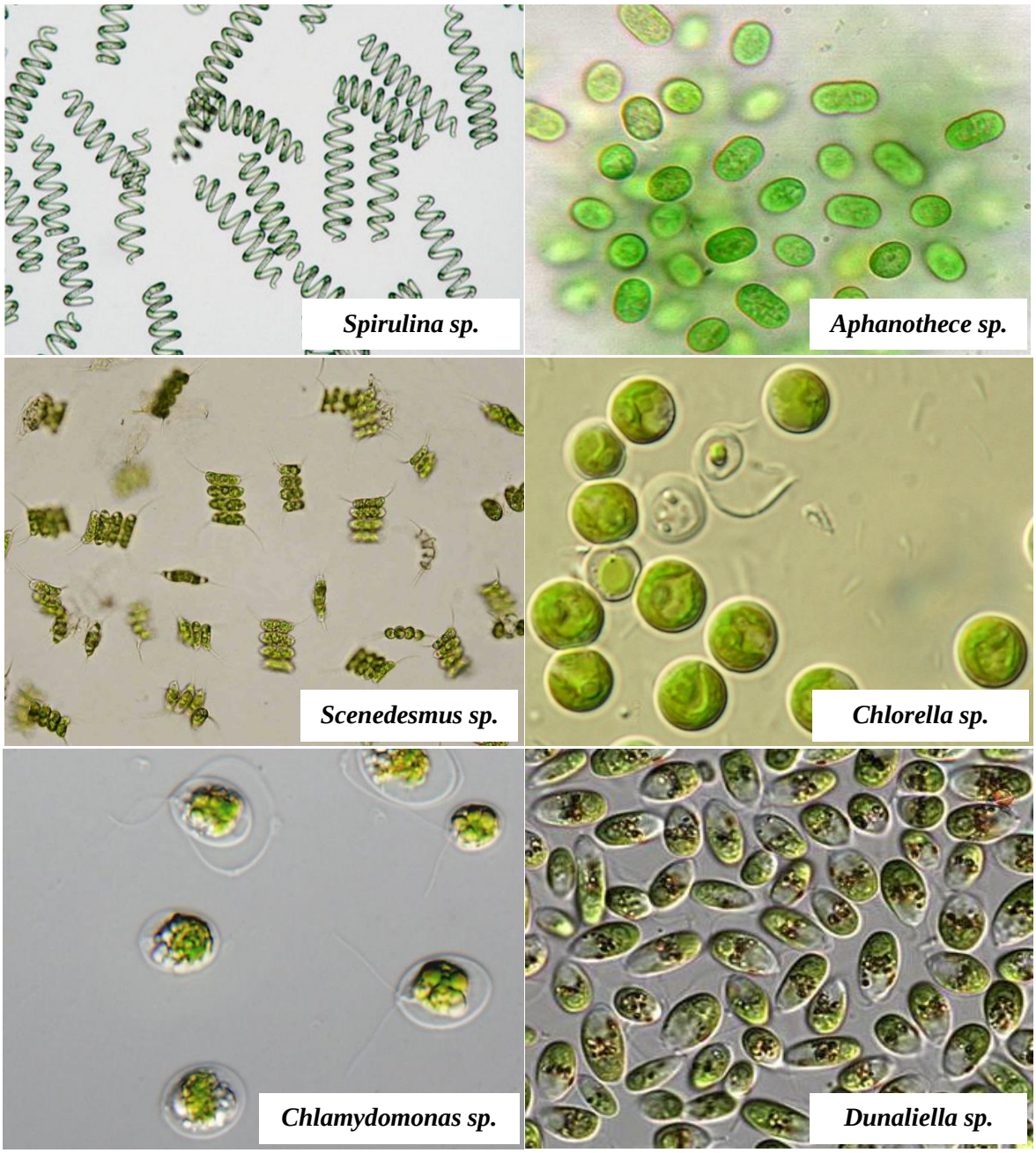

Figure 1 : *Vue microscopique de deux cyanobactéries (Spirulina sp et Aphanothece sp.) et quatre microalgues (Scenedesmus sp., Chlorella sp., Chlamydomonas sp. et Dunalilla sp.)*
***Sources d'Images** : diet-health.info/en/recipes/ingredients/-spirulina, cfb.unh.edu/Ralf Wagner, Denmark, Michael Hanophy-Pinterest, toddcaldecott.com/herbs/chlorella/, ccala.butbn.cas.cz/en/chlamydomonas-dorsoventralis-pascher et cfb.unh.edu Dunaliella sp. tropicalfish.it, respectivement.*

Les phytoplanctons, comme les microalgues et les cyanobactéries, fournissent à la fois de la nourriture et de l'oxygène à de nombreuses espèces du milieu aquatique. Leur capacité à atténuer le CO_2 par photosynthèse oxygénique est plus écologique et durable que l'élimination chimique/physique du CO_2

(Zhang 2015).

Depuis de nombreuses années, ces micro-organismes aquatiques sont une source de molécules très précieuses telles que les polysaccharides, les protéines, les PUFAs (acides gras polyinsaturés), les polyols et les antioxydants (Lordan et al. 2011; Ibañez et al. 2012). En raison de leurs diverses activités biologiques (anticancéreux, antioxydant, anti-hypertension, immunomodulateur et prévention des maladies cardiovasculaires), ces composés trouvent de nombreuses applications dans les industries alimentaire, pharmaceutique et cosmétique (Spolaore et al., 2006).

De nombreuses espèces de microalgues et de cyanobactéries (*Cyanidium* sp., *Chlorococcum littorale*, *Euglena gracilis*, *Arthrospira* sp., les diatomées et les espèces de Chlorelles) pourraient être utilisées pour la bio-fixation du CO_2 (Ono and Cuello 2003; Hopkinson et al. 2011; Johnson et al. 2013) et comme sources d'énergie - bioéthanol, biohydrogène et biodiesel - (Chisti 2007; Rodolfi et al. 2009; Hannon et al. 2010; Slade and Bauen 2013). Ils peuvent également permettre la récupération du phosphore et de l'azote des eaux usées, et l'utilisation du CO_2 des usines (par exemple des gaz de combustion). Le CO_2 converti en biomasse microalgale pourrait ensuite être utilisé pour produire des produits agricoles de valeur commerciale tels que des biostimulants des plantes et des biofertilisants.

En raison de la grande diversité des produits qui peuvent être développés à partir de leur biomasse, les microalgues et les cyanobactéries sont la clé aux défis accompagnant la production alimentaire et les problèmes émergents dans l'agriculture durable (Stephens et al. 2013; Saifullah et al. 2014).

3.1 Culture de microalgues et de cyanobactéries

La culture des microalgues et des cyanobactéries peut être photoautotrophe, hétérotrophe ou mixotrophe. La production photoautotrophe est caractérisée par une photosynthèse autotrophe dans laquelle la lumière est utilisée comme la seule source d'énergie, alors que des substances organiques telles que le glucose sont nécessaires dans le métabolisme hétérotrophe pour stimuler la croissance. Dans la culture mixotrophe, les microorganismes combinent à la fois la photosynthèse autotrophe et l'assimilation hétérotrophe des composés organiques (Chojnacka and Noworyta 2004; Chojnacka and Zielińska 2012). Cependant, dans la nature, toutes les cyanobactéries sont essentiellement des photoautotrophes oxygénés, à l'exception possible de leur capacité de photosynthèse anoxygénique facultative (Garcia-Pichel 2009).

Dans cette étude, nous nous concentrons particulièrement sur la culture photoautotrophe des microalgues et des cyanobactéries. Jusqu'à présent, des études ont montré que les microalgues et les cyanobactéries sont des microorganismes photosynthétiques oxygénés qui nécessitent essentiellement de la lumière, du CO_2 et du H_2O pour leur culture. D'autres nutriments nécessaires à la croissance et à la fonction cellulaire sont également ajoutés au milieu de culture. La culture de photoautotrophes oxygénés à grande échelle est étudiée depuis plusieurs décennies (Lee 1997). La première culture en laboratoire des microalgues a été

réalisée sur *Chlorella vulgaris* dans le but d'étudier sa physiologie (Beijerinck 1890).

Il est possible de classer la culture des microalgues à grande échelle en deux systèmes : les systèmes ouverts où la culture est directement exposée à l'air libre et les systèmes fermés où la culture est entièrement enfermée dans un photobioréacteur (Dormido et al. 2014). Ces systèmes de culture sont décrits ci-dessous, et une description comparative des systèmes fermés et ouverts en termes de productivité, de contamination, d'évaporation de l'eau et d'investissement dans les coûts est résumée dans le **tableau 2**. A part leur coût élevé d'installation, d'entretien et d'énergie, les photobioréacteurs sont les meilleurs systèmes de culture pour optimiser la production de culture et la qualité de la biomasse.

Table 2 : *La description comparative des systèmes fermés et ouverts.*

Facteur	Systèmes fermés (Photobioréacteurs)	Systèmes ouverts
Reproductibilité de la qualité de la biomasse	Reproductible	Variable
Espace requis	Grande surface	Modéré
Productivité	Élevé (en raison de paramètres de croissance hautement optimisés)	Faible (difficultés à réguler les paramètres de croissance)
Contrôle des paramètres de culture	Elevé	Faible
Risques de contamination	Elevé	Faible
Évaporation	Elevé	Faible
Coût d'investissement	Coûts élevés d'installation, d'entretien et d'apport d'énergie	Relativement bon marché

3.1.1 Les systèmes de culture ouverte

Les systèmes ouverts peuvent être divisés en bassins naturels et artificiels. Les bassins naturels comprennent les lacs eutrophes ou les petits bassins naturels, tandis que les bassins artificiels décrivent les bassins circulaires et les bassins « raceway » **Figure 2**.

3.1.1.1 Les bassins naturels

Les lacs eutrophes, les petits bassins naturels sont exploités pour la production de microalgues depuis de nombreuses années. La croissance des microalgues dans les étangs ouverts remonte à l'époque des Aztèques où, des milieux naturels avec des conditions climatiques appropriées et des nutriments suffisants favorisent la croissance des microalgues (Hamed 2016). Il est logique de suggérer que le terme "biomasse algale" aurait pu être en fait des cyanobactéries, car de nombreuses études antérieures n'ont pas séparé les cyanobactéries des microalgues en raison de leurs similitudes morphologiques. En fait, il a été rapporté que

les Aztèques récoltaient l'Arthrospira (spirulina), une souche de cyanobactéries, du lac Texcoco dans l'actuel Mexique (Borowitzka 2013; Chisti 2018). En Afrique, les lacs avec des compositions chimiques et des conditions de croissance appropriées favorisent la croissance de la spiruline, localement appelée « Dihé » par la population locale du Tchad (**Figure 2 A**).

La biomasse fleurit dans ces systèmes naturels hautement productifs, est récoltée et utilisée comme nourriture (Thein 1993; Abdulqader et al. 2000; Godinez et al. 2001; Batello et al. 2004). Des exemples de ces étangs naturels incluent également les proliférations du Lac Kossorom exploitées par le peuple Kanembu (Abdulqader et al. 2000) et du *Dunaliella salina* produites dans 2 lagunes en Australie: Hutt Lagoon (Australie Occidentale) et Whyalla (Australie Méridionale) (Tredici 2004).

3.1.1.2 Les bassins artificiels

La culture des algues dans les systèmes de production artificiels est utilisée depuis les années 1950 (Brennan and Owende 2010). Cependant, la production d'algues dans les systèmes ouverts pour la nourriture n'a commencé que dans les années 1970, en Europe de l'Est et au Japon (Ugwu et al. 2008; Costa and Morais 2013). Pendant ce temps, la culture en masse de microalgues pour la séquestration du CO_2 dans l'environnement a également été lancée au début de l'industrialisation au Carnegie Institute de Washington (Witsch and Harder 1953), puis des recherches approfondies ont finalement été menées pour cultiver des microalgues dans des systèmes de culture ouverte : bassins circulaires puis les bassins « Raceway».

a. Les bassins circulaires

Les bassins circulaires pourraient être les plus anciens systèmes de culture de microalgues à grande échelle. Ces systèmes auraient été utilisés il y a de nombreuses années au Japon, à Taïwan et en Indonésie pour la production de biomasse de *Chlorella* sp. (Lee 1997; Tredici 2004). Les bassins circulaires nécessitent une construction en béton coûteuse et un apport énergétique élevé pour mélanger le milieu de culture de microalgues par un bras rotatif monté au centre du bassin (**Figure 2 B**). Le coût de ces systèmes dans la production de microalgues a conduit au développement des bassins « raceway », comme une alternative réussie.

b. Les bassins raceway

Les bassins raceway sont les systèmes les plus utilisés pour la production commerciale de microalgues. Ils sont généralement les moins chers à construire et leur fonction est assez simple (Borowitzka 2013; Enzing et al. 2014). Ce sont des bassins de forme ovale constitués de canaux de recirculation, dans lesquels l'écoulement est guidé autour des coudes par des chicanes placées dans le canal d'écoulement. La culture est continuellement homogénéisée à l'aide d'une roue à aubes montée pour assurer l'homogénéisation de la culture et empêcher la sédimentation des cellules, comme illustré à la **Figure 3 B**. Les bassins raceway peuvent être construits en béton, ou en fibre de verre (Borowitzka 2013). Bien que les systèmes de culture

ouverte nécessitent des coûts d'investissement et d'exploitation minimes avec un besoin moindre en énergie pour le mélange des cultures, ils présentent quelques inconvénients : les systèmes ouverts nécessitent de grandes surfaces pour se développer et sont très sensibles aux contaminations par les moisissures, les bactéries, les protozoaires et la concurrence d'autres microalgues (Mendes et al. 2013). En outre, les conditions météorologiques défavorables deviennent critiques en raison du manque de contrôle des paramètres de croissance, y compris la température et l'évaporation du milieu de culture (Rupprecht 2009; Mata et al. 2010; Oyler 2011). Par conséquent, les systèmes fermés sont une meilleure alternative pour optimiser la production de microalgues et la qualité de la biomasse.

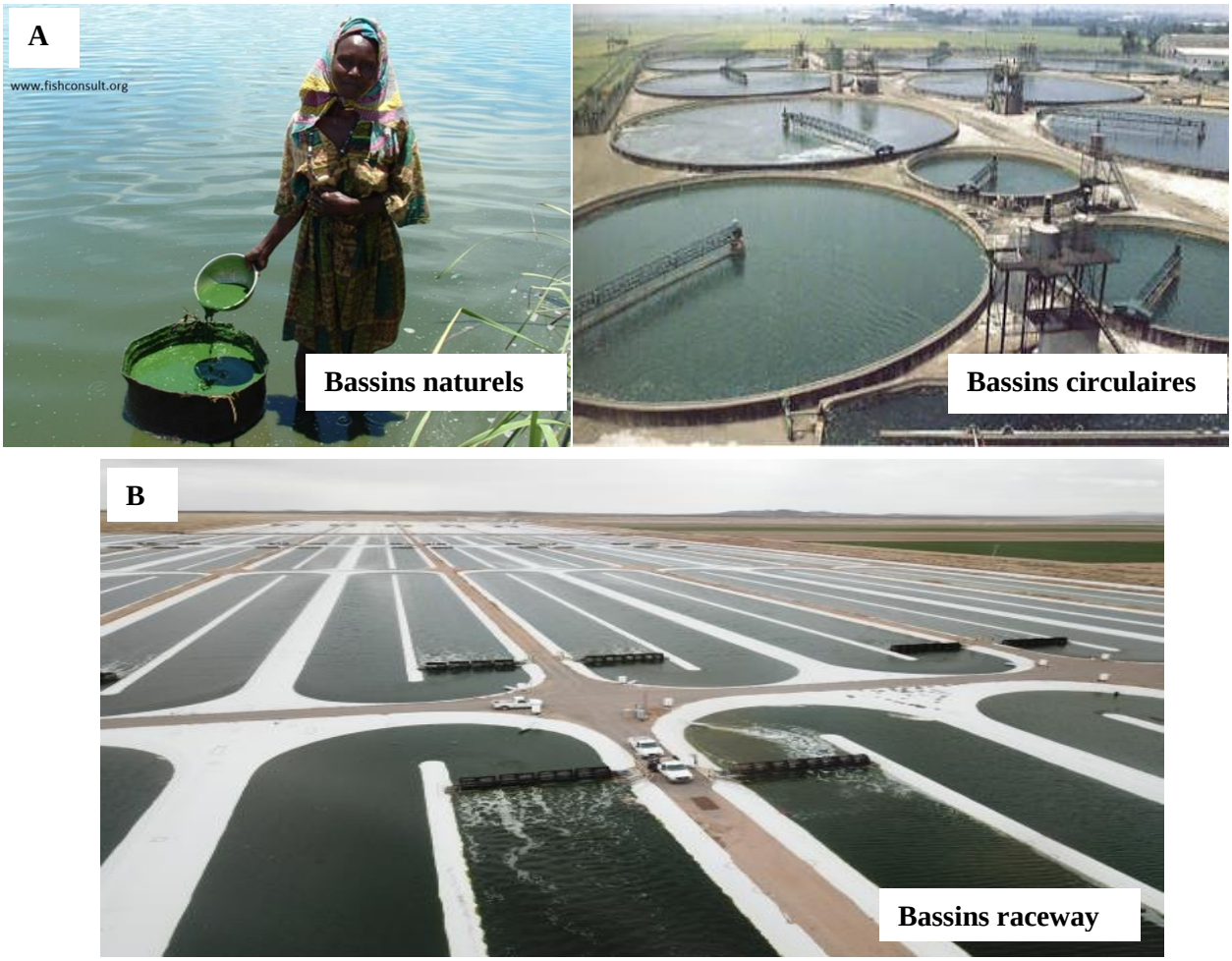

Figure 2 A : *Systèmes de cultures ouverts. Les lacs eutrophes ou les petits bassins naturels où pousse les cyanobactéries_* ***Sources d'Image****: www.fishconsult.com et les bassins circulaires artificiel* ***Source d'Image*** *: Elisa Allen, article making-biodiesel-books.com. B* **:** *Bassins raceway_Source d'Image : Qualitas, https://www.energy.gov/eere/bioenergy/algal-production*

3.1.2 Systèmes fermés (photobioréacteurs)

Les photobioréacteurs (PBR) sont des réacteurs qui offrent un environnement de culture fermée dans lequel des phototrophes sont cultivés ou utilisés pour effectuer des réactions photobiologiques (Tredici 2004). Les PBR permettent un meilleur contrôle des conditions de croissance de la culture, et surtout, ils sont protégés et relativement à l'abri de l'invasion par des microorganismes concurrents (Vishwanath Patil1 et al. 2005; Narala et al. 2016). Ils peuvent être situés à l'intérieur ou à l'extérieur, mais les emplacements extérieurs sous la lumière du soleil sont plus courants en raison de leur bas coût et de leur efficacité. Les PBR peuvent être classés en PBR tubulaires à plaques plates, verticales ou à colonnes (Dragone et al. 2010).

Les PBR à plaques plates sont quelques-unes des premières formes de systèmes fermés datant du début des années 1950. Ils sont composés de boîtes rectangulaires ouvertes à une extrémité. Ils ont des panneaux de grande surface en matériau transparent, généralement en verre ou en film optique pour une utilisation maximale de la lumière (Yen et al. 2013), ce qui est une raison pour laquelle ils reçoivent beaucoup d'attention (Brennan and Owende 2010). Aujourd'hui, la configuration tubulaire des PBR (**Figure 3 A et D**), est apparemment la plus courante, elle consiste en une série de tubes transparents en verre ou en plastique (polypropylène, acrylique ou polychlorure de vinyle) qui peuvent être alignés horizontalement, verticalement, inclinés ou en hélice. L'énergie solaire est absorbée par l'écran tubulaire (capteur solaire)

D'autres systèmes fermés comprennent des photobioréacteurs à colonne verticale composés de verres verticaux comme illustré à la **Figure 3 C**, ou de tubes acryliques. Le milieu de culture est agité par un bouillonnement conséquent (Carvalho et al. 2006).

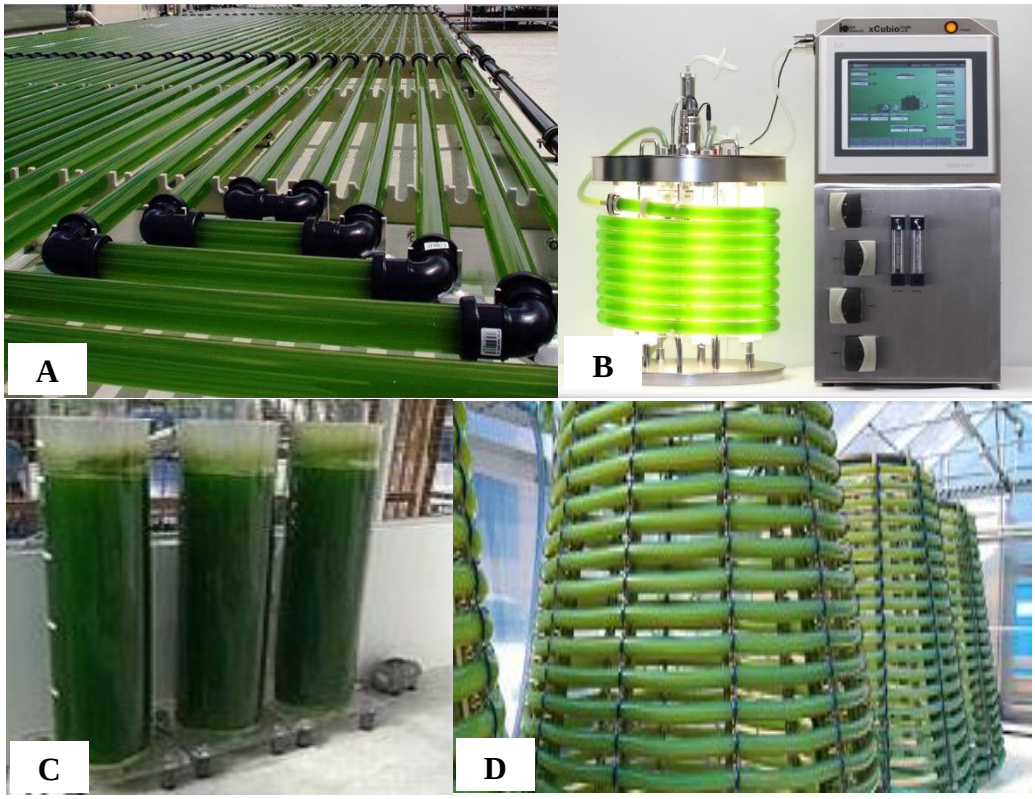

Figure 3 : *Les systèmes de culture fermés nommés « photobioréacteurs » à configuration tubulaire (**A :D**), spiralé à l'échelle laboratoire. **B** et **C :** colonne verticale**. Image source**_ Young-Lorenz and Lumley, 2013, Satpati and Pal, 2018, http://algenbioreaktor.de/en/algenbioreaktoren/referenzen/, and Koller, 2015 respectively.*

4 Extraction des biostimulants de plantes à partir des microalgues

Le développement de biostimulants à partir de microalgues ou de cyanobactéries consiste en une lyse de la biomasse et l'extraction du contenu cellulaire total. Les composés actifs de ces phytoplanctons sont enfermés dans les parois cellulaires et/ou liés à diverses structures (Craigie 2011). Ainsi, différents processus ont été décrits pour une récupération élevée des composés actifs et leur libération à partir des structures cellulaires (Sharma et al. 2014). Ces méthodes d'extraction peuvent être physiques, chimiques ou enzymatiques.

4.1 Extraction mécanique ou physique

Cette méthode inclut des techniques telles que l'autoclavage, le micro-onde, la technologie du champ électrique pulsé, la sonication, le broyage par l'azote liquide et le cryo-traitement. Une rupture cellulaire avec des traitements à haute pression a également été décrite, où la biomasse est traitée à haute pression et ensuite soumise à une réduction de pression, provoquant la rupture des parois cellulaires, libérant ainsi leur contenu (Sharma et al. 2014).

4.2 Extraction chimique

Cette méthode est basée sur les techniques impliquant la rupture cellulaire par des moyens chimiques tels que l'utilisation d'hydroxyde de sodium, d'acide chlorhydrique ou sulfurique, d'acide nitreux ou de choc osmotique.

4.3 Extraction enzymatique.

Dans ce cas, des enzymes telles que la protéase, la cellulase, la β-glucosidase, la xylanase, la β-glucanase ou la pectinase sont utilisées pour dégrader les parois cellulaires pour la lyse cellulaire (Gómez-García et al. 2012; Oancea et al. 2013; Labrou et al. 2020).

L'extraction enzymatique a un avantage sur les méthodes chimiques ou physiques en raison de la perturbation douce de la paroi cellulaire, qui permet la conservation de la structure et l'activité de composés bioactifs (Michalak and Chojnacka 2014; Michalak et al. 2015).

Le principal inconvénient des méthodes chimiques et physiques est la consommation du temps, associée à la dégradation possible des composés bioactifs utiles. Par conséquent, de nouvelles méthodes d'extraction telles que l'extraction assistée par micro-ondes, l'extraction liquide sous pression, l'extraction CO_2 sous-critique et supercritique, ou l'extraction assistée par ultrasons ont été adoptées (Joana Gil-Chávez et al. 2013; Michalak and Chojnacka 2014; Michalak et al. 2015). L'extraction de fluide supercritique (pression ~500 bar, Température ~50 ◦C et ~100 kg de CO_2 par kg de biomasse microalgale de charge) a attiré beaucoup d'attention en raison de la meilleure qualité des extraits obtenus grâce à cette technique (concentration de composés biologiquement actifs dans un environnement sans solvant) (Chojnacka et al. 2015).

Les microalgues et les cyanobactéries produisent une diversité de métabolites bioactifs. Cependant, la quantité et la qualité des métabolites bioactifs extraits dépendent largement de la technique d'extraction et des espèces de microalgues ou de cyanobactéries (Puglisi et al. 2018). Le choix de la méthode d'extraction utilisée pour obtenir les composés bioactifs à partir de la biomasse de microalgues ou de cyanobactéries est généralement dicté par la nature de la biomasse et les molécules cibles (Michalak and Chojnacka 2014). Mis à part les méthodes d'extraction et le contenu biochimique des extraits, l'efficacité des biostimulants dépendra également de la dose et du temps d'application (conditions normales/ avant, pendant ou après la soumission au stress) (Chiaiese et al. 2018). La dose optimale d'application est un facteur important. En fait, différentes concentrations peuvent influencer positivement ou négativement la croissance des plantes (Vernieri et al. 2006). En outre, les études sur le fractionnement biochimique des extraits bruts de cyanobactéries et de microalgues et les analyses agronomiques de leurs composés bioactifs purifiés peuvent constituer une nouveauté principale utile pour une étude approfondie de leurs mécanismes d'action sur les plantes.

5 Les effets biostimulants des microalgues et de leurs dérivés sur les plantes

5.1 Effet sur la croissance et le développement des plantes

Les microalgues et les cyanobactéries ont des propriétés biostimulantes sur la croissance et le développement des plantes en facilitant l'absorption des nutriments et en améliorant la performance des plantes et leur état physiologique (De Morais et al. 2015; Chiaiese et al. 2018). Au cours des dernières années, plusieurs études expérimentales, en plein champ et sous serre, ont montré l'effet biostimulant de plusieurs produits issus de ressources biologiques, y compris les microalgues et les cyanobactéries (Garcia-Gonzalez and Sommerfeld, 2016; Puglisi et al., 2018), indiquant leur utilisation potentielle en agriculture comme stimulateurs de croissance des plantes. Par exemple, la germination de la laitue dans un sol traité d'extraits de microalgues dérivés de *C. vulgaris* a favorisé la croissance des plantes aux premiers stades de leur développement (Faheed and Fattah 2008). Dans la même étude, l'augmentation de la croissance chez les plantes traitées a été corrélée à l'augmentation des pigments photosynthétiques.

Les semis de betterave à sucre cultivés en hydroponique dans une solution Hoagland et complétés par des extraits de microalgues *Chlorella vulgaris* ou *Scenedesmus quadricauda* ont présenté une taille et un volume racinaire plus élevés (Barone et al. 2018). Les différents changements dans l'architecture/morphologie des racines induits par les extraits de microalgales ont été attribués à la régulation ascendante de plusieurs gènes qui peuvent intervenir dans diverses voies métaboliques.

5.2 Effet sur la tolérance des plantes au stress abiotique

Les plantes sont exposées à des conditions environnementales difficiles telles que la sécheresse, la salinité, des températures extrêmes et une carence en nutriments qui nuisent à la croissance et à la productivité des plantes (Van Oosten et al. 2017; Bai et al. 2018). Certaines réponses générales de la défense comprennent une cascade hautement régulée d'événements de transduction de signal, à savoir : l'accumulation de ROS et de mécanismes antioxydants et la production de métabolites liés à la défense (Chaves et al. 2003; Bai et al. 2018). L'un des principaux événements liés aux stress abiotiques implique aussi un réseau hautement régulé et coordonné des phytohormones telles que l'acide abscissique (ABA), les auxines (acide indole acétique, IAA), l'éthylène (ET), les cytokinines (CK), les gibbérellines (GA), l'acide salicylique (SA), l'acide jasmonique (JA) et les brassinostéroïdes (BRs). Ces phytohormones agissent souvent comme des molécules de signalisation primaires qui déclenchent des cascades de signalisation complexes, conduisant par la suite à la stimulation de l'expression de gènes liés au stress et à l'induction des changements physiologiques et morphologiques, qui conduisent éventuellement à une tolérance ou une résistance au stress abiotique (**Figure 4**) (Cia et al. 2012; Redondo-Gómez 2013; Sah et al. 2017).

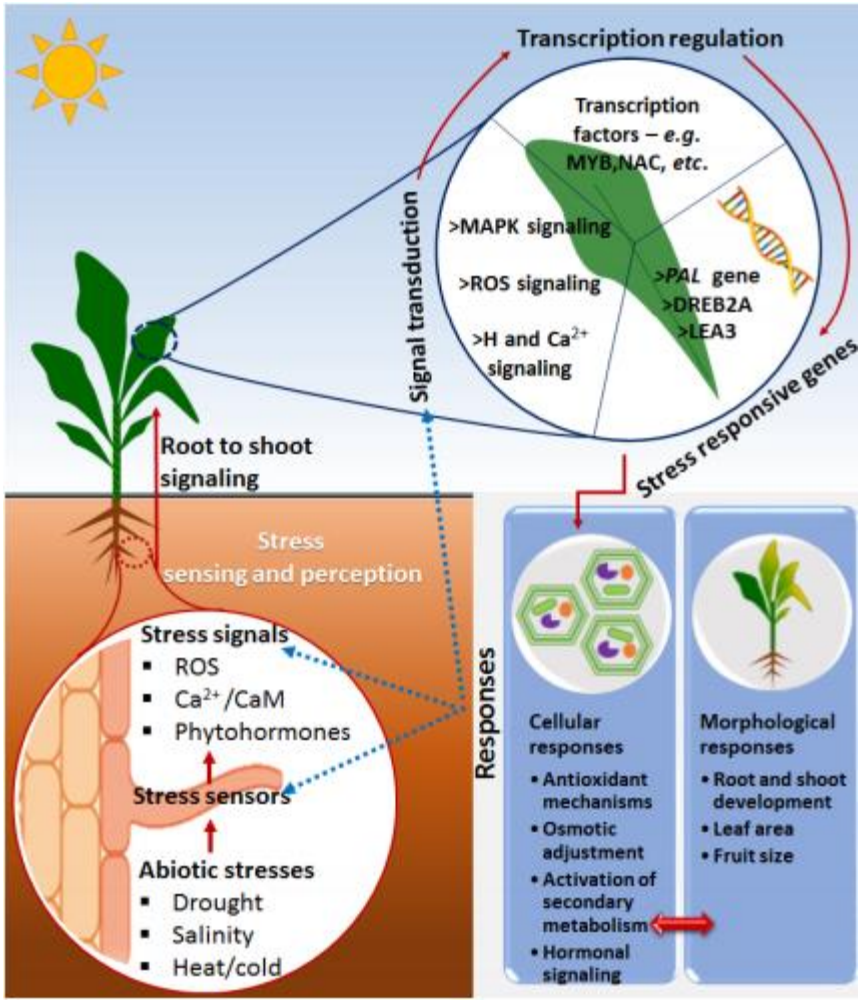

Figure 4 : *Les réponses générales de la tolérance des plantes au stress abiotique. Une cascade d'événements de transduction de signal hautement régulée: activation de gènes liés à la défense, accumulation de ROS et de mécanismes antioxydants, production de métabolites liés à la défense et les changements physiologiques et morphologiques ultérieurs* ***Image source*** _(Nephali et al. 2020)

La salinisation du sol est une contrainte agricole majeure (Davidson 2000; Shrivastava and Kumar 2015; Ronga et al. 2019) affectant environ 800 millions d'hectares dans le monde (Munns and Tester 2008). Elle est exacerbée par la gestion inappropriée de la fertilisation chimique qui conduit à un déséquilibre osmotique dans la solution nutritive, avec des dommages ultérieurs à la croissance et le rendement des cultures (Goykovic Cortés and Saavedra del Real 2007). Plusieurs études ont montré que l'application d'extraits de microalgues et de cyanobactéries pourrait améliorer la tolérance des plantes au stress. Guzmán-Murillo et al., (2013) et Abd El-Baky et al., (2010) ont montré que *Dunaliella* spp. et *Phaeodactylum* spp. atténuent le stress salin sur la germination des graines de poivron (*Capsicum annuum* L.), en réduisant la production de superoxyde O_2 et la peroxydation des lipides. Les extraits bruts issus de la cyanobacterium

Spirulina spp. et la microalgue *Chlorella* spp. stimulent la tolérance du blé au stress salin *via* l'amélioration de la capacité antioxydante et de la teneur en protéines des grains dans les plantes traitées, suggérant que l'application d'extraits de microalgues et de cyanobactéries aux plantes de blé (*Triticum aestivum* L.) pourrait améliorer la tolérance du blé à la salinité (Abd El-Baky et al. 2010). Aussi, les extraits de *Nannochloris* spp. ont déclenché la réduction du stress hydrique chez les plantes de tomates, déterminé par l'amélioration de la longueur des racines, l'augmentation de la surface foliaire et de la taille des plantes (Oancea et al. 2013).

L'application des exopolysaccharides (EPS) à partir de la microalgue Halophile (*Dunaliella salina*) a amélioré la germination des graines et la croissance des semis causées par le stress salin sur le blé (*Triticum aestivum*) ((EL Arroussi et al. 2016). Les EPS de *Dunaliella salina* ont également atténué l'effet du stress salin sur la croissance de la tomate (*Solanum lycopersicum L.)* (EL Arroussi et al. 2018). Le traitement des plantes par les EPS a déclenché l'accumulation de la proline, des composés phénoliques et du K^+, et a amélioré l'activité antioxydante de Catalase (CAT), de peroxydase (POD) et de superoxyde dismutase (SOD), qui sont les mécanismes biochimiques liés à la tolérance au stress salin.

5.3 Les extraits de microalgues et de cyanobactéries comme éliciteurs de plantes

Le terme éliciteur était à l'origine utilisé pour les molécules capables d'induire la production de phytoalexines, mais il est maintenant couramment utilisé pour les composés stimulant tout type de défense des plantes (Ebel and Cosio 1994). Aujourd'hui, les éliciteurs sont définis comme des molécules ayant la capacité d'induire des réponses de défense conduisant à une résistance élevée chez les plantes traitées. Ces molécules comprennent à la fois des substances d'origine pathogène (éliciteurs exogènes) et des composés libérés par les plantes par l'action de l'agent pathogène (éliciteurs endogènes) (Boller 1995). Les éliciteurs sont classés comme physiques ou chimiques, biotiques ou abiotiques, complexes ou définis, en fonction de leur origine et de leur structure moléculaire. Les éliciteurs peuvent être divisés en deux groupes, éliciteurs généraux et éliciteurs spécifiques à la race. Les éliciteurs généraux sont capables de déclencher la défense chez les plantes hôtes et non hôtes, tandis que les éliciteurs spécifiques à la race induisent des réponses de défense (conduisant à une résistance aux maladies) uniquement chez des cultivars hôtes spécifiques.

Les polysaccharides d'algues peuvent agir comme éliciteurs de la défense des plantes (Mercier et al. 2001). D'ailleurs, (Paulert et al. 2009) a montré l'implication de ces molécules dans l'activation des voies métaboliques secondaires ainsi que dans la mobilisation des molécules de signalisation. Selon Calvo et al. (2014), et conformément à la définition d'EBIC 2012, les biostimulants sont distingués des éliciteurs biologiques de la défense au stress biotique. Les effets des biostimulants sont essentiellement liés à l'amélioration de la croissance, de la tolérance au stress et de la qualité des plantes. Cependant, de nombreux biostimulants tels que le chitosane, la laminarine, certains PGPRs, etc., stimulent les mécanismes

biochimiques et la régulation des gènes liés aux voies de la réponse des plantes aux agents pathogènes (Du Jardin 2015). De plus, la réponse des plantes aux stress biotiques et abiotiques est un réseau complexe de réactions qui implique différentes voies physiologiques du métabolisme primaire et secondaire (Mittler et al. 2004; Rejeb et al. 2014). La transduction de ces signaux est différente d'une plante à l'autre selon la constitution génétique et les réponses adaptatives des plantes (Yoshida et al. 2014; Pereira 2016). Néanmoins, certaines voies à savoir la voie de phénylpropanoïdes et de la lipoxygénase (LOX), ainsi que les mécanismes de réponse y compris la surproduction d'espèces réactives de l'oxygène (ROS) et d'autres réponses physiologiques telles que l'épaississement de la paroi cellulaire et la formation de cire cuticulaire sont les mécanismes généraux de défense chez la plupart des plantes (Fujita et al. 2006; Rejeb et al. 2014). La signalisation lipidique se produit en réponse à divers stress environnementaux y inclus l'attaque par des agents pathogènes (Okazaki and Saito 2014).

6 Les paramètres de réponse chez la plante

La plante a plusieurs paramètres de réponse à son environnement tels que les paramètres morphologiques, métaboliques, biochimiques et moléculaires.

6.1 Paramètres morphologiques

Un certain nombre de caractéristiques des plantes telles que l'architecture globale de la plante, à savoir la structure et la taille foliaire et racinaire, ainsi que l'architecture vasculaire sont des déterminants importants de la performance globale des plantes cultivées (Lonhienne et al. 2015; Hussain et al. 2019).

Les feuilles par exemple sont le site principal de la photosynthèse. Ainsi, la taille des feuilles et le volume des tiges peuvent déterminer la quantité d'interception de la lumière, la capacité photosynthétique et la production des sucres (par conséquent la biomasse) des plantes. D'autre part, l'augmentation du volume racinaire crée une plus grande surface pour l'assimilation des nutriments et de l'eau. Ces caractéristiques morphologiques peuvent donc être considérées comme faisant partie d'un module de développement qui dicte la performance et le rendement des cultures. L'optimisation de ces caractéristiques est essentielle pour la performance efficace des plantes cultivées.

6.2 Profils des métabolites

Le volet métabolique est un paramètre très important pour étudier et comprendre les réactions de la plante à son environnement via le suivi et la quantification des empreintes chimiques laissées par des processus cellulaires spécifiques (Schauer and Fernie 2006; De Vos et al. 2007). Par exemple, la réponse à l'augmentation du sel dans le milieu de culture des plantes implique des changements dans l'activité d'un certain nombre de gènes et de protéines qui entraînent des changements dans le métabolisme des plantes. L’analyse d’un ensemble de métabolites peut fournir des informations sur de nombreux processus

physiologiques particulièrement associés à l'application de biostimulants dans des conditions normales ou de stress.

6.3 Changements biochimiques

Les plantes sont fréquemment exposées à diverses conditions environnementales défavorables appelées « stress abiotiques ». La plupart des cultures faites en plein champ sont fréquemment exposées à divers stress abiotiques. Ces stress modifient le métabolisme des plantes, entraînant des effets négatifs sur la croissance, le développement et la productivité. Le stress comme la salinité entraînent un déséquilibre ionique chez les plantes en raison d'une accumulation excessive du Na^+ et de Cl^-, ce qui réduit l'absorption d'autres nutriments minéraux tels que le K^+, Ca^{2+} et Mn^{2+}. De plus, l'accumulation excessive de Na^+ entraîne un déséquilibre de la perméabilité membranaires (résultant du déplacement de Ca^+ par Na^+), et une surproduction des ROS, qui par conséquent causent des dommages oxydatifs en oxydant les protéines, les lipides, l'ADN etc (Zhao et al. 2020). Ces altérations biochimiques limitent la croissance et la performance des plantes. Pour contrecarrer le stress salin, les plantes initient une cascade de réponses spécifiques comprenant le transport de K^+, la signalisation par Ca^{2+}, le transport de H^+ et les modifications des phospholipides. Ces premiers signaux induits par le Na^+ comprennent des changements en aval des niveaux de phytohormones (Van Zelm et al. 2020; Arif et al. 2020). Les niveaux d'expression des gènes sont également modifiés. Cette cascade de signalisation entraîne des réponses adaptatives, y compris la modulation de la croissance et du développement des plantes, le contrôle de l'absorption des ions, l'activation des enzymes anti-ROS comme la superoxyde dismutase (SOD), Catalase (CAT) et peroxydases (POD) ainsi que la production de solutés compatibles, pour compenser la pression osmotique de Na^+ (Acosta-Motos et al. 2017; Yang and Guo 2018; Van Zelm et al. 2020). Ces réponses biochimiques naturelles adéquates au stress jouent un rôle important dans l'induction de la tolérance des plantes sur les sols salins.

L'étude de l'effet des extraits microalgues sur les changements biochimiques des plantes fournit un aperçu des propriétés biostimulantes de ces extraits sur la croissance des plantes et la tolérance au stress abiotique.

6.4 Absorption des nutriments

La croissance et le développement des plantes dépendent en grande partie de la combinaison et de la concentration des nutriments minéraux disponibles dans le sol. Les plantes ne peuvent pas fabriquer d'ADN, d'ARN, d'enzymes, d'ATP et de nombreux autres composants clés, en l'absence des nutriments clés comme de l'azote (N) et du phosphore (P). Le Potassium (K) joue également un rôle essentiel dans la régulation des stomates et les activités enzymatiques. Cependant, les plantes sont souvent confrontées à des défis importants pour obtenir un approvisionnement adéquat en ces nutriments pour répondre aux exigences des processus cellulaires de base (Morgan and Connolly 2013). La présence de quantités totales suffisantes de

nutriments essentiels dans le sol ne garantit pas leur disponibilité aux plantes, car d'autres facteurs tels que la teneur en humidité du sol, l'aération, la température et le pH, ou les conditions physiques du sol et/ou la salinité peuvent être limitants (Marschner and Rengel 2011 ; Hillel 2004)

Le phosphore du sol est relativement stable dans le sol et se déplace très peu par rapport à l'azote, car il se fixe facilement au sol. Dans le secteur agricole, ce manque de mobilité et de faible solubilité réduit la disponibilité des engrais phosphatés. D'autre part, l'azote est en grande partie perdu par le lessivage. À l'échelle mondiale, seulement 50% des engrais azotés appliqués aux sols sont absorbés par les cultures, tandis que les 50% restants provoquent une pollution par émissions gazeuses et ruissellements (Brackin et al. 2015). Jusqu'à présent, les stratégies les plus évidentes adoptées pour remédier à la disponibilité du NPK dans les sols agricoles et augmenter les rendements des cultures est l'ajout de grandes quantités d'engrais chimiques. Toutefois, cela est nocif pour l'environnement. Les méthodes durables par lesquelles il est possible de surmonter cet inconvénient sont la cultivation des cultures avec des systèmes racinaires plus robustes et une plus grande efficacité d'absorption des nutriments.

Dans la présente étude, les extraits de microalgues et de cyanobactéries sont appliqués au sol dans le but de stimuler l'absorption du NPK afin d'augmenter le pourcentage d'engrais absorbés par les plantes et réduire la quantité perdue au sol. Maximiser le taux d'absorption des nutriments et l'efficacité d'utilisation par les plantes minimise par la suite l'ajout continu de grandes quantités d'engrais aux sols.

6.5 Les phytohormones

Les phytohormones font partie intégrante des réseaux de signalisation des plantes et jouent donc de multiples rôles dans le contrôle de la croissance, du développement et de la réponse aux altérations génétiques, biotiques et abiotiques (Santner and Estelle 2009; Rubio et al. 2009; Depuydt and Hardtke 2011). Les hormones régissent tous les aspects de la biologie des plantes et des études récentes ont révélé une diaphonie étendue entre différentes voies de signalisation hormonale. Les phytohormones classiques initialement découvertes comprennent les gibbérellines (GAs), les cytokinines (CKs), les auxines (Auxs), l'acide abscissique (ABA) et l'éthylène (ET). Et les hormones découvertes par la suite à savoir, l'acide salicylique (SA), les brassinostéroïdes (BRs), les jasmonates (JAs) et les strigolactones (SLs) (Kende and Zeevaart 1997; Ross and O'Neill 2001; Verma et al. 2016). Ces phytohormones peuvent agir au sein des réseaux de signalisation complexes qui contrôlent divers processus biologiques via des actions antagonistes et/ou synergiques. Il est donc essentiel d'étudier l'effet des extraits sur les différentes hormones intervenant dans la croissance et développement des plantes.

6.6 Les protéines transporteuses de la famille PhT1

Le phosphore est un nutriment majeur acquis par les racines *via* des transporteurs de phosphate inorganique (Pi) de haute affinité PHT (Liu et al. 1998). La famille de gènes PHT1 a un plus grand nombre de membres

que les trois autres familles PHT et se localise au niveau de la membrane plasmique (Gu et al. 2016). Dans certains cas, la transcription de certains membres du Pht1 est plus largement répartie dans les tissus des plantes. Ces gènes ont montré une faible réponse à la carence en phosphate inorganique Pi. Ces gènes prouvent que certains membres du Pht1 peuvent être impliqués dans la mobilisation interne du Pi, comme le chargement, le déchargement du xylème ou du phloème et le dépôt dans les graines ou d'autres organes de stockage (Gordon-Weeks et al. 2003; Jia et al. 2011; Nagarajan et al. 2011; Nussaume et al. 2011; Sun et al. 2012). Tous les gènes Pht1 n'étant pas sensibles à la concentration du P dans le sol, un nombre croissant de transporteurs du P de la famille Pht1 sont induits par la mycorhize arbusculaire dans plusieurs familles de plantes et leurs fonctions ont été associées à l'absorption de P à l'interface symbiotique intraradique (Rausch et al. 2001; Harrison et al. 2002; Paszkowski et al. 2002; Maeda et al. 2006; Guether et al. 2009).

Dans la présente étude, les profils d'expression de cinq (*Le*PT1; *Le*PT2; *Le*PT3; *Le*PT4 et *Le*PT5) de huit séquences identiques aux gènes pht1 de la tomate ont été étudiés. L'analyse des changements dans la transcription a été basée sur deux facteurs : la concentration du P dans le sol et le traitement par l'extrait de cyanobactérie.

CHAPITRE II-1

CHAPITRE II-1

Les polysaccharides de microalgues en tant que nouveaux produits bioactifs durables pour le développement de bio-stimulants de plantes.

II-1. 1 INTRODUCTION

Les microalgues sont une bioressource économique, renouvelable et durable qui sont exploitées dans différents domaines en tant que biocarburants, produits bioactifs et ingrédients alimentaires. Plusieurs espèces de microalgues ont été étudiées pour leur potentiel en tant que produits à valeur ajoutée avec des qualités pharmacologiques et biologiques. Les microalgues sont exploitées dans plusieurs domaines industriels pour leur capacité à convertir le CO_2 atmosphérique en produits utiles tels que les glucides, les lipides et d'autres métabolites bioactifs (Almomani et al. 2019; Bhagea et al. 2019; Khan et al. 2018; Molazadeh et al. 2015; Nie et al. 2018; Shakibaie et al. 2010; Villarruel-López et al. 2017). En agriculture, les microalgues peuvent être utilisées pour différentes applications, telles que les amendements du sol ou les biostimulants de plantes. (Chiaiese et al. 2018 ; Renuka et al. 2018; Ronga et al. 2019).

Les biostimulants de plantes sont définis comme des micro-organismes ou des substances qui, lorsqu'ils sont appliqués aux plantes, sont capables d'améliorer la croissance des plantes, l'efficacité de l'utilisation des nutriments, la tolérance aux facteurs de stress abiotiques et la qualité des cultures. Cette définition englobe diverses substances organiques et inorganiques, y compris les algues. (Calvo et al. 2014; Chiaiese et al. 2018; du-Jardin 2015; Rouphael et Colla 2018). Les microalgues produisent une diversité de molécules bioactives, y inclus les polysaccharides (Cuellar-Bermudez et al. 2015; Renuka et al. 2018; Ronga et al. 2019). Les polysaccharides sont généralement l'un des principaux composants d'extraits de microalgues, représentant jusqu'à 46% du poids sec chez certaines espèces telles que *Chlorella* spp., *Chlamydomonas* sp., *Dunaliella* spp. et *Spiruline* spp., (Pinzón et al. 2014; Spolaore et al. 2006; Tibbetts et al. 2015).

L'objectif de cette revue est de donner un aperçu de l'état actuel de l'utilisation des polysaccharides de microalgues et de leur potentiel en tant que biostimulants de la croissance des plantes, de l'absorption des nutriments et de la tolérance aux stress biotiques et abiotiques. La comparaison des activités biologiques entre les extraits totaux de microalgues et les extraits polysaccharidiques purifiés ou totaux de microalgues est également mise en évidence et brièvement discutée.

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REVIEW

Microalgae polysaccharides: the new sustainable bioactive products for the development of plant bio-stimulants?

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Abstract

Plant biostimulants are defined as materials containing microorganisms or substances whose function when applied to plants or the rhizosphere is to stimulate natural mechanisms to enhance plant growth, nutrient use efficiency, tolerance to abiotic stressors and crop quality, independent of their nutrient content. In agriculture, seaweeds (Macroalgae) have been used in the production of plant biostimulants while microalgae still remain unexploited. Microalgae are single cell microscopic organisms (prokaryotic or eukaryotic) that grow in a range of aquatic habitats, including, wastewaters, pounds, lakes, rivers, oceans, and even humid soils. These photosynthetic microorganisms are widely described as renewable sources of biofuels, bioingredients and biologically active compounds, such as polyunsaturated fatty acids (PUFAs), carotenoids, phycobiliproteins, sterols, vitamins and polysaccharides, which attract considerable interest in both scientific and industrial communities. Microalgae polysaccharides have so far proved to have several important biological activities, making them biomaterials and bioactive products of increasing importance for a wide range of applications. The present review describes microalgae polysaccharides, their biological activities and their possible application in agriculture as a potential sustainable alternative for enhanced crop performance, nutrient uptake and resilience to environmental stress. This review does not only present a comprehensive and systematic study of Microalgae polysaccharides as plant biostimulants but considers the fundamental and innovative principles underlying this technology.

Keywords Microalgae · Polysaccharides · Agriculture · Bio stimulants

Introduction

Microalgae are photosynthetic microscopic organisms (prokaryotic or eukaryotic) that grow in a range of aquatic habitats, including ponds, rivers, lakes, oceans, wastewaters and even humid soils. Microalgae are an economical, renewable and sustainable bioresource that are exploited in different fields as biofuels, bioactive products and food ingredients. Several microalgae species have been investigated for their potential as value-added products with remarkable pharmacological and biological qualities, which come from their significant ability to convert atmospheric CO_2 to useful products such as carbohydrates, lipids, and other bioactive metabolites (Almomani et al. 2019; Bhagea et al. 2019; Khan et al. 2018; Molazadeh et al. 2019; Nie et al. 2018; Shakibaie et al. 2010; Villarruel-López et al. 2017). In agriculture, microalgae can be used for different applications, such as soil amendments or plant biostimulants. (Chiaiese et al. 2018; Renuka et al. 2018; Ronga et al. 2019).

Plant biostimulants are defined as microorganisms or substances which when applied to plants in finite quantities, are able to improve plant growth, nutrient use efficiency, tolerance to abiotic stressors and crop quality. This definition entails diverse organic and inorganic substances and/or microorganisms including algal extracts (Calvo et al. 2014; Chiaiese et al. 2018; du-Jardin 2015; Rouphael and Colla 2018). Today, seaweeds more than microalgae, have been largely exploited for the production of plant growth biostimulants and represent an important category of organic

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biostimulants (Battacharyya et al. 2015; Colla et al. 2017; Khan et al. 2011). However, microalgae could also be largely exploited in agriculture as a renewable bioresource for plant biostimulants, considering their capacity to produce a remarkable diversity of biologically active molecules including fatty acids, osmolytes, phytohormones, polysaccharides and phenolics (Cuellar-Bermudez et al. 2015; Renuka et al. 2018; Ronga et al. 2019). In addition, other studies have reported that microalgae can also produce plant growth-promoting substances such as auxins, cytokinins, betaines, amino acids, vitamins, polyamines and gibberellins (Stirk et al. 2013a, b; Tate et al. 2013; Plaza et al. 2018). Nevertheless, carbohydrates, including polysaccharides are usually one of the major components of microalgal extracts, accounting for up to 46% of the dry weight (DW) in certain species such as *Chlorella* spp., *Chlamydomonas* spp., *Dunaliella* spp. and *Spirulina* spp., (Pinzón et al. 2014; Spolaore et al. 2006; Tibbetts et al. 2015).

In recent years, an increasing amount of research has been undertaken to study the effect of microalgae-based products (proteins hydrolysates, polysaccharides/exopolysaccharides and cell wall extracts) on the growth and development of several crops such as tomato, wheat, lettuce and pepper, under optimal and stressful conditions (Barone et al. 2018; Chiaiese et al. 2018; El Arroussi et al. 2016, 2018; Garcia and Sommerfeld 2016). The objective of this review is to report an overview of the current status of microalgae polysaccharide use and their potential as biostimulants of plant growth, nutrient uptake and tolerance to both biotic and abiotic stresses. Comparison of the biological activities between microalgae total extracts and purified or total microalgae polysaccharide extracts is also highlighted and briefly discussed.

Biochemical composition and functional groups of Microalgae polysaccharides

Polysaccharides are polymeric carbohydrate macromolecules with complex structures, and have various functional activities (Nie et al. 2018). Both biochemically and structurally, polysaccharides greatly differ from one specie to another. These complex differences are also reflected in the high number of enzymes involved in polysaccharide synthesis and modification (Rossi and Philippis 2016). With the exception of *Gyrodinium impudicum* and *Chlorella vulgaris* that produce homopolymer polysaccharides of galactose (Yim et al. 2007) and B-(1,3)-glucan respectively, microalgae polysaccharides are heteropolymers of mainly galactose, xylose and glucose in different proportions, linked together by glycosidic bonds (Raposo et al. 2015). This proportion difference in constituent neutral sugars can modify the polysaccharide's bio stimulant properties, although other biostimulatory properties are also largely attributed to the polysaccharide's sufation, uronic acid content and molecular weight (MW) (Farid et al. 2019; Mishra and Jha 2013; Ponce et al. 2003;Sangha et al. 2010; Qi et al. 2005; Zha et al. 2016). Other sugars such as rhamnose, fucose, fructose and methyl sugars can also be constituents of microalgae polysaccharides (Raposo et al. 2015). Table 1 illustrates some strains of microalgae/blue-green algae producing polysaccharides and their main neutral sugars.

There are few studies on microalgae polysaccharides exhibiting biostimulant and plant- protector properties. Hence, the direct relationship between the molecular structure of polysaccharides and their bio-stimulant activity is yet to be understood. However, the knowledge of seaweed-extracted polysaccharides and their effects on plant mechanisms (Michalak et al. 2017; Michalak and Chojnacka 2015), could help predict microalgae polysaccharides and their possible elicitor effects on plant mechanisms. For instance, recent research had unraveled that molecular mechanisms by which carrageenan polysaccharides from seaweed can mediate plant growth and plant defense responses is by modulating various metabolic processes such as photosynthesis, nitrogen and sulfur assimilation and plant defense pathways (Shukla et al. 2016). Nevertheless, the functional activities of polysaccharides differ according to their constituent monosaccharides, molecular weight and degree of sulfation, thus, a study of the molecular structure of microalgae polysaccharides and their specific elicitor effects on plant growth and defense mechanism might be a helpful tool in plant biotechnology.

Microalgae polysaccharides as plant bio stimulants

With an ever-increasing global population estimated to expand to more than 9.7 billion by 2050, modern agriculture is compelled to become increasingly efficient by producing more food in a sustainable and eco-friendly way. One of the innovative technologies addressing these challenges involves the development of novel plant biostimulants and effective methods for their application (Bulgari et al. 2015; Calvo et al. 2014; Yakhin et al. 2017). Plant biostimulants including algal polysaccharides have been described since the beginning of modern agriculture, and could be considered as a good production strategy for obtaining high yields of nutritionally valuable food with lower impact on the environment.

The definition of plant biostimulants is based on scientific principles as microorganisms or substances which when applied to plants are able to stimulate nutrient uptake, promote seed germination, improve crop's yield and tolerance to abiotic stress. Some important concepts have been proposed so far to define and classify plant biostimulants.

Table 1 Species of microalgae/blue-green algae producing Polysaccharide and their main neutral sugars

Microalgae	Polysaccharide	Main neutral sugars	Biological activity	References
Chlorella stigmatophora	Sulfated Polysaccharides	Glucose, xylose, fucose	Anti-inflammatory, immunomodulator	(Guzmán et al. 2003)
Chlorella vulgaris	B-(1,3)-glucan	Glucose	Antitumor, infection preventive agent	(Ogawa et al. 1997, 1999)
	Polysaccharides	Rhamnose, galactose		
Dunaliella salina	Exo-Polysaccharides	Xylose, galactose, glucose, fructose		(Mishra et al. 2011)
Tetraselmis sp.	Sulfated Polysaccharides		Anti-adhesive	Guzman and Ascencio (2000)
Porphyridium sp.	Sulfated Polysaccharides	Xylose, galactose, glucose	Anti-inflammatory, immunomodulator, prevention of tumor cell growth, anti-adhesive, antiviral, biolubricant	(Geresh and Arad (1991); Matsui et al. 2003; Shoshana et al. 2006; Talyshinsky et al. 2002)
Porphyridium cruentum	Sulfated Polysaccharides	Xylose, galactose, glucose, Glucuronic acid 3-O-methyl-xyl.	Antioxidant and free radical scavenging, immunomodulator, antiviral, antibacterial, antilipidemic, antiglycemic	(Raposo et al. 2014; Huang et al. 2001; Sun et al. 2009, 2012)
Rhodella reticulata	Sulfated Polysaccharides	Xylose, rhamnose, 3-*O*-methyl-rham, 4-*O*-methyl-gal	Antiviral, antilipidemic, antiglycemic, prevention of tumor cell growth	(Geresh and Arad 1991; Arad and Levy-Ontman 2010)
Cyanobacteria				
Arthrospira platensis	Exo-Polysaccharides s-Spirulan	galactose, fructose, xylose glucose Rhamnose, fucose, glucose, 3-*O*-methyl-rham	Antiviral, antibacterial, prevention of tumor cell growth Anti-proliferative, anti-adhesive, anti-metastatic	(Challouf et al. 2011; Hayashi et al. 1996; Jung-Bum et al. 2000; Kaji et al. 2004)

For instance (Basak 2008) proposed that biostimulants could be classified depending on the mode of action and the origin of the active ingredient. However, years later (du-Jardin 2015), suggested that "any definition of biostimulants should focus on the agricultural functions of biostimulants, which is improvement in plant yield or quality or increased efficiency of plant productivity, and not on the nature of their constituents nor on their modes of action". Similarly, (Bulgari et al. 2015) highlighted that "biostimulants should be classified on the basis of their action on plants or on the physiological plant responses rather than on their composition." Today, microlgae can be classed among seaweed extracts and botanicals. But compared with seaweeds, the practical applications of microalgae in agriculture is still very limited and much less is known regarding their biostimulant mechanisms in plants, the attention being much focused on their functional activities in Nutraceutical, pharmaceutical and cosmetic products. However, there seems to be opportunities to largely exploit them as plant biostimulants. In recent years, researchers have pushed the barrier by looking into exploiting microalgae total extracts and/or Polysaccharides as potential plant bio-elicitors and/or bio stimulants in agriculture. So far, Microalgae polysaccharides have demonstrated a broad range of biological activities on higher plants (El Arroussi et al. 2016, 2018; Farid et al. 2019) and could have multi-functional properties in agriculture, facilitating nutrient uptake, improving crop performance, physiological status and tolerance to abiotic stress (Barone et al. 2018, 2019; Borowitzka 2016; de Morais et al. 2015; Faheed and Fattah 2008; Garcia and Sommerfeld 2016; Renuka et al. 2018; Stadnik and Freitas 2014).

The outstanding challenge in promoting microalgae polysaccharide as plant biostimulants in agriculture is understanding their specific structure–function bioactivities on plants. Some studies demonstrated that besides neutral sugars and sulfate content, low (MW) polysaccharides exhibit greater effects on plant mechanisms (Stasio et al. 2018; Zha et al. 2016). This can be explained by the fact that a lower (MW) is generally important for the permeability of compounds. Therefore, it is not only the bioactivity of molecules or their specific target/function, but their permeability and capacity to reach the target also remains essential.

Some studies have also unraveled the molecular mechanisms by which seaweed polysaccharides such as carrageenans and their oligomeric forms mediate plant growth and plant defense responses; by regulating photosynthesis and carbon fixation in plants, enhancing ribulose 1, 5 bisphosphate carboxylase/oxygenase (Rubisco) activity, ancillary

pathways, cell division, purine and pyrimidine synthetic pathways as well as metabolic pathways involved in nitrogen and sulfur assimilation. It was also observed that carrageenans and their oligomeric forms modulate the activity of different defense pathways, including jasmonate, salicylate and ethylene signaling pathways (González et al. 2013; Mercier et al. 2001; Shukla et al. 2016; Jiang et al. 2012).

Today, significant advancements including, the application of the *omics* strategies; transcriptomics (Goñi et al. 2016; Wilson et al. 2015), and metabolomics (Ertani et al. 2014) or other molecular (Petrozza et al. 2014) studies, have been proposed in order to shed light on the modes of action of plant biostimulants. In addition, (Conan et al. 2015) at the 2nd World Congress on the use of Biostimulants in Agriculture also highlighted the identification of the bioactive compounds responsible for plant growth responses using metabolomic profiling of biostimulant products and analysis of their physiological effects on plants through transcriptomic and metabolomic strategies. This methodology would allow the determination of metabolite pathways in plants, modulated by biostimulants and could provide insight into gene regulations. Figure 1 depicts the bio-stimulant property of algal polysaccharides on some of the key mechanism related to plant growth.

Microalgae polysaccharides on plant growth and Nutrient uptake

Microalgae Polysaccharides can stimulate plant growth and nutrient uptake by modulating various physiological and biochemical processes. Some studies have demonstrated that microalgae extracts, tested both under open-field and greenhouse conditions are able to stimulate germination, seedling growth, shoot, and root biomass in plants.

In his study, El Arroussi et al. (2016) showed that polysaccharides extracted from *S. platensis* significantly promoted plant growth in *Capsicum annuum* and *Solanum lycopersicum*, which was demonstrated in terms of plant weight, plant size, and size/number of leaves. In the same way, seaweed polysaccharides have demonstrated a number of biological activities on plant growth (Castro et al. 2012; González et al. 2013; Khan et al. 2011; Sarfaraz et al. 2011; Sharma et al. 2012, 2014; Vera et al. 2012). It was speculated by González et al. (2013) that oligo-alginate and oligo-carrageenan may interact with plasma membrane receptors that use a co-receptor involved in signal transduction leading to simultaneous activation of plant growth. In addition, macroalgal-extracted polysaccharides have been confirmed to be used as perfect metal ion chelators. However, without in-depth study of polysaccharide mechanisms on nutrient uptake, it is possible to assume that the increased nutrient uptake might not be directly modulated by polysaccharides but could be a result of improved root surface of treated plants, which may automatically improve nutrient accessibility and uptake. It has also been reported that polysaccharides are rich in functional groups having the ability to bind to some micro elements with important plant nutritional value (Kaplan et al. 1987), hence improving the roots' nutrient availability. Therefore, there is need to study microalgae polysaccharides and their specific effects on nutrient uptake by plants and soil nutrient availability.

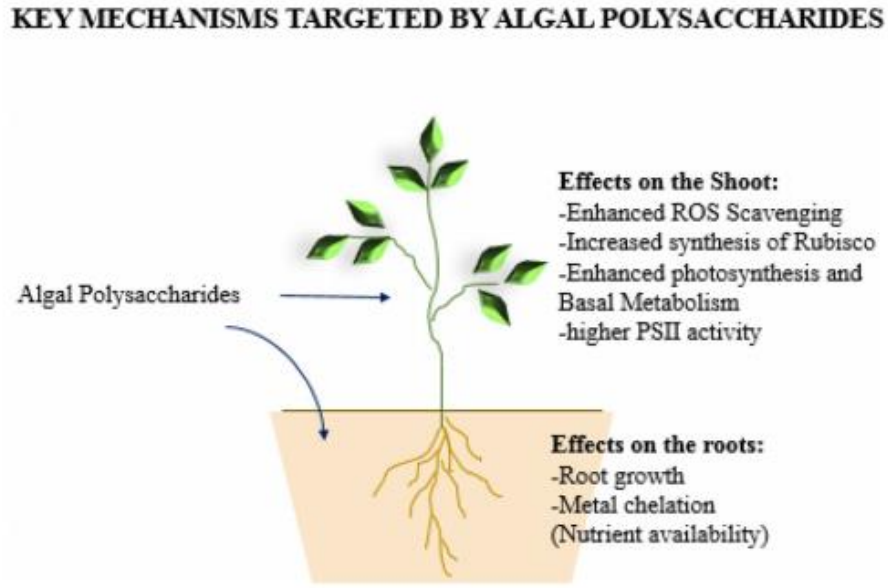

Fig. 1 The application of Algal polysaccharides could enhance plant growth and resistance by targeting mechanisms on both the shoot and root systems. The root target includes root growth and metal chelation leading to increased root surface and nutrient access. The effects on shoot growth include: enhanced ROS Scavenging, increased synthesis of Rubisco, higher PSII activity, enhanced photosynthesis and basal Metabolism

Microalgae polysaccharides on abiotic stress

Algal compounds exhibit great potential to enhance not only plant growth and nutrient uptake but resistance to abiotic and biotic stresses. During their entire life cycle, plants are exposed to several environmental stresses, which negatively affect their productivity and survival (Shaik and Ramakrishna 2014). One of the most common indication of stress is the production of reactive oxygen species (ROS). ROS are constantly produced from metabolic processes such as photosynthesis and respiration but are also formed in plant cells as a consequence of myriad stimuli ranging from abiotic and biotic stress. ROS constitute an ambiguous role during stress responses. Being toxic molecules, they are able to oxidatively injure cells (Mittler et al. 2004). To enable them to tolerate stress factors and survive, plants have developed a range of morphological, physiological and biochemical mechanisms such as the production of ROS scavenging enzymes in order to mitigate ROS potential toxic effects. (Gill and Tuteja 2010; Potters et al. 2004; You and Chan 2015). Interestingly, it has been demonstrated that Microalgae Polysaccharides can mitigate ROS toxicity in plants by enhancing the production of ROS antioxidant enzyme activities.

This mechanism can be explained by the fact that Polysaccharides contain neutral sugars which may interact with plasma membrane receptors as signal molecules and thereby leading to the activation of a series of biochemical reactions. But more importantly, algal polysaccharides could be perceived by membrane receptors as microbial-associated molecular patterns (MAMPs) and thereby inducing the MAMPs-dependent signaling pathways which involves the activation of Ca^{2+} influx, ROS production, Octadecanoid and Phenylpropanoid pathways with Lipoxygenase (LOX) and Phenylalanine ammonia lyase (PAL) respectively as key enzymes, and consequently ROS scavenging enzymes (Farid et al. 2019; Kemmerling et al. 2011). In his study, El Arroussi et al. (2016) demonstrated that the application of microalgae sulfated exopolysaccharides derived from *D. salina* enhanced the accumulation of proline and ROS antioxidant enzyme activities of Catalase (CAT), Peroxidase (POD) and Superoxide dismutase (SOD) in plants subjected to saline stress. Farid et al. (2019) also reported that treatment with *C. vulgaris* polysaccharides stimulated the enzymatic activity of Ascorbate Peroxidase (APX) in tomato plants. In the same study, microalgae polysaccharides extracted from *C. reinhardtii* and *C. sorokiniana* significantly enhanced POD activity. The ability of Algae polysaccharides to enhance ROS scavenging was also reported by Zou et al. (2019), who demonstrated that *Lessonia nigressens* polysaccharides (LPNs) effectively induced ROS scavenging in wheat seedlings by modulating their antioxidant enzyme activities under salt stress. Figure 2 illustrates the possible cellular mechanisms triggered by algal polysaccharides.

The activation of the MAMPs-dependent signaling pathways also trigger other mechanisms such as wax formation on the cuticle and synthesis of lignin, leading to the thickening of the cell walls (Fujita et al. 2006; Rejeb et al. 2014), which may reduce water loss through transevaporation and alleviate drought stress in plants.

Microalgae polysaccharides on biotic stress

As described in Fig. 2, plants deploy a wide range of defense mechanisms induced by elicitors from pathogen's cell wall components including chitin, lipo-polysaccharides, proteins such as bacterial flagellin and other chemicals of natural and synthetic origin.

Microalgae could be a promising source of polysaccharide elicitors. Molecules of polysaccharides perceived by plants' membrane receptors may trigger a series of defense mechanisms. Farid et al. (2019) indicated that microalgae crude polysaccharide extracts can induce plant innate immunity, depending on the microalgae strain. In his study, polysaccharides extracted from *C. vulgaris* and *C. sorokiniana* exhibited a significant increase in β-1,3-glucanase activity, which is one of the pathogenesis-related (PR) proteins, grouped in the PR-2 family that break down the cell wall components of pathogens. Induction of PR proteins is a consequence of the activation of plant defensive pathways, which limit the entry, or the further spread of the pathogen (Gupta et al. 2013). In the same study, *C. sorokiniana* crude polysaccharides had a significant stimulatory effect on Phenylalanine ammonia lyase (PAL) activity, indicating an up-regulation of the phenylpropanoid pathway.

The products of phenylpropanoid pathway in plants are thousands of compounds, that may end up in the composition of phenolic substances such as phytoalexins, which are toxic molecules against pathogens (Fesel and Zuccaro 2016). Moreover, sulfated polysaccharides from seaweed exhibited similar effects on phytoalexin accumulation and on the expression of genes involved in Salicylic acid (SA) and Jasmonic acid (JA) signaling pathways that induce plants' resistance against pathogens (Ghannam et al. 2013; Klarzynski et al. 2003). The activation of these signaling pathways leads to an increased expression of genes encoding PR proteins, PAL, lipoxygenase (LOX) and ROS Scavenging enzymes and other enzymes involved in synthesis of terpenoids and/or alkaloids having antimicrobial activities (Vera et al. 2011, 2012).

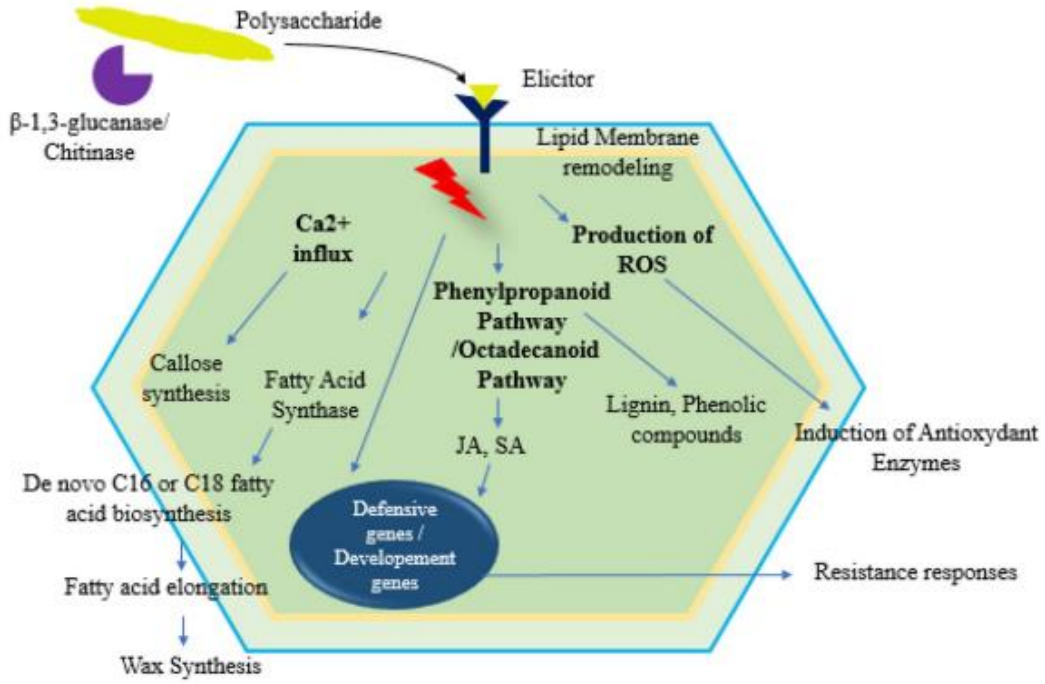

Fig. 2 Polysaccharides are broken-down into smaller fragments of oligosaccharides or simple sugars by Hydrolases (β-1,3-glucanase/chitinase). These fragments could be recognized by receptor membranes and induce a cascade of biochemical reactions; Ca^{2+} influx, ROS production (resulting in the induction of ROS scavenging enzyme activities), Octadecanoid and Phenylpropanoid pathways involving Lipoxygenase (LOX) and Phenylalanine ammonia lyase (PAL) respectively, as key enzymes and de novo synthesis of Fatty acids (C16 and C18) leading to the formation of cuticular Wax. A series of biochemical reactions also induces Transcription factors leading to the expression of defensive genes. Another hypothesis is that neutral sugars from polysaccharides could directly act as signal molecules leading to the induction of development genes

Comparison of the biological activity of Microalgae total crude extracts and purified polysaccharides extracts

The biostimulant properties of total algae extracts have generally been attributed to the presence of many bioactive ingredients such as phytohormones, phenolic compounds, amino acids organic molecules, and bioactive secondary metabolites (Di Stasio et al. 2018). Nonetheless, it is difficult to determine the active compounds of total extracts and their mode of action due to their diversity and complexity. On the other hand, polysaccharides are purified compounds and much easier to exploit, their biostimulant properties usually being attributed to constituent neutral sugars, sulfate content and MW (Ponce et al. 2003; Qi et al. 2005; Sangha et al. 2010; Zha et al. 2016). Moreover, polysaccharides are one of the major components of total algal extracts that contribute to the observed beneficial effects of total extracts on plants (Di Stasio et al. 2018). However, the application of total crude microalgae extracts may prove more effective in stimulating plant growth as compared to their purified polysaccharides, attributing to the fact that total crude extracts contain several other bioactive molecules that may work in synergy to promote plant growth (Rouphael and Colla 2018). But while several bioactive molecules might exhibit greater effects, we cannot annul the possibility for their contrarious and *antagonistic* interactions, which could result in less effect on plant growth as compared to purified compounds. Thus, an in-depth comparative study of microalgae extracts and their purified polysaccharides is necessary to shed more light on this hypothesis. The comparison of total algae extracts and their purified compounds is summarized in Table 2.

Future perspective

Studies on microalgae polysaccharides in agriculture have so far highlighted their biostimulant properties on plants in laboratory conditions. However, On-farm trials and whole-field studies are needed to provide support information for practical microalgae polysaccharide applications and valorize the studies conducted so far. There is also need to study the effect of microalgae polysaccharides on a broader range

Table 2 comparison of total algae extracts and their purified compounds

Microalgae total extract		Polysaccharide extracts	
Advantages	Constraints	Advantages	Constraints
Contain several other bioactive molecules that may work in synergy to promote plant growth.	Not easy to decipher the active compounds leading to the observed effects.	Easier to study the structure and mode of action of purified compounds.	No other bioactive molecules that may work in synergy to amplify the bio stimulant effects.
	Possible *antagonistic* interactions of bioactive compounds present in the total extract.	Depending on the molecular weight (MW) and sulfate content. Purified polysaccharides may exhibit diverse biological activities, which may be easier to study.	

of plant species to understand their biostimulant effects on different plant types.

Conclusion

Microalgae could be the new sustainable bioproducts for the development of plant biostimulants. These photosynthetic microorganisms are not only renewable but sustainable and economical sources of bioactive products and food ingredients. Microalgae polysaccharides have so far exhibited bio-stimulant properties that have proven useful for diverse industrial applications, although only a few studies have demonstrated their potential as plant bio-elicitors and/or biostimulants in agriculture. The modes of action of Microalgae polysaccharides have also been poorly studied. Understanding their specific elicitor effects on plant growth and defense mechanism might not only be a helpful tool in plant biotechnology but a big step in addressing modern agriculture challenges faced with an ever-increasing food demand and a changing climate.

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II-1. 3 SYNTHÈSE

Les polysaccharides sont des macromolécules de polymères glucidiques qui ont des structures complexes et diverses activités fonctionnelles (Nie et al. 2018). Il existe peu d'études sur les polysaccharides de microalgues présentant des propriétés biostimulantes et protectrices des plantes. Par conséquent, la relation directe entre la structure moléculaire des polysaccharides et leur activité bio-stimulante reste à comprendre. Néanmoins, la connaissance sur les extraits polysaccharides de macroalgues et leurs effets sur les voies biochimiques des plantes pourrait aider à comprendre les effets éliciteurs potentiels des polysaccharides de microalgues. (Michalak et al., 2017; Michalak et Chojnacka 2015). Par exemple, des recherches récentes ont révélé que les polysaccharides (carraghénane) des algues peuvent indirectement déclencher la croissance et la défense des plantes en modulant diverses voies biochimiques tels que la photosynthèse, l'assimilation de l'azote et du soufre et les voies biochimiques de la défense (Shukla et al. 2016). Les activités fonctionnelles des polysaccharides dépendent de leur composition monosaccharidique, de leur poids moléculaire et de leur degré de sulfatation. Ainsi, l'étude de la structure moléculaire des polysaccharides de microalgues, de leurs effets éliciteurs spécifiques sur la croissance des plantes ainsi que de leurs mécanismes de défense pourrait être un outil utile en biotechnologie végétale.

Les polysaccharides des microalgues ont montré une large gamme d'activités biologiques sur les plantes supérieures (El-Arroussi et al. En 2016, 2018; Farid et al. 2019) et pourraient avoir des propriétés multifonctionnelles en agriculture. Elles permettent de faciliter l'absorption des nutriments et d'améliorer la performance, l'état physiologique et la tolérance des plantes au stress abiotique (Barone et al. 2018, 2019; Borowitzka. 2016; de-Morais et al. 2015; Faheed et Fattah 2008; Garcia et Sommerfeld 2016; Renuka et al. 2018; Stadnik et Freitas 2014).

Le défi majeur dans la promotion des polysaccharides microalgales en tant que biostimulants des plantes en agriculture est de comprendre leurs activités biologiques et les fonctions spécifiques sur les plantes, dépendant de leur structure spécifique. Certaines études ont montré qu'en plus des sucres neutres et de la teneur en sulfate, les polysaccharides à faible poids moléculaire (PM) ont des effets plus importants sur les mécanismes des plantes (Stasio et al. 2018; Zha et al. 2016). Cela peut s'expliquer par le fait qu'un PM faible est généralement important pour la perméabilité des composés. Par conséquent, ce n'est pas seulement l'activité biologique des molécules ou leurs groupes fonctionnels spécifiques qui sont essentiels, mais aussi leur perméabilité.

Certaines études ont également montré que les mécanismes par lesquels les polysaccharides d'algues telles que les carraghénanes et leurs formes oligomères stimulent la croissance et les réponses de défense chez les plantes ; est en régulant la photosynthèse et la fixation du carbone chez les plantes, et en améliorant l'activité de la Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), ainsi que la division cellulaire. Il a également été observé que les carraghénanes et leurs formes oligomères modulent l'activité de

différentes voies de défense, y compris les voies de signalisation du jasmonate, du salicylate et de l'éthylène (González et al. 2013; Mercier et al. 2001; Shukla et al. 2016; Jiang et al. 2012).

Les polysaccharides de microalgues peuvent stimuler la croissance des plantes et l'absorption des nutriments en modulant diverses voies physiologiques et biochimiques. Dans son étude, González et al (2013) ont émis l'hypothèse que l'oligo-alginate et l'oligo-carraghénane pourraient interagir avec les récepteurs de la membrane plasmique qui utilisent un co-récepteur impliqué dans la transduction du signal conduisant à l'activation simultanée de la croissance des plantes.

Les polysaccharides sont également riches en groupes fonctionnels ayant la capacité de se lier à certains micro-éléments qui ont des valeurs nutritives importantes chez les plantes (Kaplan et al. 1987), améliorant ainsi la disponibilité des nutriments aux racines. Il a aussi été confirmé que les polysaccharides extraits des macroalgues peuvent agir comme des chélateurs d'ions métalliques parfaits. Cependant, Il est également possible de supposer que l'augmentation de l'absorption des nutriments pourrait être le résultat d'une meilleure surface racinaire observée chez les plantes traitées avec les polysaccharides. Ceci pourrait automatiquement améliorer l'accessibilité et l'absorption des nutriments. Par conséquent, il est nécessaire d'étudier les polysaccharides de microalgues et leurs effets spécifiques sur l'absorption des nutriments par les plantes et la disponibilité des nutriments dans le sol.

Les composés algaux présentent un grand potentiel pour améliorer non seulement la croissance des plantes et l'absorption des nutriments, mais aussi la résistance aux stress abiotiques et biotiques. Ce mécanisme peut s'expliquer par le fait que les polysaccharides contiennent des sucres neutres qui peuvent interagir avec les récepteurs de la membrane plasmique en tant que molécules de signal et conduire ainsi à l'activation d'une série de réactions biochimiques. Les polysaccharides d'algues pourraient être perçus par les récepteurs membranaires comme des modèles moléculaires associés aux microorganismes (MAMPs), induisant ainsi les voies de signalisation dépendantes des MAMPs. Cela implique l'activation de l'afflux de Ca^{2+}, la production de ROS, les voies octadécanoïdes et Phénylpropanoïdes, dont la Lipoxygénase (LOX) et la Phénylalanine ammoniac lyase (PAL) qui sont des enzymes clés (Farid et al. 2019; Kemmerling et al. 2011). Les polysaccharides d'algues peuvent aussi améliorer les activités antioxydants contre les ROS. Zou et al (2019), a montré que les polysaccharides de *Lessonia nigressens* (LPNs) induisaient efficacement l'activité des enzymes antioxydantes dans les semis de blé soumis au stress salin.

La différence en composition biochimique d'extraits bruts totaux de microalgues et d'extraits polysaccharidiques purifiés pourraient influencer leur activité biologique et effets biostimulants sur les plantes. Les propriétés biostimulantes des extraits totaux d'algues sont généralement attribuées à la présence de nombreux ingrédients bioactifs tels que les phytohormones, les composés phénoliques, les molécules organiques d'acides aminés et les métabolites secondaires bioactifs (Di Stasio et al. 2018). Néanmoins, il est difficile de déterminer les composés actifs des extraits totaux et leur mode d'action en raison de leur

diversité et de leur complexité. D'autre part, les polysaccharides sont des composés purifiés et beaucoup plus faciles à simuler, leurs propriétés biostimulantes étant généralement attribuées aux principaux sucres neutres, à la teneur en sulfate et au MW (Ponce et al. 2003; Qi et al. 2005 ; Sangha et al. 2010; Zha et al. 2016).

II-1. 4 CONCLUSION

Les microalgues pourraient être les nouveaux bioproduits durables pour le développement des biostimulants de plantes. Ces microorganismes photosynthétiques ne sont pas seulement des sources renouvelables mais durables et économiques de produits bioactifs et d'ingrédients alimentaires. Les polysaccharides de microalgues ont jusqu'à présent montré des propriétés bio-stimulantes qui se sont révélées utiles pour diverses applications industrielles. Bien que quelques études aient montré leur potentiel en tant que bio-éliciteurs des plantes et/ou biostimulants en agriculture, les modes d'action des polysaccharides de microalgues ne sont pas largement étudiés. Comprendre leurs effets spécifiques sur la croissance et le mécanisme de défense des plantes pourrait non seulement être un outil utile dans la biotechnologie végétale, mais aussi un grand pas pour relever les défis de l'agriculture moderne face à une demande alimentaire toujours croissante et au changement climatique.

CHAPITRE III-2

CHAPITRE II-2

Le criblage d'extraits liquides de microalgues pour leurs propriétés biostimulantes de la croissance des plantes, l'absorption des nutriments et le profil des métabolites de Solanum lycopersicum L.

II-2.1 INTRODUCTION

L'agriculture est continuellement confrontée à des contraintes causées par la population humaine croissante et les impacts écologiques du changement climatique, tels que les stress abiotiques, qui entravent la croissance et le développement des cultures agricoles (Parry, 2019; Wallace et al., 2003). Afin de maximiser la productivité et d'optimiser les conditions de croissance des plantes, des approches conventionnelles telles que l'irrigation, la mécanisation et l'application d'engrais NPK et de biocides ont été adoptées (Tilman et al., 2002). Cependant, en raison de l'impact néfaste de ces intensifications sur l'environnement et l'écosystème en général (Foley et al., 2011; Tilman et al., 2002), l'agriculture moderne est mise au défi d'adopter de nouvelles approches respectueuses de l'environnement. Aujourd'hui, les technologies innovantes basées sur les bioressources, y compris les biostimulants, se sont avérées être des méthodes efficaces pour améliorer les performances des cultures. Les biostimulants ne peuvent pas seulement modifier les processus physiologiques pour optimiser la productivité des cultures (Yakhin et al., 2017) mais peuvent améliorer l'absorption des nutriments, ce qui optimise finalement la consommation d'engrais et l'efficacité d'utilisation (Halpern et al., 2015). Ainsi, l'utilisation des biostimulants est une stratégie alternative durable pour améliorer le rendement des cultures et éviter la pollution de l'environnement. Un biostimulant n'est pas un "engrais" au vrai sens du terme, car sa fonction et son rôle dans la croissance des plantes sont indépendants de sa teneur en nutriments (Calvo et al., 2014), mais c'est un produit composé de substance(s) et/ou de micro-organismes qui stimulent les processus naturels chez les plantes conduisant à l'amélioration de la croissance, à l'efficacité d'utilisation des nutriments, la tolérance aux stress abiotiques et/ou à la qualité des cultures (European Biostimulant Industry Council EBIC, 2016). Yakhin et al., (2017) a également défini les biostimulants de plantes comme "un produit formulé d'origine biologique qui améliore la productivité des plantes en raison des propriétés nouvelles ou émergentes du complexe de constituants, et non en raison de la présence de nutriments essentiels de la croissance des plantes". Parmi les différentes catégories, les algues se sont jusqu'à présent révélées utiles pour la production de biostimulants de croissance végétale (Calvo et al., 2014; Craigie et al., 2009; Sharma et al., 2014). Cependant, moins d'attention est déployée sur les microalgues et les cyanobactéries dans ce domaine. Les microalgues (eucaryotes) et les cyanobactéries (procaryotes) sont des organismes photosynthétiques microscopiques unicellulaires qui poussent dans divers habitats aquatiques. Les microalgues et les cyanobactéries sont renouvelables et durables, et sont des bioressources économiques exploitées pour

plusieurs applications industrielles en tant qu'ingrédients alimentaires, produits bioactifs et matières premières pour la production de biodiesel (Khan et al., 2018). Plusieurs espèces de microalgues et de cyanobactéries ont présenté des qualités pharmacologiques et biologiques remarquables. Le potentiel de ces micro-organismes en tant que ressources pour les composés bioactifs est attribué à leur capacité de synthétiser des produits utiles tels que les polysaccharides, les protéines, les acides gras polyinsaturés, les lipides et d'autres métabolites bioactifs (Khan et al., 2018). De plus, certaines études ont signalé la présence de phytohormones (cytokinine, auxine, gibbérellines et brassinostéroïdes) dans les extraits cellulaires et le milieu de croissance de plusieurs espèces de microalgues (Bajguz, 2009; E et al., 2007). Ces phytohormones jouent un rôle clé dans la croissance et le développement des plantes. En outre, les produits de microalgues tels que les polysaccharides, peuvent améliorer la croissance des plantes et atténuer les effets du stress salin sur les plantes (El Arroussi et al., 2016).

Dans la présente étude, nous avons étudié les Bio-Extraits bruts (CBEs) de 18 espèces de microalgues et de cyanobactéries pour leurs effets biostimulants sur la croissance des plantes, l'absorption des nutriments et le profil des métabolites de *Solanum lycopersicum* L. Une étude comparative entre les propriétés biostimulantes des microalgues et des cyanobactéries d'eau douce et d'eau de mer a également été mise en évidence. L'objectif de cette étude est de montrer le potentiel des microalgues en tant que matériaux bioactifs pour le développement de bioproduits en agriculture.

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Screening of microalgae liquid extracts for their bio stimulant properties on plant growth, nutrient uptake and metabolite profile of *Solanum lycopersicum* L.

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The present study investigates the biostimulant effects of 18 Crude Bio-Extracts (CBEs) obtained from Microalgae and Cyanobacteria on tomato plant growth, chlorophyll content, nutrient uptake and metabolite profile. Significant root and shoot length improvement (112.65%, 53.70%); was recorded at treatment with *Aphanothece* sp and *C. ellipsoidea* CBEs respectively. Meanwhile, the highest root and shoot dry weight (DW) (34.81%, 58.69%) were obtained at treatment with *Aphanothece* sp. The latter also displayed the maximum uptake of Nitrogen, phosphorus and potassium, which increased by 185.17%, 119.36% and 78.04% respectively compared with non-treated plants. Principal Component Analysis (PCA) confirmed that Phosphorus and Potassium levels in roots were closely related to enhanced Root length, whereas Nitrogen and chlorophyll b were closely related to Shoot and root DW. Additionally, Gas Chromatography-mass spectrometry (GC-MS) indicated that treatment with CBEs, induced the production of a vast array of metabolites. Treated plants recorded higher accumulation of palmitic and stearic acids, which could indicate a stimulation in *de novo* Lipid synthesis. CBEs also triggered the accumulation of pyridine-3-carboxamide (an amide active form of vitamin B3) and Linolenic acid; one of the key precursors in the biosynthetic pathway leading to plant jasmonates. Our results are a first step towards understanding the effects of microalgal extracts on plant physiology and biochemical pathways. Further investigations on biochemical fractionation of microalgal extracts and agronomic tests of their purified bioactive compounds could be a useful principal novelty for in-depth study of CBE action mechanisms. Other useful tools include; Comparative hormone profiling of treated and non-treated plants accompanied with combined High-Throughput Plant Phenotyping, transcriptomics and metabolomics analysis.

As the world population increases, concomitantly with the ecological impacts of climate change, agriculture is continuously faced with constraints, such as abiotic stresses, which severely hamper plant growth and development[1,2]. In order to maximize crop productivity and optimize plant growth conditions, conventional approaches such as irrigation, mechanization and the application of NPK fertilizers and biocides have been adopted[3]. However, due to the harmful impact of these intensifications on the environment and the ecosystem at large[3,4], modern agriculture is challenged to adopt novel eco-friendly approaches. Today, the development of innovative technologies based on bioresources including plant biostimulants, have proven to be effective methods for improved crop performance. Plant biostimulants cannot only modify physiological processes to optimize productivity in crops[5] but can enhance nutrient uptake, which ultimately optimize fertilizer consumption and use

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efficiency[6]. Thus, the use of plant biostimulants is a sustainable alternative strategy to improve crop yield and avert environmental pollution. A plant biostimulant is not a "fertilizer" in the true sense because it's function and role in plant growth is independent of its nutrient content[7], but it's a material composed of substance(s) and/or microorganisms that stimulate natural processes in plants, leading to enhanced plant growth, nutrient use efficiency, tolerance against abiotic stresses and/or crop quality (European Biostimulant Industry Council EBIC, 2016)[5]. also defined plant biostimulants as; "*a formulated product of biological origin that improves plant productivity as a consequence of the novel, or emergent properties of the complex of constituents, and not as a sole consequence of the presence of known essential plant nutrients, plant growth regulators, or plant protective compounds*". Among the various categories, seaweeds have so far proven useful for the production of plant growth biostimulants[7–9]. However, far less attention has been focused on Mmicroalgae and cyanobacteria in this field. Microalgae (eukaryotic) and cyanobacteria (prokaryotic), are unicellular microscopic photosynthetic organisms that grow in diverse aquatic habitats and even humid soils. Renewable and sustainable, microalgae and cyanobacteria are economical bioresources that have been exploited for several industrial applications as food ingredients, bioactive products and raw materials for biodiesel production[10]. Several microalgae and cyanobacteria species have exhibited remarkable pharmacological and biological qualities. The potential of these microorganisms as resources for bioactive compounds is attributed to their remarkable capacity to synthesise useful products such as polysaccharides, Proteins, Polyunasturated fatty acids, lipids and other bioactive metabolites, from atmospheric CO_2[10]. Moreover, some studies have reported the presence of phytohormones (cytokinin, auxin, gibberellins, and brassinostreroids) in cellular extracts and growth medium of several microalgae species[11,12]. These phytohormones play key roles in plant growth and development. Also, microalgae products such as polysaccharides, can enhance plant growth and alleviate the effects of saline stress on plants[13–15]. In the present study, we investigated Crude Bio-Extracts (CBEs) of 18 Microalgae and Cyanobacteria species for their biostimulant effects on plant growth, nutrient uptake and metabolite profile of *Solanum lycopersicum* L. In addition, biochemical pathways of fresh and sea water organisms and subsequently their biochemical composition, may differ due to diverse adaptation mechanisms. Thus, a comparative study between the biostimulant properties of fresh and sea water Microalgae and Cyanobacteria was also highlighted. The aim of this research is to demonstrate the potential of microalgae as bioactive materials for bioproduct development in agriculture.

Results

Hydrolysis type and partial characterisation of the 18 shortlisted CBEs. CBEs of the 18 species of Microalgae and Cyanobacteria were extracted by acid hydrolysis at three different Sulfuric acid (H_2SO_4) concentrations (0M, 0.1M and 0.2M) and tested on plant growth parameters of tomato (*Solanum lycopersicum* L.) at three different doses (0.1, 0.5, and 1 g/L), under laboratory conditions. Therefore, a total of 162 Extracts were tested on plants, from where 18 CBEs were shortlisted based on their positive effects on plant development.

Partial characterization of the shortlisted CBEs revealed that they contained polysaccharides, soluble sugars, amino acids as well as diverse mineral elements such as nitrogen (N), phosphorus (P) and potassium (K). Table S1 shows the composition and hydrolysis type of the 18 shortlisted CBEs.

Growth and Biomass concentration of the selected microalgae and Cyanobacteria species. All microalgae and cyanobacteria species showed growth increase during the 30-day culture period, no decline in cell density was recorded (Table 1). The mean growth rate, doubling times and biomass accumulation of all microalgae and cyanobacteria species were evaluated. Table 1 is presented in two groups for easier comparison: freshwater and seawater species of microalgae and cyanobacteria.

Cyanobacteria and freshwater microalgae species exhibited the highest growth rates, ranging from 0.093 d^{-1} to 0.135 d^{-1}. Whereas, seawater microalgae species exhibited lower growth rates varying from 0.067 d^{-1} to 0.096 d^{-1}. Additionally, the final biomass concentrations of microalgae and cyanobacteria greatly varied from one specie to another.

Effect of CBEs on tomato root growth under laboratory conditions. To investigate the effect of Microalgae and Cyanobacteria crude extracts (CBEs) on tomato growth, the extracts were applied to tomato seedlings. Except for *Tetreselmis sp* and *I. gabana*, all CBEs displayed significant ($p < 0.05$) effect on root length compared with control plants (Fig. 1a). The highest percentage increases on root length (112.65%, 89.87%, 84.80%, and 70.88%) were recorded in plants treated with, *Aphanothece sp.*, *A. maxima*, *C. pyrenoidosa* and *C. ellipsoidea* extracts of freshwater species respectively. Whereas the highest root length increase from seawater species was recorded at *N. gaditana* CBE treatment (90.38%) (Fig. 1a). Also, *D. salina*, *I. galbana* and *C. sorokiniana* CBEs exhibited the lowest effects on root length.

The maximum root dry weight (DW) increases (34.81%, 29.11%, 28.48% and 27.21%) were obtained respectively at *Aphanothece sp.*, *C.ellipsoidea*, *A. maxima* and *C. pysrenoidosa* CBE treatments, for freshwater species while the highest root DW increase (24.68% and 19.62%) for seawater species was recorded respectively at *T. suecica* and *Porphyridium* sp. CBEs treatments (Fig. 1b).

Effect of CBEs on shoot growth under laboratory conditions. CBE treatment exhibited relatively lower effects on shoot length as compared to root length. In the case of tomato plants treated with cyanobacteria and freshwater microalgae, we observed an increase of 53.6%, 45.68% and 35.8% in shoot length for *C. ellipsoidea*, *Aphanothece* sp. and *S. obliquus* CBEs respectively, compared to non-treated control plants (Fig. 2a). Whereas, highest increases in shoot lengths (45.67% and 35.18%) were recorded at treatment with *Porphyridium* sp. and *C. marina* CBEs respectively for seawater microalgae. No significant differences in shoot length were observed in tomato plants treated with *I. galbana* or *C. sorokiniana* extracts (Fig. 2a).

	Mean Growth rate ((μ) day^{-1})	Doubling time (days)	Biomass Concentration (gL^{-1})
Cyanobacteria			
Aphanothese sp.	0.283 ± 0.075	2.445	0.236
Arthrospira maxima	0.213 ± 0.103	3.253	0.886
Arthrospira platensis	0.201 ± 0.093	3.449	0.936
Chlorophyta			
Chlorella pyrenoidosa	0.159 ± 0.054	4.359	0.598
Chlorella vulgaris	0.224 ± 0.050	3.089	0.548
Chlorella ellipsoidae	0.154 ± 0.034	4.486	0.49
Chlorella sorokiniana	0.205 ± 0.043	3.376	0.562
Chlorella marina	0.128 ± 0.049	5.421	0.237
Scenedesmus dimorphus	0.225 ± 0.082	3.072	0.59
Scenedesmus obliquus	0.225 ± 0.029	4.688	0.979
Chlamydomonas reinhardtii	0.264 ± 0.086	2.625	0.574
Dunaliella salina	0.115 ± 0.035	5.983	0.736
Tetraselmis marina	0.137 ± 0.030	5.061	0.735
Tetraselmis sp.	0.108 ± 0.021	6.383	0.506
Tetraselmis suecica	0.111 ± 0.022	6.219	0.4
Rhodophyta			
Porphyridium sp.	0.245 ± 0.073	2.82	0.283
Haptophyta			
Isochrysis galbana	0.131 ± 0.046	5.255	0.643
Ochrophyta			
Nannochloropsis gaditana	0.127 ± 0.025	5.473	0.39

Table 1. Growth and Biomass concentration of the selected microalgae and cyanobacteria at 30-days old. The growth rate is represented as the mean of 3 replicate cultures ± the standard error (n = 3), doubling times were evaluated from the mean growth rate.

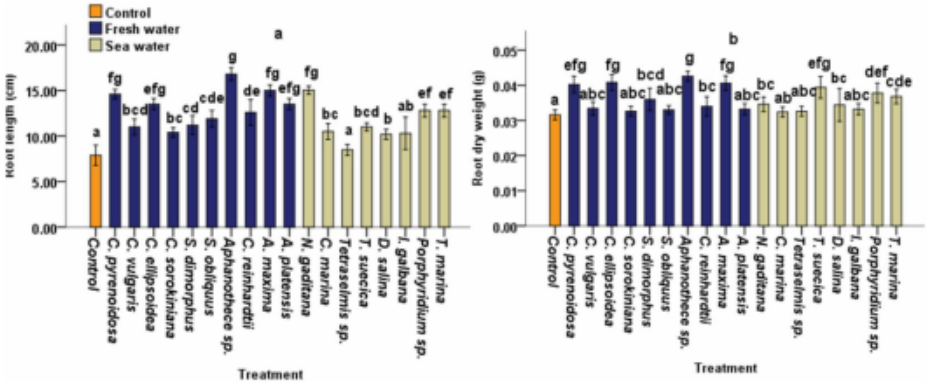


Figure 1. *Effects of CBEs* applications on (**a**) root length (**b**) root DW of 40 days old tomato plants. Data represent means and standard errors (error bars) of five biological replicates. Different letters indicate significant difference ($p < 0.05$), according to Turkey, One-way ANOVA.

The highest increases in shoot DW (58.69%, 55.66% and 49.60%) for cyanobacteria and freshwater microalgae were obtained at treatment with *Aphanothece sp*, *C. ellipsoidea* and *C. pyrenoidosa* respectively, while seawater microalgae recorded a 40.70%, 40.22% and 38.09% increase at *Porphyridium sp.*, *N. gaditana* and *T.* marina treatment respectively, compared with non-treated control plants (Fig. 2b).

Leaf concentrations of photosynthetic pigments. Chlorophyll a, Chlorophyll b and Carotenoid concentrations in tomato leaves were evaluated 35 days after treatment. The results illustrated in Fig. 3a,b show a significant change in photosynthetic pigments in the majority of treated plants.

Chlorophyll a. CBEs displayed highest values at treatment with *Aphanothece* sp. and *C. ellipsoidea* extracts, which increased by 42.00% and 40.36% respectively compared with the control. Whereas the highest increase in

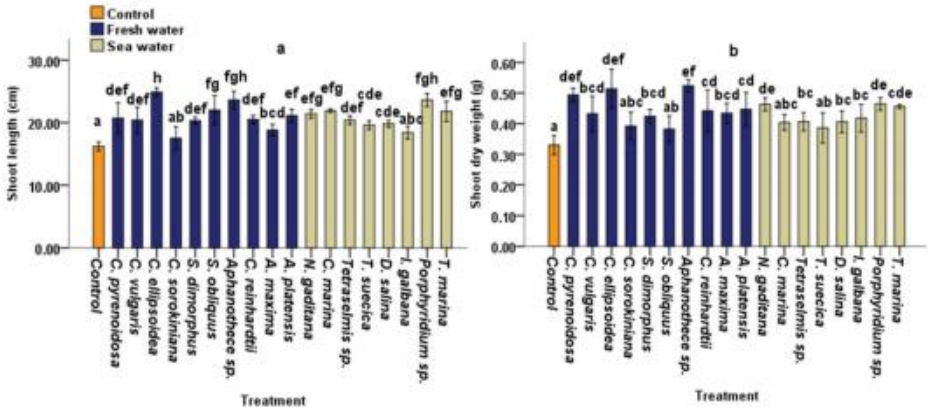


Figure 2. Effects of CBEs applications on (**a**) shoot length, (**b**) shoot DW of 40 days old tomato plants. Data represent means and standard errors (error bars) of five biological replicates. Different letters indicate significant difference ($p < 0.05$), according to a Turkey, One-way ANOVA.

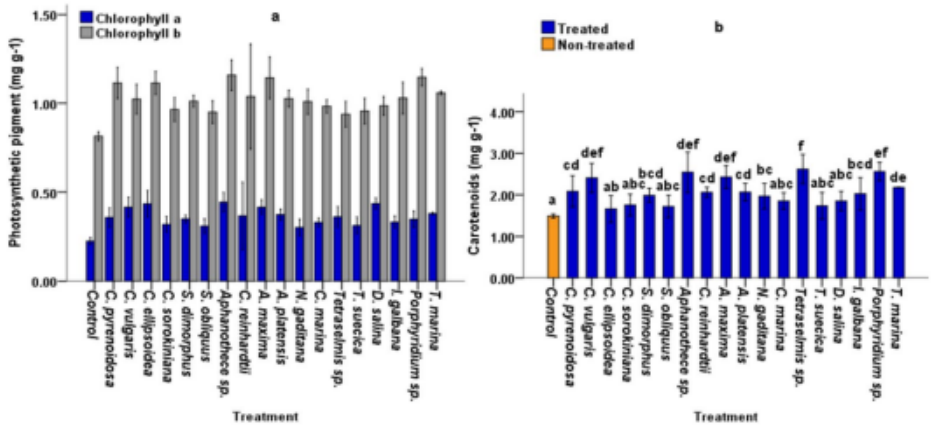


Figure 3. Leaf concentrations of photosynthetic pigments; chlorophyll a (mg g−1), chlorophyll b (mg g−1) (**a**); and Carotenoids (mg g−1) (**b**) in 40 days old tomato plants under laboratory conditions. five replicates were used. Different letters indicate significant difference ($p < 0.05$), according to a Turkey, One-way ANOVA.

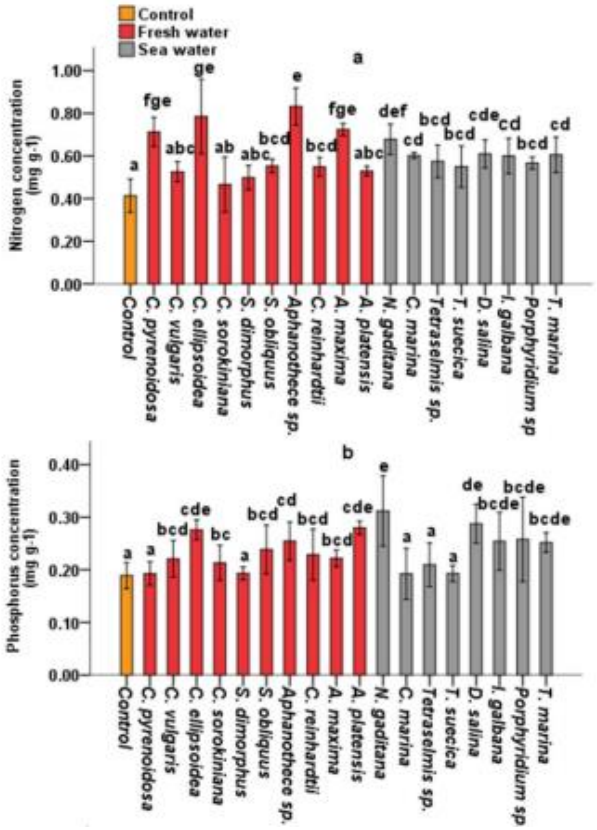
Chlorophyll a content (40.63% and 36.70%) of seawater microalgae were obtained with *D. salina* and *Tetraselmis* sp crude extracts (Fig. 3a).

Chlorophyll b. A similar trend was observed in Chlorophyll b content, the maximum increase in chlorophyll b content (92.45%, 92.28% and 83.95%) across all treatments was observed in plants treated with *Aphanothece sp.*, *A. maxima* and *C. pyrenoidosa* extracts respectively compared with non-treated control plants (Fig. 3a) while the highest Chlorophyll b content for seawater microalgae was observed at treatment with *Tetraselmis sp.* and *N. gaditana* extracts, which increased by 93.31% and 83.95% respectively compared to control plants (Fig. 3a).

Carotenoids. All CBEs displayed significant increases in carotenoid content (Fig. 3b) ($p < 0.05$). The highest values of the carotenoids content were obtained at treatment with *Tetraselmis sp, N. gaditana* and *Aphanothece* sp, extracts, which increased by 129.83, 124.75 and 123.05% respectively compared with control plants (Fig. **b**).

Conclusion. On plant growth parameters, we did not observe a clear-cut difference between the bio-stimulatory effects of CBEs extracted from fresh and seawater Microalgae or Cyanobacteria. These results illustrate that the bio-stimulant properties of microalgae and cyanobacteria are independent of their water sources.

Root concentrations of Nitrogen, Phosphorus and Potassium. *Nitrogen (N).* Among the three macro elements, N was highly enhanced in all treated plants compared with non-treated plants. The highest N concentrations were recorded in plants treated with *Aphanothece* sp, *C. ellipsoidea* and *A. maxima* extracts, which



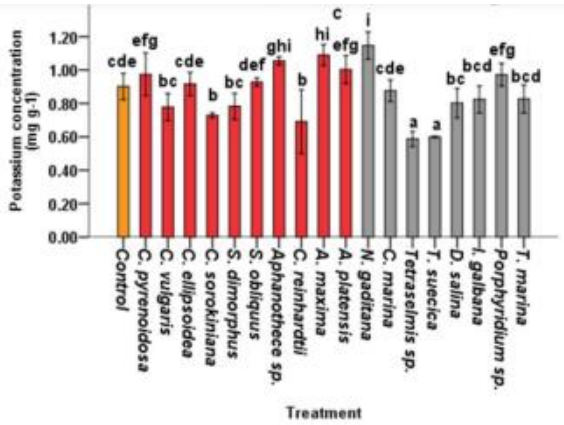


Figure 4. Root concentrations of Nitrogen (mg g^{-1}) (**a**), Phosphorus (mg g^{-1}) (**b**) and Potassium (mg g^{-1}) (**c**) in 40-day old tomato plants under laboratory conditions. Five replicates were used. Different letters indicate significant difference ($p < 0.05$), according to a Turkey, One-way ANOVA.

increased by 101.07%, 89.79% and 75.04%, respectively compared to non-treated control plants. Whereas the lowest percentage increase (12.64%) was recorded at treatment with *C. sorokiniana* extracts (Fig. 4a).

Phosphorus (P). Tomato plants treated with *Aphanothece sp, C. ellipsoidea* and *C. pyrenoidosa* extracts exhibited the highest P increase (65.08%, 52.15% and 48.11%) compared to control plants, whereas treatment with *S. dimorphus*, *C. marina* and *T. suecica* extracts, recorded the lowest effect on P concentration in tomato root biomass (1.72%, 2.13% and 4.54%) respectively (Fig. 4b).

Potassium (K). CBE application had the least effect on K concentration in roots. (Fig. 4c) illustrated that the treatment of *C. sorokiniana*, *S. dimorphus, Tetraselmis sp*. and *T. suecica* extracts decreased K concentrations in

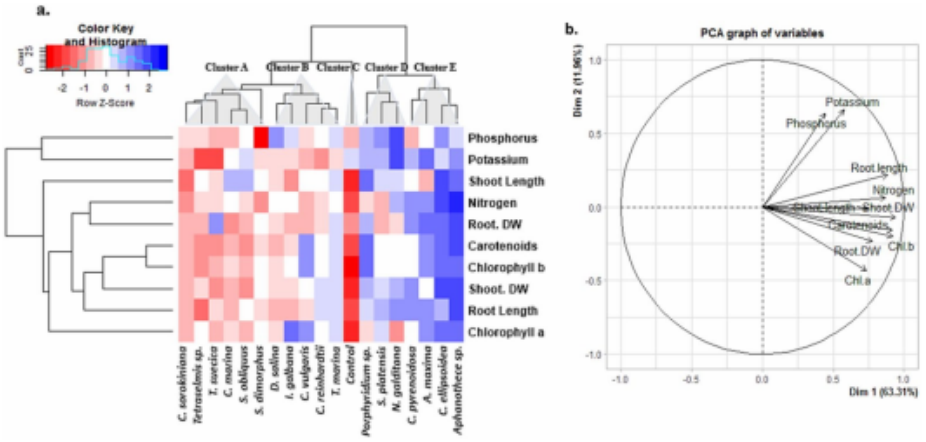


Figure 5. Hierarchical clustering and principal component analysis (PCA) to understand variable associations in treated tomato plants. CBEs were associated with five major clusters. (**a**) The entire data were analysed using PCA. The variables included root DW (root dry weight), shoot DW (shoot dry weight), Root length, Shoot length, Chl a (chlorophyll a), Chl b (chlorophyll b), Carotenoids (Carotenoids), Nitrogen, Phosphorus and Potassium. The lines originating from central point of PCA plot indicate positive or negative correlations of different variables (**b**); where their closeness indicates correlation strength with particular treatment S3.

roots by 16%, 20.53% and 27. 36.97% compared to non-treated control plants (0.902 mg g^{-1}). The highest K levels were observed at treatment with *Aphanothece sp, C. ellipsiodea* and *T. marina* extracts which increased by 78.04%, 67.37% and 38.50% compared to the control.

Treatment-variable Interactions through hierarchical clustering and PCA analysis. Hierarchical clustering and principal component analysis (PCA) were performed to understand treatment-variable relationships in treated plants (Fig. 5b). The data of different parameters were normalized. The clustering analysis represented the normalized average mean values of all the studied parameters in both treated and control plants. PCA analysis revealed that Phosphorus and Potassium levels in root biomass were positively associated to Root length. This could indicate that Phosphorus and Potassium uptake was influenced by the Root length (Fig. 5b). PCA also showed that root Nitrogen concentration was more closely associated to Shoot DW, which was associated to chlorophyll b, indicating a correlation between Nitrogen uptake, chlorophyll b and shoot biomass accumulation in treated plants (Fig. 5b).

Metabolomics using GC-MS approach. To further evaluate the effect of CBEs on plant growth and development, a comparative metabolite profile analysis of treated and non-treated control plants, was performed using GC-MS. CBEs effect on plant growth were clustered in 5 major groups (Fig. 5a). For GC-MS comparative metabolite analysis, 9 CBEs were randomly selected across the 5 cluster groups:

- Control plants
- *1 CBE (Tetraselmis* sp.) from cluster A (CBEs with the least effect on the majority of the studied parameters),
- *2 CBEs (D. salina* and *I. galbana)* from cluster B,
- *2 CBEs (Porphyridium* sp. and *N. gaditana)* from the cluster D.
- All CBEs from cluster E*; Aphanothece sp., C. ellipsoidea, A. maxima and C. pyrenoidosa* (CBEs with the highest on the majority of the studied parameters).

Treatment with CBEs, induced the production of a vast array of metabolites (Table. S6).

Comparative metabolite profile of treated and non-treated control plants. Treatment with CBEs significantly influenced the profile of detected metabolites compared with the non-treated plants, a larger spectrum and higher concentration of metabolites was detected in all treated plants (Table. S6). Some metabolites including palmitic acid, stearic acid and linolenic acids, eicosane, phytol, pyridine-3-carboxamide were detected in significantly higher concentrations compared to non-treated control plants (Fig. 6). We observed the highest enhancement of palmitic acid (9372%, 4117%, 1539, 1110% and 1022%), stearic acid (12510%, 2635%, 2343%, 734% and 1137%) and linolenic acid accumulation (5185%, 2186%, 778%, 673% and 561%) at treatment with *Tetraselmis* sp, *D. salina*, *N. gaditana*, *Aphanothece* sp and *A. maxima* CBE respectively (Fig. 6). A similar trend was observed in phytol concentrations where the highest phytol enhancements (8947%, 5329%, 3310%, 2044% and 1973%) were recorded at Treatment with *Tetraselmis* sp, *D. salina*, *N. gaditana*, *Aphanothece* sp and *A. maxima* CBEs respectively (Fig. 6). The highest Pyridine-3-carboxamide enhancements (15227%, 10808%, 5440, and 3012%) were also recorded at treatment with *D. salina*, *Tetraselmis* sp, *N. gaditana*, and *I. galbana* CBEs respectively

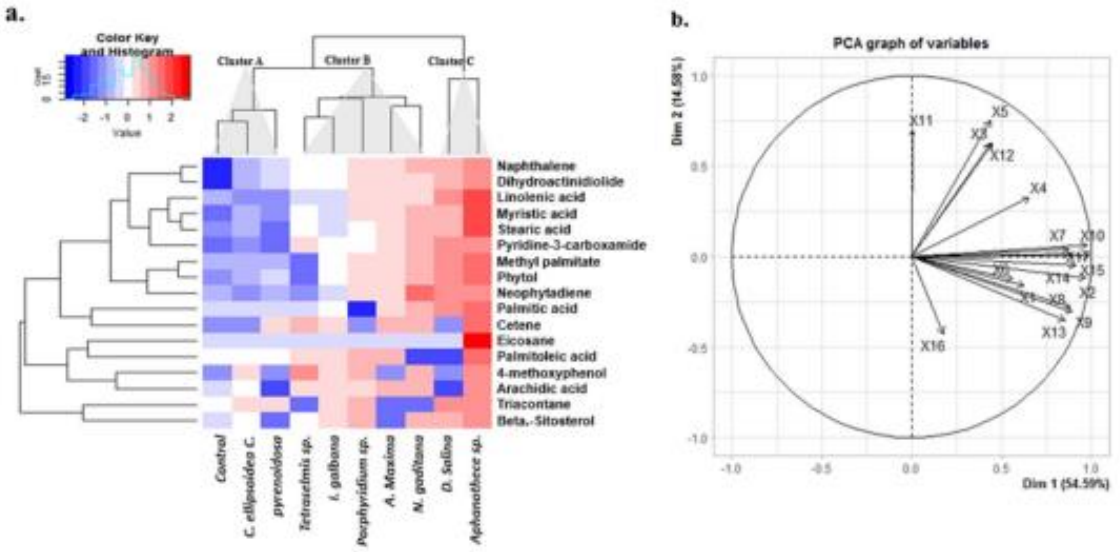


Figure 6. Hierarchical clustering and principal component analysis (PCA) of 17 metabolites detected in high concentration in 40 day old treated and non-treated tomato plants, under laboratory conditions. The Heat-map represents the normalized mean values (centered and scaled) of leaf metabolites of three replicates. The variables included: X1 = Beta.-Sitosterol, X2 = Linolenic acid, X3 = Cetene, X4 = Eicosane, X5 = Arachidic acid, X6 = Palmitic acid, X7 = Naphthalene, X8 = Neophytadiene, X9 = Methyl palmitate, X10 = Stearic acid, X11 = Palmitoleic acid, X12 = 4-methoxyphenol, X13 = Phytol, X14 = Pyridine-3-carboxamide, X15 = Myristic acid, X16 = Triacontane, X17 = Dihydroactinidiolide. Refer to Fig. S5 for variable -treatment b-Plot and Table S6 for all metabolites detected under different treatments.

Conclusion. *Although CBEs extracted from Tetraselmis sp. exhibited less effect on root and shoot sizes*, Fig. 6 *illustrates that Tetraselmis sp. CBE treatment recorded the highest effect on plant metabolite profile. Thus, the effect of plant biostimulants cannot only be evaluated on the basis of plant growth. Our results suggest that besides plant growth, some extracts trigger other biochemical processes such as the accumulation of lipophilic metabolites, which are related to plant abiotic stress tolerance and defence. The accumulation of Pyridine-3-carboxamide could also reflect more on crop quality as opposed to crop productivity. Therefore, microalgae and/or cyanobacteria based bio-products could be orientated for diverse application purposes in agriculture.*

Discussion

Our study demonstrated that CBEs can enhance growth and nutrient uptake in treated plants. Understanding the effects of CBEs on regulating physiological and biochemical pathways related to plant growth, nutrient uptake and Metabolomic profile can play a vital role in improving agriculture production. Our study is the first step towards such understanding.

The application of CBEs to tomato seedlings improved chlorophyll content, nutrient uptake from the soil and eventually, the root and shoot length and dry weight. Notably *Aphanothece* sp CBEs, significantly enhanced root length (112.65%), root and shoot DW (34.81% and 58.69%) respectively (Figs. 1 and 2b) while *C.ellipsoidea* CBEs played a significant positive role in improving the shoot length (53.70%) (Fig. 2a). Previous studies have shown that algal extracts and their purified compounds can induce strong physiological responses in plants and increase shoot and/or root weight[5,16–20]. In addition, microalgae extracts such as polysaccharides have proven to enhance growth of agricultural crops[13,15,21,22]. Sugars for instance act both as effective signalling molecules and as immediate substrates for intermediary metabolism, and could therefore promote plant growth and development[20,23]. A role for macro- and microelements, vitamins and phytohormones in alga extracts may also be suggested[11,12,20,24]. For instance[16], reported that Potassium in seaweed extracts have a positive effect on regulating water status in treated plants and enhancing photosynthesis and meristematic growth. In the present study. CBEs contained various concentrations of sugars, proteins and NPK (Table 1). However, taking into consideration the CBE concentrations applied to plants, we could rule out the contribution of macroelements: Thus, the beneficial effects may involve several plant growth-promoters working synergistically.

N, P and K are three important macro elements frequently added as fertilizers in modern agricultural schemes. Therefore, this study targeted NPK concentrations in roots to evaluate the effect of CBEs on nutrient uptake. PCA analysis confirmed that improved P and K levels in roots was closely associated with enhanced root length (Fig. 5b), indicating that improved nutrient uptake observed in treated plants could also be attributed to increased root size. This is explained by the fact that enhanced root growth increases the root surface area and directly improves nutrient accessibility and uptake.

Treatment of plants with CBEs could also increase chlorophyll concentration in leaves (Fig. 3). The maximum increase in chlorophyll b content (92.45%, 92.28% and 83.95%) across all treatments were observed at treatment with *Aphanothece sp*., *A. maxima* and *C. pyrenoidosa* extracts for freshwater species respectively, and *Tetraselmis sp*. and *N. gaditana* extracts for seawater species, which increased by 93.31% and 83.95% respectively compared with control plants (Fig. 3). Our results are coherent with previous studies; which reported increased chlorophylls

in plants treated with seaweed extracts. This increase in chlorophylls was attributed to reduced chlorophyll degradation and delayed plant senescence[7,25]. Increased chlorophyll content was also reported in *Brassica chinensis* L. treated with Kelp water extracts[20]. In addition, CBEs extracts might have several effects on photosynthetic pathways[26]. reported that genes involved in carbon fixation, including rubisco and carbonic anhydrase were upregulated by algal extracts. Besides chlorophyll content, improved nutrient uptake highly influence photosynthetic efficiency and consequently, plant biomass accumulation[27,28]. reported that N levels in plants is strongly correlated with photosynthetic rate while improved photosynthesis is recognized as one of the most efficient ways to increase nitrogen use efficiency and crop yield[29]. Additionally[30], proposed that further increases in crop yield potential will rely in large part on improved photosynthesis. In this study, N concentration in root biomass was closely associated with Root DW, Shoot DW and Chlorophyll b (Fig. 5b), indicating that N taken up by roots was partly invested in photosynthetic machinery. Our results are coherent with[31], who reported that the majority of assimilated N in plants is invested in photosynthetic machinery and is therefore fairly strongly positive correlated with photosynthetic rate[27,28]. Apart from directly taking part in photosynthesis, N also acts as an important regulator in manipulation of CO_2 diffusion[32–34].

Primary and secondary metabolite profiles are another indispensable component of crop performance, photosynthesis and biomass accumulation[35]. The major phytochemical groups detected by GC–MS analysis in this study included fatty acids, alkanes, alkenes, alcohols, terpenes, tocopherols and sterols (Fig. 6). Groups found in very high concentrations included fatty acids, alkanes, alkenes and alcohol terpenes. In the present study, phytols were the most abundant alcohol terpenes detected in all plants, with the highest phytol enhancement (8947%, 5329%, 3310%, 2044% and 1973%) recorded at Treatment with *Tetraselmis* sp, *D. salina*, *N. gaditana*, *Aphanothece* sp and *A. maxima* CBEs respectively (Fig. 6). Phytol is an acyclic diterpene alcohol and a constituent of chlorophyll that is generated from chlorophyll breakdown[36–38]. Phytols are essential in the biosynthesis of tocopherols which is a well-known lipid antioxidant and contribute to the protection of photosystem II (PSII) against photodamage under environmental stress[37,39]. On the other hand, a large proportion of phytol and *fatty acids* is converted into *fatty acid phytyl* esters in chloroplasts, which are involved in maintaining the integrity of the photosynthetic membrane during abiotic stress and senescence in *Arabidopsis thaliana*[40]. However, very high accumulation of phytyl fatty acids was only recorded in plants treated with *Aphanothece* sp and *A. maxima* CBEs (1088% and 1008%) respectively (Table S6). It could be suggested that the accumulated phytol observed in treated plants and coherent with chlorophyll content, could have been generated from chlorophyll breakdown during plant metabolite extraction. Phytol accumulation was also closely associated with palmitic acid (C16:0), linolenic acid (C18:3) and stearic acid (C18:0) concentration (Fig. 6). Increased palmitic C16:0 and stearic C18:0 acid accumulation in treated plants might indicate enhanced lipid biosynthesis. Literature has reported that the first stage of *de novo* lipid synthesis in plants commences with the generation of C16 and C18 fatty acids. These fatty acids are then elongated to very long chain fatty acids (VLCFAs), and mainly incorporated in sphingolipids (structural elements in lipid bilayers of the cell membrane) and cuticular wax layers[41,42], thereby helping plants reduce water

loss and pathogen invasion. In addition, our study demonstrated that all treated plants exhibited higher total fatty acid concentrations, indicating the biostimulant effects of CBEs on plants' lipid profile.

CBEs also enhanced the accumulation of pyridine-3-carboxamide in all treated plants. Pyridine-3-carboxamide, also known as Nicotinamide or niacinamide is an amide active form of Vitamin B3[43]. Pyridine-3-carboxamide is the primary precursor of an essential coenzyme in ATP production known as nicotinamide adenine dinucleotide (NAD+), and the sole substrate of poly-ADP-ribose polymerase-1 (PARP-1)[43]. Today, Pyridine-3-carboxamide is commercially exploited in vitamin supplements and cosmetic products. In the present study, higher accumulation of metabolites such as pyridine-3-carboxamide in treated tomato plants indicates that CBEs may improve the biochemical composition and subsequently, the nutritional quality of crop products.

In conclusion, our study reveals the remarkable biostimulant properties of crude microalgae and cyanobacteria extracts. The comparative study between the biostimulant properties of fresh and seawater microalgae and cyanobacteria did not highlight any clear-cut differences, suggesting that the bio-stimulant properties of microalgae and cyanobacteria is independent of their water sources. PCA analysis illustrated a close relation between enhanced shoot DW and chlorophyll b content, indicating that shoot DW was attributed to improved photosynthetic capacity. In addition, CBEs also improved N uptake, which may contribute to an improved nitrogen metabolism and consequently enhance photosynthetic efficiency. In addition, our research demonstrated that treatment with CBEs induces the production of a vast array of plant metabolites. CBEs triggered the accumulation of linolenic acid (C18:3), one of the key precursors in the biosynthetic pathway leading to plant jasmonates, and pyridine-3-carboxamide which is an amide active form of vitamin B3. Treated plants also exhibited higher concentrations of palmitic (C16:0) and stearic (C18:0) acids, suggesting a stimulation in *de novo* Lipid synthesis. Some CBEs such as *Tetraselmis* sp., with the lowest effect on plant growth, triggered the production and accumulation of several plant metabolites, indicating that the effect of plant biostimulants cannot only be evaluated on the basis of plant growth, some extracts trigger other biochemical processes such as the accumulation of lipophilic metabolites, which are related to plant abiotic stress tolerance and defence.

The present study highlights the potential of CBEs for enhancing plant growth, nutrient uptake and metabolomic pathways. However not all microalgae species have significant biostimulant activities on plant growth parameters, suggesting that the biostimulant properties of CBEs are due to "specie-specific" metabolites produced by particular microalgae or cyanobacteria species. Our results are a first step towards understanding the effects of microalgal extracts on plant physiology and biochemical pathways. Further investigations on biochemical fractionation of microalgal extracts and agronomic tests of their purified bioactive compounds could be a useful principal novelty for in-depth study of CBE action mechanisms. Other useful tools include; Comparative hormone profiling of treated and non-treated plants accompanied with combined High-Throughput Plant Phenotyping, transcriptomics and metabolomics analysis.

Materials and Methods

Culture of microalgae. In this study, we used 18 Microalgae and Cyanobacteria species from the AlgoBioTech collection of the MAScIR Foundation (Moroccan Foundation for Advanced Science, Innovation and Research). we selected *Chlorella pyrenoidosa, Chlorella ellipsoidea, Chlorella vulgaris, Chlorella sorokiniana, Chlamydomonas reinhardtii, Aphanothece sp., Scenedesmus obliquus, Scenedesmus dimorphus, Arthrospira platensis, spirulina maxima, Dunaliela salina, Porphyridium sp., Tetraselmis suecica, Tetraselmis marina, Tetraselmis sp., Nanochloropsis gaditana, Isochrysis galbana* and *Chlorella marina*. The microalgae and Cyanobacteria species were cultivated in 3 replicates using 250 mL Erlenmeyer flasks containing culture mediums, according to the optimised standard culture medium protocols of MAScIR. The culture mediums were first selected based on the water source of Microalgae and Cyanobacteria. All softwater microalgae species and *Aphanothece* sp. were cultured in BG11 medium at pH = 7.2 whereas *Arthospira plantesis* and *Arthospira maxima* were cultured in Zarrouk's medium at pH = 9.5. All sea water microalgae species were cultured in F2 medium at pH = 7.8, with the exception of Dunaliela salina, which was cultured in Walne's medium. The culture mediums for all seawater species were made with sea-salt solution. The Microalgae and Cyanobacteria species were grown in their respective culture mediums at a standard temperature of 25 °C, 140 rpm of agitation and 135 µmol photons m^{-2} s^{-1} of continuous white fluorescent light. The harvesting of the Microalgae biomass was done by centrifugation at 6000 rpm or 5 minutes and the biomasses collected was dried at 50 °C for 7 days.

Determination of microalgae growth characteristics. The growth of microalgae and cyanobacteria was monitored every 2 days by measuring the optical density of the culture at 680 nm, using a UV/VIS spectrophotometer. The initial optic density for all cultures was = 0.1 (±0.02). However, according to cell size and specific growth rates of Microalgae and Cyanobacteria species in the respective culture mediums used in this study, the final optic density and fresh biomass varied from one specie to another. The total fresh biomass for each specie, was harvested by centrifugation at 6000 rpm for 5 minutes and dried at 50 °C for 7 days.

The measured optical density was used to study the growth kinetics via the increase in density (in logarithmic scale) versus time. Growth rates were calculated using the following equations[44]:

$$G = \ln(C2/C1)/(t2 - t1) \quad (1)$$

Where C1 and C2 indicates cell Concentrations at time 1 (t1) and time 2 (t2), respectively. Doubling time was also calculated once the specific growth rate was known.

$$\text{Doubling time} = \ln 2/G \quad (2)$$

Preparation of microalgae extracts by Acid hydrolysis. 0.3 g dry biomass of each one of the 18 Microalgae and Cyanobacteria species was grinded in liquid nitrogen and hydrolysed in 20 mL of H_2SO_4 at three different concentrations (0M, 0.1M and 0.2M). The mixtures were then heated for 2 h at 95 °C with constant stirring (interrupted every 30 min by; 1 min vortexing and 10 min sonification). The resulting slurries were then

autoclaved at 121 °C for one complete cycle. After the acid hydrolysis, the samples were cooled to room temperature, centrifuged for 10 min at 4 °C and 4000 rpm. The supernatants were collected as microalgae crude extracts and stored at −20 °C.

The crude bio-extracts (CBEs) of microalgae and Cyanobacteria were each tested on plant growth parameters of tomato (*Solanum lycopersicum* L.) under laboratory conditions at three different concentrations of CBEs (0.1, 0.5, and 1 g/L).

Therefore, a total of 162 crude extracts were tested on plants: The effect of the 162 CBEs was tested on tomato seedlings. For each microalgae and cyanobacteria specie, 9 different extracts based on the H_2SO_4 hydrolysis concentration (0M, 0.1M and 0.2M) and application dose (0.1, 0.5, and 1 g/L) were tested. For each specie, only 1 out of 9 tested extracts was retained, based on their positive effects on plant development. Therefore, a total 18 CBEs were shortlisted

The 18 selected CBEs were then re-tested for their effect on plant growth, chlorophyll content, nutrient uptake and eventually, on the metabolomic profile of plants.

Partial characterization of the crude microalgae and cyanobacteria bio-extracts. Protein content in crude extracts was determined by using the 96 Well Plate Assay Protocol according to Bradford method[45]. The quantitative determination of total sugars was done according to the protocol described by[46]. The crude extracts were analysed subsequently for $P\text{-}PO_4$, $N\text{-}NO_3$, $N\text{-}NH_4$ and K^+ using a skalar nutrient auto analyser at MAScIR.

The biochemical composition of the general dry mass of microalgae and cyanobacteria was not studied, our focus mainly being projected on the effect/bio-stimulant properties of the end product of the biomass hydrolysis (Crude Extracts) of microalgae and cyanobacteria on plant morphological, physiological and biochemical mechanisms.

Culture and treatment of tomato plants. The plant model used in this study was *Solanum lycopersicum* L. seeds, JANA F1 provided by BAYER Nunhems Netherlands BV. The Tomato seeds were sown on 24 cell seed trays filled with peat moss and were irrigated with distilled water. The seedling trays were then covered with aluminium foil to optimize germination and placed in a growth chamber at (MAScIR). The growth chamber conditions were set at 25 °C, 16:8 day/night photoperiod, 240 µmol photons m^{-2} s^{-1} and 60–70% relative humidity.

After germination (5 days after sowing), CBEs were applied as soil drench. Treatment with CBE was applied three times at an interval of 10 days. Also, two weeks after sowing, both treated and non-treated control plants

Solution _A		Solution_B		Solution_C	
Element	g/L	Element	g/L	Element	mg/L
Mg (NO3)26H2O	4.94	KH2PO4	2.67	H3BO3	128.8
NH4NO	8.48	K2HPO4	1.64	CuCl2_2H2O	4.84
KNO3	2.28	K2SO4	6.62	MnCl2_4H2O	81.1
		Na2SO4	0.6	(NH4)6 Mo7O24_4H2O	0.83
		NaCl,	0.33	ZnCl2	23.45
				Ferric citrate pentahydrate	809.84

Table 2. The salts used to make up the nutrient solution. The applied dilute solution was prepared by mixing 100 mL of each of the macronutrient stock solution (A and B) with 50 mL of the micronutrient (solution C) supplement and diluting to 4.5 L with deionized distilled water. The pH was adjusted to 6.0 by adding HCl or KOH.

Major Constituents:	Mixture of weakly-moderate decomposed bog peat (H3-H5), strongly decomposed bog peat (H5-H8), with further additions like clay and perlite.	
Organic substances:	70–90% (Dry masses)	
Contents filling:	70L (EN 12580)	
Chemical properties:	pH ($CaCl_2$):	5.7
	Salt g/L KCl	0.9
	N mg/L	140
	P_2O_5 mg/L	100
	K_2O mg/L	180

Table 3. The Peat moss chemical and physical characteristics.

were irrigated with 10 mL of Raukura nutrient solution every two days, alternated with distilled water. All plant cultures (Treated and non-treated control plants) were supplied with equal recommended doses of nutrients, according to[47,48] Table 2. The resulting differences observed in N, P, K concentration and accumulation on the root biomass was therefore an indicator of their uptake by roots.

At 40 days old. The plants were harvested and measured for plant size. For each treatment, five replicates were dried in an oven at 50 °C while five other replicates were weighed and stoked at −80 °C.

Peat moss Mixture. The floragard- "SPECIAL-MIXTURE" peat moss was obtained from Flora-gard Company, Floragard Vertriebs-GmbH.

Origin: Gebr.Brill Substrate GmbH & Co.KG – D-49828 Georgsdorf, Germany.

The physical-chemical characteristics of the peat moss are shown in Table 3.

Measurement of the growth and pigments. The root and shoot lengths were measured manually with a ruler. Tomato leaves (0.1 g) sampled in the middle part of each plant with uniform size and color were homogenized in 4 mL of 80% acetone and 0.1% (w/v) of CaCO3 and left overnight at 4 °C. The pigment contents were measured using spectrophotometric technique and calculated using the method described by[49,50]

Analysis of plant metabolomics profile using GC-MS. Extraction method was modified from the method previously described by[51]. 400 mg of the plant leaves was grinded in liquid nitrogen and transferred in a glass vial with a cap. 10 µl of internal standard Dodecane and 4 mL of $CHCl_3$ (pre-cooled at −20 °C) were added to the vial. The mixture was thoroughly vortexed for 1 min. The vials were then transferred to a heat block (Labet International, Edison, USA) pre-set at 85 °C and left for 2hrs. After 2hrs, the vials were transferred to an ultrasound bath (Branson ultrasonic Sonifier 450, Danbury, USA) and sonicated at 60 °C for 1 hr. 2 mL of methanol was then added to the mixture. The vials were thoroughly vortexed, transferred back into the ultrasound bath and sonicated at 60 °C for 2 hrs. 1 mL of distilled water (dH_2O) was then added to the vials (for phase separation), and the bottom organic phase was transferred to a fresh vial with the help of a separating funnel. The $CHCl_3$ solvent was then evaporated completely under nitrogen flow. After that, 500 µl of 6% methanolic KOH (w/v) was added to the dried residue and the mixture was heated for 2 hrs at 85 °C and sonicated for 1 hr at 60 °C. The mixture was dried again under nitrogen flow, then, 250 µL of distilled H_2O and 750 µL of $CHCl_3$ was added to the dried residue and vortexed for 1 min. The bottom organic phase was transferred to a fresh vial with the help of a separating funnel and conserved at −20 for GC-MS analysis. **Note:** The mixtures were thoroughly vortexed for 1 min every 30 mins of the procedure.

Metabolomics analysis was carried out using gas chromatography (GC) (Agilent 7890A Series GC, USA) coupled to mass spectrometry (MS) equipped with multimode injector and BD-ASTMD6584 column (15 m × 0.320 mm × 0.1 µm) and electron impact ionization.

Statistical analysis. Statistical analyses were performed by SPSS and R scripts. Descriptive statistics and statistically significant differences between the mean values from control and treated plant samples were determined using One-way ANNOVA and Turkey via SPSS (IBM SPSS statistics 22). Data presented are means ± standard errors of five replicates at the significant level of $p < 0.05$.

Statistical calculation and graphical generation were performed using the R programming environment. The PCA and heatmap was generated using RStudio, visualization of corrplot and ggplot packages, integrated into the R software. In order to perform the analysis, the data was centered and scaled. Principal component analysis PCA was calculated based on a Euclidean distance matrix and as a hierarchical ascendant classification (HAC) using the prcomp function and MixOmics package where each variable was centered by subtracting from the mean = True), but not by the standard deviation (scale = False). The first two components explained the maximum variance in the datasets (Table. S3).

Data availability

The data generated and/or analyzed during current study are available from the corresponding author on reasonable request

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Author contributions
E.A.H., K.L. and Y.Z. conceived the experiments, C.M.J., E.M.N. and B.R. conducted the experiments, C.M.J., L.S. and K.Y. analyzed the results. All authors reviewed the manuscript. The authors declare that they have no conflict of interest.

Competing interests
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II-2. 3 SYNTHÈSE

La présente partie de notre étude concerne l'évaluation des effets biostimulants de 18 Bio-Extraits bruts (**CBE** *pour* Crude Bio-Extracts) obtenus à partir de microalgues et de cyanobactéries, sur la croissance, la teneur en chlorophylle, l'absorption des nutriments et le profil des métabolites chez la tomate. L'application des CBEs aux plantes de tomates a amélioré la teneur en chlorophylle, l'absorption des nutriments par les racines, et la longueur et le poids sec des racines et tiges. Les CBEs d'*Aphanothece* sp, ont significativement amélioré la longueur des racines de 112,65% ainsi que le poids sec racinaire et de tiges respectivement de 34,81% et 58,69% (**Fig. 1 et Fig 2b**). Pareillement, les CBEs extrait de *Chlorella ellipsoidea* ont joué un rôle positif significatif dans l'amélioration de la longueur des tiges (53,70%) (**Fig. 2a**). Des études antérieures ont montré que les extraits d'algues et leurs composés purifiés peuvent induire de fortes réponses physiologiques chez les plantes et augmenter le poids des tiges et / ou des racines (Ertani et al., 2018; Hamed et al., 2018; Hernández-Herrera et al., 2014; Michalak et al., 2016; Yakhin et al., 2017; Zheng et al., 2016). En outre, les extraits de microalgues tels que les polysaccharides peuvent améliorer la croissance des cultures agricoles (El Arroussi H et al., 2016; Garcia-Gonzalez et Sommerfeld, 2016; Ronga et al., 2019). Les sucres, par exemple, agissent à la fois comme des molécules de signalisation efficaces et comme des substrats immédiats pour le métabolisme intermédiaire, et pourraient donc favoriser la croissance et le développement des plantes (O'Hara et al., 2013; Zheng et al., 2016). Les vitamines et les phytohormones dans les extraits d'algue peuvent également améliorer la croissance des plantes (Bajguz, 2009; E et al., 2007; Ghaderiardakani et al., 2019; Zheng et al., 2016). Par exemple le potassium dans les extraits d'algues (Hernández-Herrera et al., 2014) a un effet positif sur la régulation de l'état de l'eau, l'amélioration de la photosynthèse et la croissance des plantes soumises au stress salin. Les CBEs contiennent diverses concentrations de sucres, des protéines, et de l'azote, du phosphore et du potassium (NPK) (**Tableau. 1**). Cependant, la concentration des extraits CBE appliqués aux plantes est très faible, ce qui exclurait leur contribution nutritionnelle à la croissance des plantes traitées : ainsi, les effets bénéfiques peuvent être attribués à plusieurs autres molécules promotrices de la croissance.

Les « N, P et K » sont trois macro-éléments importants fréquemment ajoutés comme engrais dans les systèmes agricoles modernes. Par conséquent, cette étude a ciblé les concentrations de NPK dans les racines pour évaluer l'effet des CBEs sur l'absorption des nutriments. L'analyse en composantes principales (ACP) a confirmé que l'amélioration de la concentration du phosphore et de potassium dans les racines était étroitement associée à l'amélioration de la longueur des racines (**Fig. 5b**), indiquant que l'amélioration de l'absorption des nutriments observée chez les plantes traitées pourrait également être attribuée à l'augmentation de la taille des racines. Cela s'explique par le fait que l'augmentation de la croissance des racines augmente la surface racinaire et améliore directement l'accessibilité et l'absorption des nutriments.

Le traitement des plantes avec les CBEs a également entrainé l'augmentation la concentration de

chlorophylle dans les feuilles (**Fig. 3**). L'augmentation maximale de la teneur en chlorophylle b (92,45%, 92,28% et 83,95%) dans tous les traitements était remarquable chez les plantes traitées avec les extraits d'*Aphanothece* sp., *A. maxima* et *C. pyrenoidosa* pour les espèces d'eau douce respectivement, et *Tetraselmis* sp. et les extraits de *N. gaditana* pour les espèces d'eau de mer, qui ont montré une augmentation respective de 93,31% et 83,95% par rapport aux plantes témoins (**Fig. 3**). Selon la littérature, cette augmentation des chlorophylles pourrait être attribuée à une dégradation réduite de la chlorophylle et à un retard de la sénescence des plantes (Blunden et al., 1996; Calvo et al., 2014). En outre, les extraits de CBEs pourraient avoir plusieurs effets sur les voies photosynthétiques. Selon Jannin et al., (2013), les gènes impliqués dans la fixation du carbone, y compris la rubisco et l'anhydrase carbonique étaient induits par les extraits d'algues. Mis à part la teneur en chlorophylle, l'absorption améliorée des nutriments influence fortement l'efficacité photosynthétique et, par conséquent, la production de biomasse. Les études de Makino (2003); Makino et al., (2000) ont montré que les niveaux d'azote dans les plantes sont fortement corrélés avec le taux de photosynthèse, tandis que l'amélioration de la photosynthèse est reconnue comme l'un des moyens les plus efficaces d'augmenter l'efficacité de l'utilisation de N et le rendement des cultures (Guo et al., 2016). Dans son étude, Zhu et al., (2010) a suggéré que l'augmentation de l'absorption en azote dépend en grande partie de l'amélioration de la photosynthèse. Dans cette étude, la concentration de N dans la biomasse racinaire était étroitement associée au poids de racines et de tiges, et à la chlorophylle b (**Fig. 5b**), indiquant que le N absorbé par les racines a été en partie investi dans la machinerie photosynthétique. Nos résultats sont cohérents avec ceux de Nunes-Nesi et al., (2010), qui a montré que la majorité de l'azote assimilé dans les plantes est investie dans la machinerie photosynthétique et est fortement corrélée avec le taux de la photosynthèse (Makino, 2003; Makino et al., 2000). En plus de participer directement à la photosynthèse, l'azote agit également comme un régulateur important dans la manipulation de la diffusion du CO_2 (Tosens et al., 2012; Xiong et al., 2015; Zhong et al., 2017).

Les profils de métabolites primaires et secondaires sont un autre élément indispensable de la performance des cultures, de la photosynthèse et de l'accumulation de biomasse (Turner et al., 2016). Plusieurs groupes phytochimiques ont été détectés par l'analyse GC–MS (**Fig. 6**). Les phytols étaient significatives améliorés chez les plantes traitées avec *Tetraselmis* sp, *D. salina*, *N. gaditana*, *Aphanothece* sp et *A. maxima* respectivement (**Fig. 6**). Le phytol est l'un des principaux alcools diterpènes acycliques qui est un précurseur des vitamines E et K et un constituant de la chlorophylle (Dorp et al., 2015; Mach, 2015; Zhang et al., 2014). Les phytols sont essentiels dans la biosynthèse des tocophérols qui est un antioxydant lipidique bien connu et contribuent à la protection du photosystème II (PSII) contre le « dommage photonique » en cas de stress environnemental (Dorp et al., 2015; Spicher et al., 2017). D'autre part, une grande proportion de phytol et d'acides gras est convertie en esters phytyliques d'acides gras dans les chloroplastes, qui sont impliqués dans le maintien de l'intégrité de la membrane photosynthétique pendant le stress abiotique et la

sénescence chez *Arabidopsis thaliana* (Lippold et al., 2012). Cependant, une accumulation très élevée d'acides gras phytyles (1088% et 1008%) n'a été enregistrée que chez les plantes traitées avec *Aphanothece* sp et *A. maxima* respectivement (**tableau S6**). L'accumulation du phytol chez les plantes traitées était cohérente avec la teneur en chlorophylle, alors les concentrations élevées du phytol chez les plantes traitées pourrait être à partir de la dégradation de la chlorophylle lors de l'extraction des métabolites. L'accumulation du phytol était aussi étroitement associée à la concentration d'acide palmitique (C16 :0), d'acide linolénique (C18 :3) et d'acide stéarique (C18 :0) (**Fig. 6**). Une augmentation de l'accumulation d'acide palmitique C16 :0 et stéarique C18 :0 dans les plantes traitées pourrait indiquer une amélioration de la biosynthèse lipidique. Selon la littérature, la première étape de la synthèse lipidique *de novo* chez les plantes commence par la génération d'acides gras C16 :0 et C18 :0. Ces acides gras sont ensuite allongés en acides gras à très longue chaîne (VLCFA), et principalement incorporés dans les sphingolipides (éléments structuraux dans les bicouches lipidiques de la membrane cellulaire), et les couches de cire cuticulaires qui aident les plantes à réduire la perte d'eau et l'invasion d'agents pathogènes (Li-Beisson et al., 2013; Troncoso-Ponce et al., 2016). De plus, notre étude a montré que toutes les plantes traitées présentent des concentrations d'acides gras totaux plus élevées, ce qui indique les effets biostimulants des CBEs sur le profil lipidique des plantes.

Les extraits bruts des microalgues et cyanobactéries ont également amélioré l'accumulation de pyridine-3-carboxamide dans toutes les plantes traitées. La pyridine-3-carboxamide, également connue sous le nom de nicotinamide ou de niacinamide, est une forme active amide de vitamine B3 (Surjana et al., 2010). La pyridine-3-carboxamide est le précurseur primaire d'une coenzyme essentielle dans la production d'ATP connue sous le nom de nicotinamide adénine dinucléotide (NAD+), et le seul substrat de la poly-ADP-ribose polymérase-1 (PARP-1) (Surjana et al., 2010). Aujourd'hui, la pyridine-3-carboxamide est exploitée commercialement dans les suppléments vitaminiques et les produits cosmétiques. Dans la présente étude, une accumulation plus élevée de métabolites tels que la pyridine-3-carboxamide dans les plantes de tomates traitées indique que les CBEs peuvent améliorer la composition biochimique et, par la suite, la qualité nutritionnelle des produits végétaux.

II-2. 4 CONCLUSION

La présente étude met en évidence les effets des Bio-Extraits bruts (CBEs) sur la croissance, l'absorption des nutriments et les voies métaboliques de la tomate. Toutes les espèces de microalgues n'ont pas eu d'activités biostimulantes significatives sur les paramètres de la croissance de la tomate, ce qui suggère que les propriétés biostimulantes des CBEs sont dues à des métabolites “spécifiques à l'espèce” produits par des microalgues ou des cyanobactéries particulières. Nos résultats constituent un premier pas vers la compréhension des effets des extraits des microalgues et cyanobactéries sur la physiologie végétale et les voies biochimiques. D’autres outils utiles, citons le profilage hormonal comparatif des plantes traitées et non traitées, accompagné d'une analyse combinée du phénotypage des plantes à haut débit, de la transcriptomique et de la métabolomique pourraient contribuer à l’étude approfondie des mécanismes d'action des CBEs.

CHAPITRE III

CHAPITRE III

L'effet du biostimulant à base des microalgues et cyanobactéries sur les mécanismes de tolérance de la tomate au stress salin.

III-1. INTRODUCTION

Les microalgues et les cyanobactéries gagnent en popularité en tant que ressources bioactives et renouvelables qui peuvent être exploitées en agriculture pour le développement de biostimulants. Ceci est dû à leur capacité à produire une diversité de molécules biologiquement actives telles que les polysaccharides sulfatés, les osmolytes, les phytohormones, les acides aminés et les phénoliques (De Morais et al. 2015; Cuellar-Bermudez et al. 2015; Renuka et al. 2018). Un nombre croissant d'études ont mis en évidence les propriétés biostimulantes d'extraits de différentes espèces de microalgues et de cyanobactéries sur différentes activités biologiques chez les plantes supérieures, y compris l'absorption des nutriments, la croissance et la tolérance au stress abiotique (De Morais et al. 2015; El Arroussi et al. 2016; Garcia-Gonzalez et Sommerfeld 2016; Barone et al. 2018; EL Arroussi et al. 2018; Farid et al. 2019).

La salinisation des sols est l'un des principaux facteurs des stress abiotiques en agriculture, affectant environ 20% des surfaces irriguées mondiales, en particulier dans les zones méditerranéennes (Zaman et al. 2018; Libutti et al. 2018). Le stress salin entraîne un déséquilibre ionique chez les plantes causé par une accumulation excessive de Na^{+} et de Cl^{-}, ce qui réduit l'absorption d'autres nutriments minéraux tels que le K^{+}, Ca^{2+} et Mn^{2+}. L'accumulation excessive de Na^{+} entraîne un déséquilibre nutritionnel, une perméabilité et une instabilité membranaires (résultant du déplacement de Ca^{+} par Na^{+}) ainsi qu'une surproduction des espèces réactives de l'oxygène (ROS), qui causent des dommages oxydatifs sur les macromolécules cellulaires (Zhao et al. 2020; Arif et al. 2020). Ces altérations biochimiques limitent la croissance et la performance des plantes, entraînant une réduction du rendement des cultures. Pour contrecarrer le stress de salinité, les plantes déploient une cascade des réponses adaptatives spécifiques notamment le transport de K^{+}, les modifications des phospholipides, l'activation des enzymes anti-oxydants et la production de solutés compatibles pour compenser la pression osmotique de Na^{+} (Acosta-Motos et al. 2017; Yang et Guo 2018; Van Zelm et al. 2020; Arif et al. 2020). Ces réponses naturelles adéquates au stress salin jouent un rôle important dans l'induction de la tolérance des plantes dans les sols salins.

La présente étude vise à étudier l'effet combiné des extraits de microalgues et de cyanobactéries en tant que stimulateurs des réponses de la tolérance au stress salin, et de l'absorption des nutriments et de la croissance végétative des plantes de tomates dans des conditions salines. L'étude met également en évidence, les mécanismes d'action possibles des formulations MEF en tant biostimulants de la croissance de la tomate dans des conditions salines.

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Microalgae-cyanobacteria–based biostimulant effect on salinity tolerance mechanisms, nutrient uptake, and tomato plant growth under salt stress

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Abstract

High soil salinity is a major abiotic stress affecting the growth, nutrition, development, and productivity of crops. This study investigated the modulating effect of combined microalgae-cyanobacteria extract formulations (MEF1%, MEF5%, and MEF10%) prepared from the species *Dunaliella salina*, *Chlorella ellipsoidea*, *Aphanothece* sp., and *Arthrospira maxima*, on tomato plant growth and tolerance under four NaCl concentrations (0, 80, 120, and 150 mM). MEF5% enhanced the vegetative growth of tomato plants, characterized by higher shoot and root weight and larger leaf area. According to principal component analysis (PCA), improved plant growth was closely associated with leaf photosynthetic pigments, which was mainly due to improved osmotic adjustment and ion homeostasis. Proline accumulation was significantly enhanced by MEF5%-treatment in plants grown under 120 mM and 150 mM NaCl conditions. MEF5%-treatment also significantly improved nitrogen (N), phosphorus (P), and potassium (K^+) absorption in plants grown at 80 mM and 120 mM NaCl levels. Leaf lipid peroxidation through ROS oxidative stress significantly decreased with enhanced CAT and SOD activities in MEF5%-treated plants. MEF5% triggered a significant decline in fatty acid content, indicating fatty acid transformation into other lipid forms such as alkanes, which are essential in the cuticular wax synthesis of hydric stressed plants. Enhanced K^+ uptake and reduced Na^+/K^+ ratio in the leaves of treated plants indicate MEF's active role in reestablishing ion homeostasis. Nutrient uptake can be improved by enhanced root biomass, which subsequently increases the roots' surface for nutrient absorption. These results indicate that MEF stimulated plant growth and tolerance responses through (i) enhanced antioxidant enzyme activities and (ii) improved root growth and nutrient uptake. Therefore, combined microalgae-cyanobacteria formulations could be another sustainable alternative to boost nutrient uptake, growth, and crop adaptability under normal and saline conditions.

Keywords Chlorophyceae · Cyanobacteria · Salt stress tolerance · *Solanum lycopersicum* · Plant growth

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Introduction

As the global population increases and negative effects of climatic change escalate, modern agriculture is challenged to search for efficient and eco-friendly methods of increasing crop productivity and tolerance against the harsh environmental conditions. This means that producers are compelled to cut-down the excessive applications of chemical fertilizers and pesticides which pose both short- and long-term threats to human health and the entire ecosystem (Carvalho 2017; Rahman and Zhang 2018).

To address this challenge, innovative sustainable agricultural products including plant growth biostimulants have been largely described (Calvo et al. 2014; Elzaawely et al. 2017; Van Oosten et al. 2017; Yakhin et al. 2017; Desoky et al. 2018). According to the European Union Fertilizing Products Regulation 2019/1009, biostimulants are defined as "*fertilizing products the function of which is to stimulate plant nutrition processes independently of the product's nutrient content with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere: i) nutrient use efficiency, ii) tolerance to abiotic stress, iii) quality traits, or iv) availability of confined nutrients in the soil or rhizosphere*" (Ricci 2020). Among the many categories of plant biostimulants, seaweeds, more than microalgae, have been exploited extensively and represent an important category of organic biostimulants (Khan et al. 2011; Battacharyya et al. 2015; Colla et al. 2017). Microalgae and cyanobacteria are now gaining popularity as renewable bioactive resources that can be exploited in agriculture for the development of plant biostimulants. Microalgae (eukaryotic) and cyanobacteria (prokaryotic) are unicellular microscopic photosynthetic organisms that grow in diverse aquatic habitats and even humid soils (Khan et al. 2018). They have their capacity to produce a diversity of biologically active molecules such as sulfated polysaccharides, osmolytes, phytohormones, amino acids, and phenolics (Cuellar-Bermudez et al. 2015; De Morais et al. 2015; Renuka et al. 2018). An increasing number of studies have been conducted to highlight biostimulant properties of extracts from different microalgae and cyanobacteria species (Chiaiese et al. 2018; Ronga et al. 2019; Carillo et al. 2020; Colla and Rouphael 2020). Such extracts have been tested on a broad range of biological activities in higher plants, including nutrient uptake, crop performance, and tolerance to biotic and abiotic stress (Garcia-Gonzalez and Sommerfeld 2016; Barone et al. 2018; El Arroussi et al. 2018; Chanda et al. 2019; Farid et al. 2019; Rachidi et al. 2020).

Soil salinization is one of the major abiotic stressors in agriculture, affecting about 20% of the world irrigated surfaces, particularly in Mediterranean zones (Libutti et al. 2018; Shahid et al. 2018). Salt stress leads to ionic imbalance in plants due to excessive accumulation of Na^+ and Cl^-, which reduces the uptake of other mineral nutrients such as K^+, Ca^{2+}, and Mn^{2+}. Excess Na^+ accumulation leads to nutritional imbalance, membrane permeability and instability (resulting from Ca^+ displacement by Na^+), and an overproduction of reactive oxygen species (ROS), which cause oxidative damage on cellular macromolecules (Arif et al. 2020; Zhao et al. 2020). These biochemical alterations limit plant growth and performance, leading to crop yield reduction. To counteract salinity stress, plants induce a cascade of specific adaptative responses including K^+ transport, phospholipid modifications, activation of ROS-scavenging enzymes, and production of compatible solutes to compensate for the osmotic pressure of Na^+ (Acosta-Motos et al. 2017; Yang and Guo 2018; Arif et al. 2020; Van Zelm et al. 2020). Such adequate natural stress responses play an important role in inducing plant tolerance on saline soils.

The present study aimed to investigate the combined effect of microalgae-cyanobacteria extracts as stimulators of salt stress tolerance responses, nutrient uptake, and vegetative growth of tomato plants under saline conditions. The study also investigates possible action mechanisms of MEF formulations as enhancers of salt stress tolerance and crop growth under saline conditions.

Material and methods

Culture of microalgae

Two microalgae (*Dunaliela salina* MSD 002 and *Chlorella ellipsoidea* BEA 0337) and two cyanobacteria species (*Aphanothece* sp. BEA O935B and *Arthrospira maxima* MSS001) were selected from the AlgoBioTech collection of the Moroccan Foundation for Advanced Science, Innovation and Research (MAScIR). They were cultivated in 3 replicates using 250 mL Erlenmeyer flasks containing using the following media: *D. salina* was cultured in F2 medium at pH = 7.8; *Arthospira maxima* in Zarrouk's medium at pH = 9.5; *Aphanothece* sp. and *C. ellipsoidea* were cultured in BG11 medium at pH = 7.2. The culture medium for *D. salina* was made with sea-salt solution. The initial optical density for all cultures was 2 ± 0.2. All cultures were placed in a photo-incubator at 25 °C under constant orbital agitation, and 135 µmol photons m^{-2} s^{-1} of continuous white fluorescent light. After 30 days, the biomass was harvested by centrifugation at 7000 *xg* for 5 min at 4 °C. The collected biomasses were dried at 50 °C for 7 days.

Preparation of the microalgae extract formulation

Based on the optimal extract concentrations previously established when screening microalgae extracts (Chanda et al. 2020), the dry biomasses of the two microalgae and cyanobacteria species were ground in liquid nitrogen. Then, 300 mg of the mixture was hydrolysed in 20 mL 2% sulfuric acid. The resultant slurry was heated for 3 h at 95 °C with constant stirring (interrupted every 30 min by 1 min vortexing and 15 min sonification). The mixture was then autoclaved at 121 °C and 106 kPa for 30 min. The total crude extract was cooled to room temperature, and stored at − 20 °C. The extract was tested at three concentrations: 1%, 5%, and 10% (v/v of MEF formulation in distilled water).

The pH of the extract formulations was adjusted to 5.8 using NaOH before application to plants by soil drenching.

Experimental design and plant growth conditions

The plant model used in this study was *Solanum lycopersicum* L. JANA F1 obtained from BAYER Nunhems Netherlands BV. Tomato seeds were sown on 24 cell seed trays (6 cm^2) filled with peat moss (Gebr. Brill Substrate, Germany) and were irrigated with deionized water. After germination (5 days after sowing), the trays were placed in a Phytotron chamber set at 26 °C, 16:8 h photoperiod, 240 µmol photons m^{-2} s^{-1}, and 60–70% relative humidity.

The experimental design consisted of four culture groups defined by NaCl concentrations. Each culture group consisted of treated and untreated plants:

$Group_{0mM}$—Control 0 mM NaCl (Untreated plants under normal condition) + treated plants under normal conditions.

$Group_{80mM}$—Control 80 mM NaCl (Untreated plants under 80 mM conditions) + treated plants at 80 mM conditions.

$Group_{120mM}$—Control 120 mM NaCl (Untreated plants under 120 mM conditions) + treated plants at 120 mM conditions.

$Group_{150mM}$—Control 150 mM NaCl (Untreated plants under 150 mM conditions) + treated plants at 150 mM conditions.

The seedling trays (one plant per pot) were arranged in a completely randomized block design in the growth chamber. Five replications were sampled in each treatment. Three most homogenous replicates were selected for biochemical studies. Plants were grown for 35 days (after germination) under controlled environmental conditions at the Moroccan Foundation for Advanced Science Research and Innovation (MAScIR). The growth chamber conditions were set at 25 °C, 16:8 day/night photoperiod, 240 µmol photons m^{-2} s^{-1}, and 60–70% relative humidity.

At 40 days old, the plants were harvested. The roots were washed under running tap water and the root and shoot lengths were measured manually with a ruler.

Salinity application

To prevent salt-exciting reactions, 50 mM NaCl was initially applied to the peat moss and the NaCl concentration was gradually increased by a unit of 50 until the predetermined application concentration for each treatment was reached. MEF were applied to plants every week by irrigation (10 mL per pot). Two weeks after sowing, all plants were irrigated every 2 days with 10 mL of a plant nutrient solution prepared according to the table presented in Supplementary information (S2) . All plant groups (treated and non-treated control plants) were supplied with equal recommended doses of nutrients according to Smith et al. (1983) and Khan et al. (2012)

The nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and sodium (Na) concentrations in the leaf biomass were analyzed using a skalar nutrient auto analyser at MAScIR; the resulting differences observed in the nutrient concentrations were therefore an indicator of the nutrient absorption by roots.

Measuring NaCl retained in the peat moss soil

The peat moss salinity was measured by determining the electrical conductivity (EC) of the peat moss at 24 °C from 1:5 peatmoss/distilled water suspension, according to the method described by Hardie and Doyle (2012) with a few modifications. The peat moss was dried at 70 °C for 24 h.

Then, 1:5 peat moss/water suspension was prepared by adding 10 g of the dried peat moss to 50 mL of deionized water in a 250-mL glass beaker. The beaker was thoroughly closed using aluminum foil and agitated on an orbital shaker at 145 rpm and 25 °C for 30 min to dissolve soluble salts. The peat moss mixture was filtered through a sterile hydrophilic cotton gauze due to the texture of the soil (peat moss) which is not easily sedimented at the ratio of 1:5. Then, the EC, soil salinity, and total dissolved solutes (TDS) were measured using an EC meter—EC300 VWR—by dipping the electrode into the supernatant. The reference solutions were prepared by dissolving 3 g dried sodium chloride (NaCl) in 1 L of distilled water for 50 mM NaCl, 6 g for 100 mM, 9 g for 150 mM, and 12 g for 200 mM. The electrode was rinsed with distilled water between samples.

Determination of photosynthetic pigments content

For each replicate, 100 mg leaf biomass ground in liquid nitrogen was homogenized in 5 mL 95% ethanol containing 0.1% (w/v) $CaCO_3$ and left overnight at 4 °C. The

homogenate was then vortexed for 30 s and centrifuged at 7000 *xg*, 4 °C for 5 min. The optic densities of all the samples were measured using a UV/visible spectrophotometer (Ultropec 3100 *pro*_ Amersham Biosciences) and calculated using the method described by Lichtenthaler (1987) and Lichtenthaler and Buschmann (2001): 95% ethanol and 0.1% (w/v) of $CaCO_3$ served as blank.

Determination of antioxidant enzyme activities

Superoxide dismutase, peroxidase, and catalase.

Determination of superoxide dismutase (SOD) antioxidant enzyme activities was performed using the method of Meloni et al. (2003). All specific activities of enzyme fractions were calculated based on the amount of protein in the fraction and the protein content was determined according to the Bradford method (Bradford 1976) using bovine serum albumin (BSA) as standard. For the extraction method, 500 mg leaf biomass was homogenized in 5 mL potassium phosphate buffer 10 mM (pH 7.0) containing 4% (w/v) polyvinylpyrrolidone. The homogenate was centrifuged at 3000 *g* for 30 min and the supernatant was used as the enzyme extract. All experiments on enzyme activities were carried out on ice.

For peroxidase (POD) antioxidant enzyme activity, the following reaction mixture was used: 3 mL containing 10 mM potassium phosphate buffer (pH 7), 600 µL guaiacol 1% (w/v), and 60 µL enzyme extract. The reaction was initiated with the addition of 150 µL 100 mM H_2O_2. The increase in absorbance due to the formation of tetraguaiacol was recorded at 470 nm; an identical mixture with no H_2O_2 was used as blank. The linear initial reaction rate was used to estimate the activity, expressed in mM of the guaiacol dehydrogenation product (GDHP) formed per milligram of protein per minute, using the extinction coefficient of 26.6 mM^{-1} $cm^{-1.57}$ (U mg^{-1} min^{-1}) (Velikova et al. 2000). The activity of catalase (CAT) was assayed by measuring the initial rate of the disappearance of H_2O_2. The CAT assay reaction mixture (3 mL) contained 10 mM potassium phosphate buffer (pH 7.0), 100 µL enzyme extract, and 0.035 mL H_2O_2 3%. The decrease in H_2O_2 was followed as decline in optic density at 240 nm, and the activity was calculated using the extinction coefficient (40 mM^{-1} cm^{-1}) for H_2O_2 (Velikova et al. 2000).

Determination of malonyldialdehyde

Lipid peroxidation in the leaf biomass was determined according to the method described by Velikova et al. (2000), using thiobarbituric acid (TBA). TBA determines malonyldialdehyde (MDA) concentration (the final product of lipid peroxidation). The leaf biomass (500 mg FW) ground in liquid nitrogen was homogenized in 5 mL 0.1% trichloroacetic acid (TCA) solution (w/v) and the homogenates were centrifuged at 10000 *g* for 20 min at 4 °C. Then, 500 µL supernatant was added to 1 mL containing 0.5% (w/v) TBA and 20% TCA. The mixture was incubated in boiling water for 30 min, and the reaction was stopped by placing the reaction tubes in an ice bath. The samples were then centrifuged at 10000 *g* for 5 min, and the supernatant absorbance was read at 532 nm. The value of the non-specific absorption at 600 nm was subtracted. The amount of complex MDA–TBA (red pigment) was calculated from the extinction coefficient 155 mM^{-1} $cm^{-1.572}$.

Determination of proline

In this method according to Carillo and Gibon (2011), 20 mg ground leaf biomass was homogenized in 1 mL ethanol:water (v / v) (70:30). For each sample, 500 µL of the ethanolic extract was added to 1 mL of the reaction mixture containing 1% (w/v) ninhydrin, 60% (v/v) acetic acid, and 20% (v/v) ethanol. The mixture was heated at 95 °C on a heat block for 20 min. After centrifugation at 10000 *g* for 1 min, the absorbance was measured at 520 nm and the proline content was calculated from the standard curve as µg mg^{-1} of protein. A standard curve for proline was prepared using L-proline.

Determination of polyphenols

Phenolics were assayed by homogenizing 20 mg leaf biomass in 2 mL 95% (v/v) methanol, according to Ainsworth and Gillespie (2007). Samples were then incubated at room temperature for 48 h in the dark and centrifuged at 13000 *g* for 5 min at room temperature; the supernatant was recovered. For each sample, 100 µL of the recovered supernatant was placed in 2 mL tubes, to which 200 µL 10% (v/v) Folin–Ciocalteu reagent was added. The reaction mixture was vortexed, and 800 µL Na_2CO_3 (700 mM) was added to each tube and incubated for 2 h at room temperature. The sample absorbance and gallic acid standard range were measured at 765 nm.

GC–MS lipidomic analysis of tomato plant

Extraction.

The extraction and transesterification of lipophilic metabolites were carried out according to Kamthan et al. (2012) with modifications optimized by MAScIR. Leaf biomass (400 mg FW) ground in liquid nitrogen was added to a glass vial. Then, 10 µL internal standard dodecane and 4 mL chloroform (pre-cooled at − 20 °C) were added to the vial. The vials were tightly covered with caps, thoroughly vortexed

for 1 min and heated at 85 °C for 30 min on a heat block. The vials were then vortexed for 1 min and sonicated for 15 min at 60 °C in an ultrasound bath (Branson ultrasonic Sonifier 450, USA). The heating and sonication process were repeated four times. Then, 2 mL methanol was added to the vials, thoroughly vortexed and transferred back into the ultrasound bath for 2 h at 60 °C. The 2 h was interrupted by 1 min vortexing every 30 min. For the separation phase, 1 mL distilled water was added to the vials, and the bottom organic phase was transferred to clean vials with the help of a separating funnel. The $CHCl_3$ solvent was then evaporated completely under nitrogen flow.

Transesterification.

For transesterification, 500 µL 6% methanolic HCl (v/v) was added to the dried residue. The mixture was heated for 30 min at 85 °C, then vortexed and sonicated for 15 min at 60 °C. The heating and sonication process was repeated four times. The mixture was dried under nitrogen flow, and 250 µL distilled H_2O and 750 µL $CHCl_3$ were added to the dried residue and vortexed for 1 min. The bottom organic phase was transferred to clean vials with the help of a separating funnel and stored at − 20 for GC–MS analysis.

Metabolomics analysis was carried out using gas chromatography (GC) (Agilent 7890A Series GC, USA) coupled to mass spectrometry (MS) equipped with multimode injector and BD-ASTMD6584 column (15 m × 0.320 mm × 0.1 µm) and electron impact ionization. The soluble extract (4 µL) was injected into the column by 1:5 split mode using helium as the carrier gas at 3 mL min^{-1}. The detection was done using full scan mode between 30 and 1000 m/z, with gain factor of 5. The temperatures of the ion source and the quadrupoles were 230 and 150 °C, respectively. The oven temperature was maintained at 30 °C for 1 min and then increased at 10 °C min^{-1} to 250 °C then at 20 °C min^{-1} until 340 °C. The identification was carried out using NIST 2017 MS Library. The amount of each compound was estimated by comparing the peak area with that of the internal standard (dodecane).

Statistical analysis

Statistical analyses were performed by IBM SPSS statistics 22 and RStudio software. Results represent the descriptive statistics and statistically significant differences between the mean values of the control and treated plant samples. Data was analyzed with two-way ANOVA. The statistically significant differences between the mean values were determined using Tukey's post hoc test. Results are expressed as the mean ± SE of three replicates. Significance levels ($p < 0.05$) are represented by different letters. The PCA and heatmap were generated using RStudio, visualization of corrplot, and ggplot packages, integrated into the R software. In order to perform the analysis, the data (mean values) was normalized into a standard range of − 1 to + 1 using the equation $x' = (x_{mean} - x_i) / (x_{max} - x_{min})$. The first two components explained the maximum variance in the datasets.

Results

Plant morphological responses to MEF treatment across different NaCl concentrations

Shoot and root lengths

One of the first observable responses in plants subjected to salinity stress is the reduction in the shoot length (SL). Non-stressed control plants exhibited the highest shoot length, which decreased with increasing NaCl concentrations (Fig. 1a). No significant root length changes were recorded across all culture groups after treatment with MEF. Treatment with MEF 1%, 5%, and 10% exhibited significant effects (22.00%, 18.22%, and 21.62% SL increase, respectively) on the shoot length of plant cultures grown at 80 mM NaCl (Fig. 1b). Unstressed control plants treated with MEF1% showed very significant increase in shoot length (14.73%). There was also significant SL increase in plants treated with MEF 1%, 10%, and 1%, grown at 120 mM, 120 mM, and 150 mM, respectively (Fig. 1b).

Shoot and root weights

There were significant improvements on shoot and root weights (Fig. 1c and d). Treatment with MEF5% significantly enhanced root weight, notably in unstressed control plants and plants grown at 80 mM NaCl, where the highest percentage improvements were 68.60% and 87.50%, respectively (Fig. 1c). The results also show extremely significant increase in shoot weight (70.19%, 54.44%, and 53.44%) for plants grown at 80 mM NaCl and treated with MEF1%, MEF5%, and MEF10%, respectively (Fig. 2d). The effects were independent of the MEF concentration for each treatment group but MEF5% exhibited the most consistent significant effects on all morphological parameters. Thus, only samples treated with MEF5% were retained for biochemical studies.

Biochemical analysis of the effects of MEF on tomato plants

The effects of MEF treatment on tomato plants subjected to salt stress were independent of the MEF concentration for each treatment group. However, MEF5% exhibited the most consistent effects on all morphological parameters.

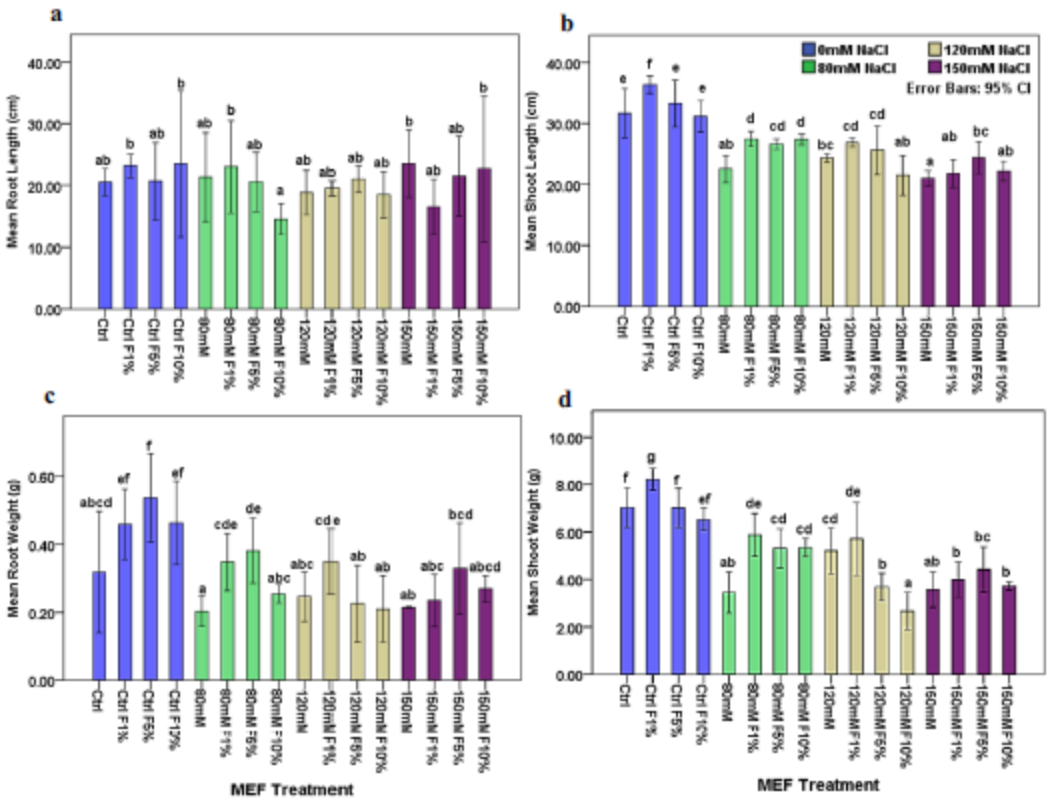

Fig. 1 Morphology and growth traits in tomato culture groups under different salt levels. Different small letters indicate significant differences between the MEF treatments across different NaCl levels

Therefore, only plants treated with MEF5% were selected for the determination of photosynthetic pigment content and the study of certain enzymatic and biochemical processes affected by salt stress.

Effect on photosynthetic pigments content

Salt stress causes chlorophyll degradation, which is one of the major limitations of photosynthesis. Photosynthetic pigments' content reduced with increasing salinity concentration. Treatment with MEF5% significantly increased pigment content in plant cultures subjected to lower salinity concentrations (80 mM NaCl) (Fig. 2a). The highest percentage increase in chlorophyll *a*, chlorophyll *b*, and β-carotene contents were 16.88%, 13.55%, and 16.62%, respectively, in plants grown at 80 mM NaCl. Application of MEF5% on plant cultures grown at 120 and 150 mM NaCl did not exhibit any effects on the leaf concentration of photosynthetic pigments. Treatment of plants with MEF5% at the lower saline levels (non-stressed and 80 mM NaCl) proved to improve the vegetative growth of tomato plants, characterized by increased shoot size and large total leaf area (Fig. 2b). See Supplementary Information S4 for statistically significant differences between the mean values (Tukey's post hoc test).

Effect of MEF5% treatment on the accumulation of proline and polyphenols

Treatment of plants with MEF5% exhibited significant effects on the accumulation of proline under normal and high saline conditions (120 and 150 mM NaCl). Significant percentage increases were up to 140.5% and 87.89%, respectively, in plants grown at 120 and 150 mM NaCl levels (Fig. 3b). Contrarily, polyphenols (Fig. 3b) were only significantly enhanced in unstressed plant cultures after MEF5%

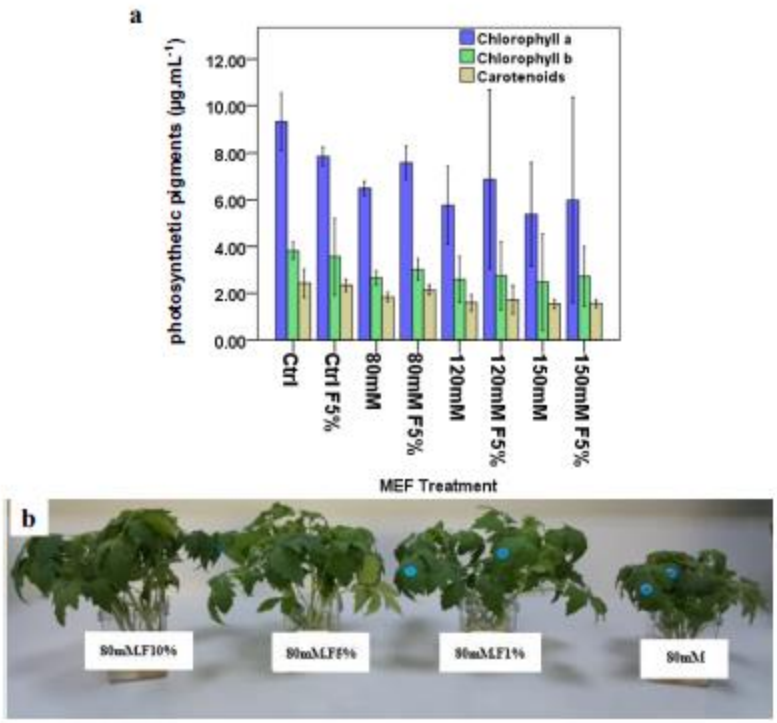

Fig. 2 Leaf chlorophyll content in tomato plants groups under different salt levels. Different small letters indicate significant differences between the MEF treatments across different NaCl levels. **b** The effect of MEF on the vegetative growth of tomato plant cultures, characterized by high shoot biomass and large total leaf area

treatment. The accumulation of proline was influenced NaCl concentration, whereas polyphenols remained unaffected by salinity increase.

Effect of MEF on lipid peroxidation and the activity of ROS scavenging enzymes

Lipid peroxidation

MEF5% exhibited very significant effects on the MDA content of plants grown at 80, 120, and 150 mM NaCl. There were no significant effects on unstressed control plants. All tomato plants treated with MEF5% except non-stressed control plants showed a significant decrease in MDA content, with the lowest decrease (– 27.49% and – 33.76%) in plants grown at 80 and 120 mM NaCl, respectively (Fig. 3c).

ROS scavenging enzyme activity

SOD and CAT enzyme activities were enhanced with increasing NaCl concentration compared to unstressed control plants. SOD activity exhibited the highest percentage increase (24.19% and 28.85%) in plant cultures subjected to 80 and 120 mM NaCl, respectively (Fig. 3d). Plant cultures grown at 80 mM exhibited the highest percentage increase in CAT activity, which increased by 144.38% after treatment with MEF5% (Fig. 3e). The highest percentage increase in POD activity was 97.59%, at 150 mM NaCl (Fig. 3f). Out of the three enzymes, SOD and CAT activities were significantly enhanced by MEF5% supplementation, notably in plants subjected to 80 and 120 mM NaCl.

Leaf concentration of nitrogen, phosphorus, and potassium

Nitrogen.

The concentration of N in the leaves decreased progressively with increasing NaCl concentrations. Supplementation of plants with MEF5% significantly enhanced N uptake by roots, with the highest N content improvement (182.95% and 21.87%) in plants grown at 80 and at 120 mM NaCl, respectively (See Supplementary Information (S3) for percentage increase values). N uptake positively correlated with MEF5% treatment in tomato culture groups grown at 80 and 120 mM NaCl (Fig. 4a).

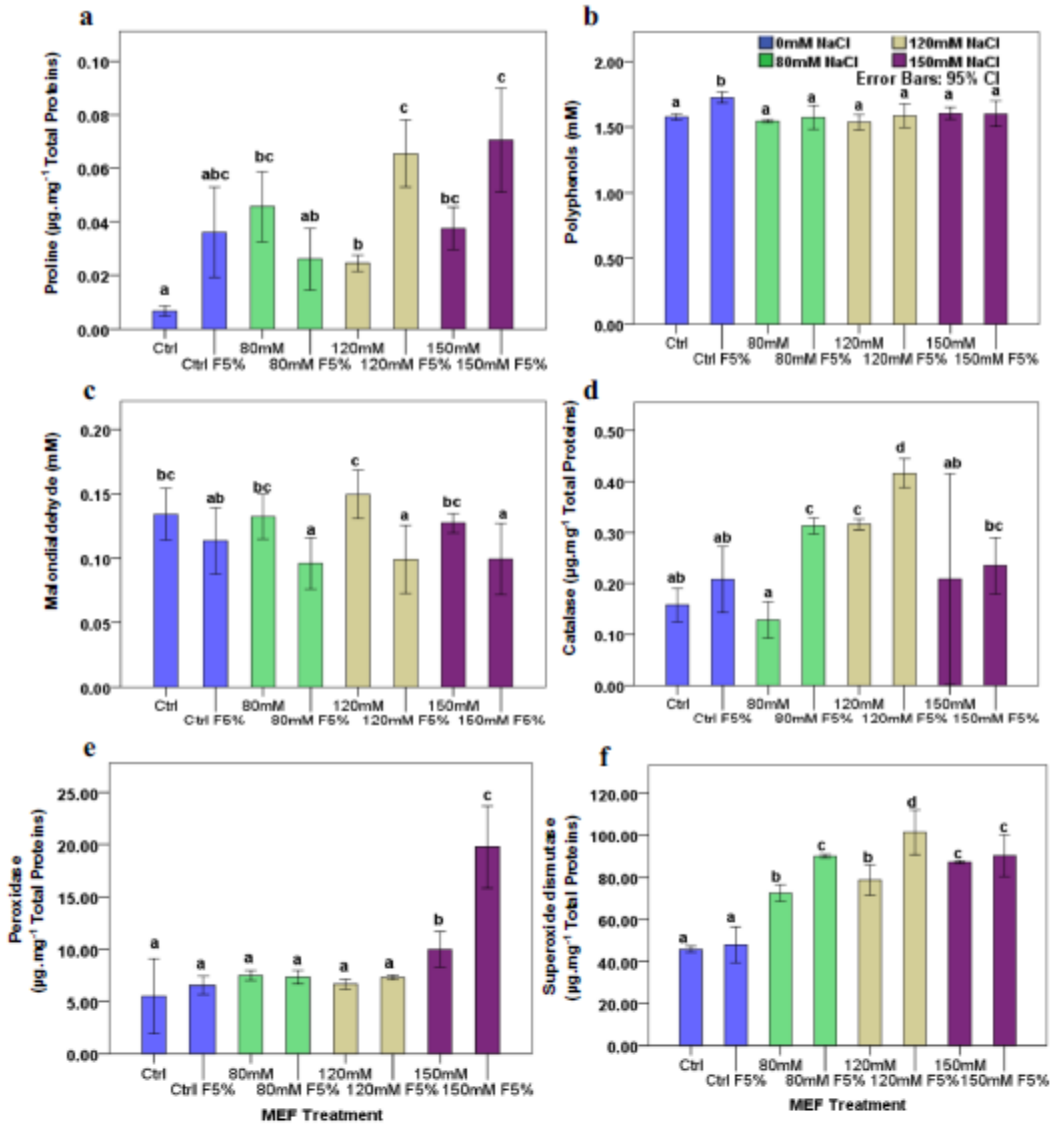

Fig. 3 Accumulation of proline, polyphenols, and MDA and anti-oxidative enzymes activities in leaves of tomato plant groups under different saline levels. Different small letters indicate significant differences between the MEF treatments across different NaCl levels

Phosphorus.

MEF5% treatment exhibited similar effects on P uptake. The highest amelioration effects (78.35% and 50%) were recorded in plants grown under 80 and 120 mM NaCl conditions (S3). P uptake was also significantly enhanced (36.49%) in unstressed control plants after treatment with MEF5%. There was a positive correlation between P uptake and MEF5% treatment in plants grown at 80 and 120 mM NaCl (Fig. 4a).

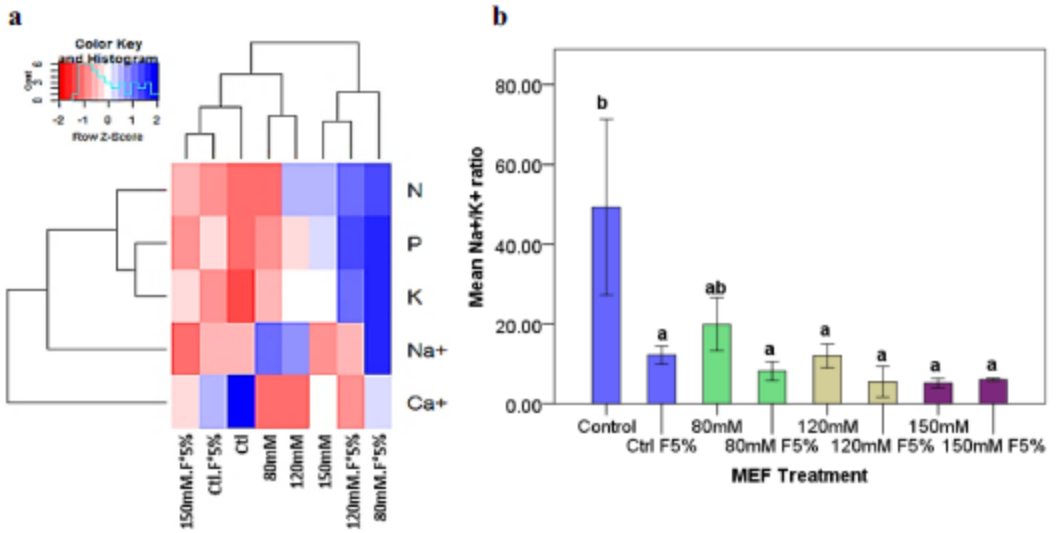

Fig. 4 Cluster analysis and comparative Na^+/K^+ ratios between treated and untreated plants under different saline levels. Different small letters indicate significant differences between the MEF treatments across different NaCl levels

Potassium.

The highest K uptake amelioration was 327.04% and 165.13% in unstressed control plants and plants grown at 80 mM NaCl, respectively. The MEF5% effect on K uptake decreased with increasing salinity (58.63% at 120 mM NaCl) and exhibited negative effects (– 26.76%) in plants grown at 150 mM NaCl (S3).

Calcium, sodium ion levels, and Na+/K+ratios

MEF5% supplementation to salt-stressed plants showed no effect on Ca+uptake expect for plants grown at 80 mM NaCl. MEF5% exhibited the least effect on Na+leaf concentration with 9.19 and 22.74% percentage increase in unstressed control plants and plants grown at 80 mM NaCl. Sodium ion levels reduced by – 41.93% and – 14.08% upon MEF5% application in plants grown at 120 and 150 mM NaCl. The enhanced K uptake in unstressed control plants and plants grown at 80 mM NaCl lowered their Na^+/K^+ ratios (Fig. 4b). There was no significant effect on Na^+/K^+ ratios in plants grown at higher salinity concentrations (120 and 150 mM) (Fig. 4b).

Treatment-variable interactions through matrix correlation and PCA

The influence of salinity and MEF treatment could be distinguished into three major groups: treated and untreated control plant groups were closely associated, followed by treated plant groups grown at 80 and 120 mM NaCl. Treatments were distinguished into three major groups (Fig. 5b). Untreated plant groups grown at 80 and 120 mM NaCl and treated and untreated groups grown at 150 mM were all closely associated and exhibited the least effect on all the studied parameters (Fig. 5b). Improved shoot length and shoot and root weights were closely associated with improved leaf content of photosynthetic pigments, which were positively correlated with unstressed control tomato plant group (Fig. 5c). Treated plants grown at 80 and 120 mM NaCl were positively correlated with NPK nutrient uptake which was closely associated with SOD and CAT activities but negatively correlated with MDA content, implying that improved SOD and CAT activities reduced MDA content. This correlation indicates that significant increase of SOD and CAT alleviated lipid peroxidation in plants subjected to 80 mM and 120 mM salinity levels (refer to Figure S5 for plot cumulative variance and scores).

Fatty acid profile analysis of MEF5%-treated plants under salt stress

These results suggest that the application of MEF5% to tomato plants induces changes in the profile of saturated fatty acids (SFA) and unsaturated fatty acids (UFA) (Table 1). Total leaf SFA content decreased by fourfold after MEF5% application in unstressed plants, by 1.8-fold

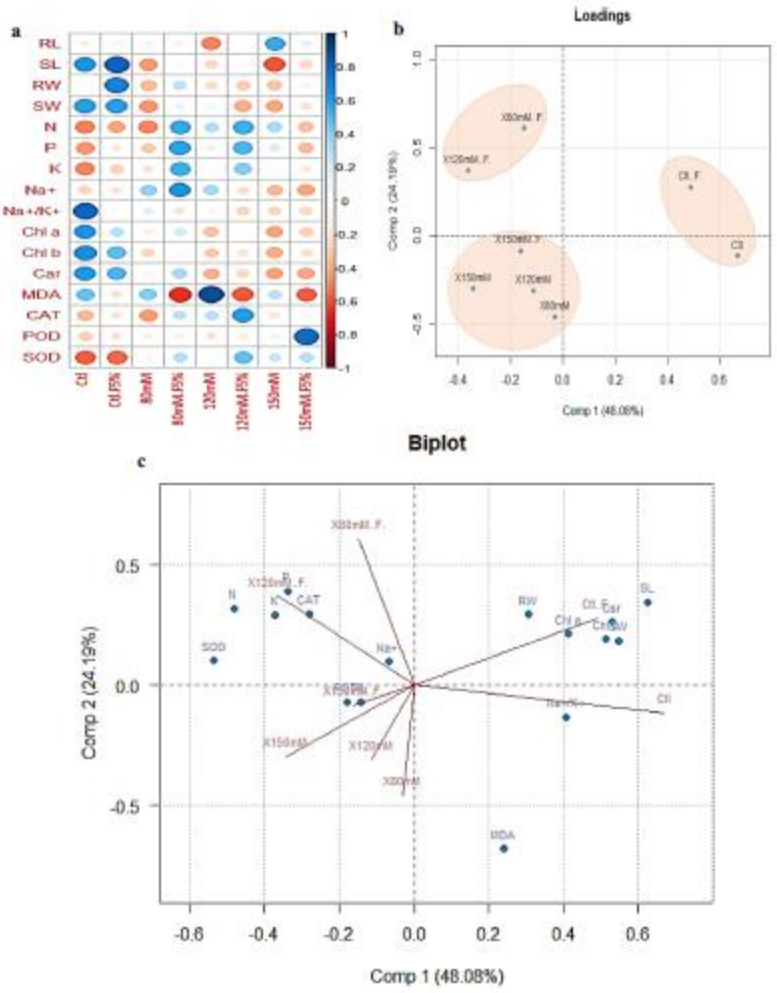

Fig. 5 Correlation matrix (Fig. 5a) and principal component analysis (PCA) to understand treatment-variable associations (Fig. 5b and c). The entire data was analyzed using PCA biplot. The variables included RW and SW (root and shoot weights), RL and SL (root and shoot lengths), Chl *a* (chlorophyll *a*), Chl *b* (chlorophyll *b*), Car (carotenoids), nitrogen, phosphorus, and potassium as N, P and K, Na^+ (sodium), Na^+/K^+ ratio, MDA (malondialdehyde), CAT (catalase), SOD (superoxide dismutase), and POD (peroxidases). The lines originating from the central point of PCA biplot indicate positive or negative correlations of different variables (Fig. 5c), where their closeness indicates correlation strength with particular treatment. Figures were generated using R Studio software

in plants grown at 80 mM NaCl, and by sevenfold in plants grown at 120 mM. The were no effects on SFA in plants subjected to 150 mM. Unsaturated fatty acids exhibited a similar profile, where UFA decreased by 3.5-fold in unstressed control plants and by 1.6-fold and 1.4-fold in plants grown at 80 and 120 mM NaCl, respectively, with no effects recorded in plants grown 150 mM NaCl. There were significant effects on the levels of very long chain fatty acids (VLCFA) in all plants, including plants grown at 150 mM, where the total VLCFA content was lowered by 1.4-fold.

MEF5% effect on the profile of sterols in tomato plants at different NaCl concentrations

Table 2 shows the sterol levels in treated and non-treated plants grown at different NaCl concentrations. Apart from plant cultures grown at 150 mM, the sterol profile was highly variable and independent of the MEF5% treatment or NaCl concentration. No sterols were detected in MEF5%-treated plants subjected to 80 and 120 mM NaCl. Moreover, besides stigmasta-3,5-diene, no sterols were detected in MEF5%-treated plants of both non-stressed

Table 1 MEF5% effect on fatty acid profiles in tomato leaves of culture groups under different saline levels. F° indicate plants treated with MEF5% formulation. The values represent the sterol content of homogenized biomass of three biological replicates. Values were normalized with an internal standard (dodecane). Margaric acid C17:0, stearic acid C18:0, oleic acid C18:1, linoleic acid C18:2, linolenic acid C18:3, arachidic acid C20:0, erucic acid C22:1, behenic acid C22:0, tricosylic acid C23:0, lignoceric acid C24:0, pentacosylic acid C25:0, cerotic acid C26:0. Refer to Table S2 for all fatty acids detected under different treatments

		Concentration µg g^{-1} FM							
		Ctrl -	Ctrl F°	80 mM	80 mM F°	120 mM	120 mM F°	150 mM	150 mM F°
SFA	C6:0	0.000	69.442	0.000	27.463	0.000	0.000	0.000	0.000
	C9:0	1253.101	298.487	138.351	49.884	2805.387	556.307	57.290	107.889
	C12:0	557.414	0.000	0.000	0.000	4088.724	0.000	0.000	0.000
	C14:0	3050.472	665.961	440.419	254.272	12,626.299	1545.959	261.254	286.115
	C16:0	88,756.399	21,011.373	8451.520	4734.680	364,616.620	45,808.759	4659.114	5628.807
	C17:0	6002.363	1731.938	0.000	195.814	0.000	5544.761	0.000	456.284
	C18:0	17,874.863	4685.496	2089.059	1444.497	71,947.898	11,340.332	1008.353	891.720
	C20:0	4169.906	1363.356	1138.506	143.870	48,487.909	5308.078	275.652	296.938
UFA	C16:1	0.000	0.000	0.000	54.534	19,340.917	0.000	0.000	0.000
	C16:3	8552.267	1680.394	3488.677	2108.289	0.000	0.000	2401.106	2414.350
	C18:1	0.000	0.000	0.000	0.000	2311.055	0.000	0.000	188.036
	C18:2	0.000	0.000	0.000	0.000	0.000	3971.558	0.000	0.000
	C18:3	48,879.291	14,515.578	16,400.765	10,078.791	0.000	10,684.092	11,398.563	11,597.446
VLCFA	C22:1	0.000	0.000	0.000	0.000	0.000	0.000	184.549	97.171
	C22:0	5004.036	883.023	612.817	0.000	9394.557	1305.277	186.525	106.592
	C23:0	1630.908	0.000	0.000	0.000	0.000	0.000	80.580	0.000
	C24:0	2770.699	727.703	356.809	0.000	10,430.802	1254.794	160.164	164.783
	C25:0	1483.958	382.394	0.000	0.000	3807.702	623.105	0.000	0.000
	C26:0	2012.692	560.915	0.000	0.000	0.000	0.000	0.000	55.931
Total	SFA	121,664.520	29,826.053	12,257.855	6850.481	504,572.830	70,104.195	6261.664	7667.752
	UFA	57,431.558	16,195.971	19,889.442	12,241.614	21,651.972	14,655.650	13,799.669	14,199.833
	VLCFA	12,902.293	2554.036	969.626	0.000	23,633.061	3183.176	611.818	424.477

control plants or plants subjected to 120 mM NaCl. However, plants grown at 150 mM NaCl exhibited higher levels of sterols. Supplementation of MEF5% to plants grown at 150 mM NaCl conditions reduced total leaf sterol concentrations by 1.2-fold. The leaf content of stigmasta-3,5-diene; stigmasta-5,22-dien-3-ol, acetate, (3β); stigmasterol, and β cholesterol also reduced upon MEF5% application.

Table 2 The profile of sterols in 40-day-old MEF5%-treated and non-treated tomato plants subjected to different NaCl concentrations under the laboratory conditions. F° indicate plants treated with MEF5% formulation. The values represent the sterol content of homogenized biomass of three biological replicates. Values were normalized with an internal standard (dodecane)

	Concentration µg g^{-1} FM							
	Ctrl -	Ctrl F°	80 mM	80 mM F°	120 mM	120 mM F°	150 mM	150 mM F°
Stigmasta-3,5-diene	2260.356	568.681	364.075	0.000	0.000	445.533	226.125	178.680
β-Sitosterol	0.000	0.000	190.972	0.000	0.000	0.000	0.000	0.000
Stigmasta-5,22-dien-3-ol, acetate, (3β)-	2721.109	0.000	0.000	0.000	0.000	0.000	200.635	148.960
Cholest-5-ene, 3-methoxy-, (3β)-	0.000	0.000	121.145	0.000	0.000	0.000	101.332	109.506
Stigmasterol	0.000	0.000	0.000	0.000	0.000	0.000	257.085	243.088
Cholesterol	417.157	0.000	300.860	0.000	0.000	0.000	218.248	160.891
Lanosterol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	18.808
Total	5398.621	568.681	977.052	0.000	0.000	445.533	1003.425	841.125

Effect of MEF5% on the profile of some alkanes detected in the leaf biomass

Certain alkane molecules were detected in high concentrations (S5). These results indicated that the application of MEF5% to tomato plants reduced the levels of nexacloroethane in the leaves of all plant groups, notably, in plants grown at 120 mM NaCl, which decreased by sixfold. Hentriacontane (n-C_{33}) content in leaves exhibited a similar pattern, where hentriacontane levels reduced by 3.6-fold, 1.6-fold, 5.4-fold, and 2.2-fold upon MEF5% application in non-stressed control plants and plants grown 80 mM, 120 mM, and 150 mM respectively. The most common cyclic siloxanes were cyclohexasiloxane, dodecamethyl-, cycloheptasiloxane, tetradecamethy, cyclooctasiloxane, hexadecamethyl, cyclononasiloxane, and octadecamethyl-. The was a significant increase (1.4-fold and threefold) in the total leaf content of these cyclic siloxanes in non-stressed control plants and plants grown at 150 mM NaCl respectively. In contrast, total content of the four cyclic siloxanes decreased (by 9.6-fold and 4.8-fold) upon MEF5% application (see data in Supplementary information (S5).

Discussion

Salinity stress exerts negative effects on the growth, development, and metabolic machinery of plants. In this study, both plant size and leaf concentrations of photosynthetic pigments significantly reduced with increasing NaCl concentrations (Fig. 2a). High NaCl concentrations lead to chlorophyll degradation through photooxidative reactions (Mitsuya et al. 2003) and low osmotic potential. Low osmotic potential triggers stomatal cloture in plants, leading to reduced CO_2 fixation, increased photorespiration, and H_2O_2 production in peroxisomes (Noctor 2002; Huang et al. 2015; Zhao et al. 2020). In the present study, treatment with MEF5% improved chlorophyll contents in unstressed plants and plants grown at 80 mM NaCl. According to PCA analysis, treatment with MEF5% was closely associated with improved nutrient uptake in plants grown at 80 and 120 mM NaCl. Improved nutrient uptake, notably K^+, in salt-stressed plants can play a major role in improving salt tolerance (Acosta-Motos et al. 2017). These results show that MEF5% can be a plant growth promoter and stress tolerance enhancer under non-saline or moderate saline conditions. The significant increase of growth and chlorophyll content in MEF5%-treated plants subjected to no stress and plants grown at 80 mM NaCl can be explained by improved osmotic adjustment.

In normal conditions, osmotic adjustment or cell turgidity is achieved by vacuolar K^+ pools. However, excessive Na^+ influx induces membrane depolarization, resulting into cytosolic K^+ efflux and subsequent liberation of vacuolar K^+ leading to decrease in cell turgor (Barragán et al. 2012; Latz et al. 2013). In salt-stress conditions, plants maintain osmotic adjustment via two major ways: (i) through de novo synthesis of compatible osmolytes and (ii) by increased uptake of inorganic ions (Na^+, Cl^-, and K^+) (Zhao et al. 2020). Compatible osmolytes including glycine-betaine and proline play an important role in osmotic adjustment by compensating for the osmotic pressure of Na^+ (Yan et al. 2013; Acosta-Motos et al. 2017; Chun et al. 2018). Osmotic adjustment via inorganic ion uptake such as Na^+ and K^+ require very low carbon cost compared to the production of organic osmolytes (Munns et al. 2020). Na^+ accumulation is generally preferred in halophytes that overcome Na^+ toxicity through vacuolar Na^+ sequestration in specialized leaf cells (Zhao et al. 2020). Induced K^+ intracellular retention and cytosolic accumulation of proline and sugars also improved salt tolerance in transgenic tomato (Leidi et al. 2010). In the present study, proline accumulation was significantly triggered by MEF5% in plants subjected to high NaCl conditions (120 and 150 Mm NaCl). Slightly significant affects were also recorded in unstressed control plants (Fig. 3a). These results indicated that improved salt tolerance in MEF5%-treated plants resulted from both enhanced proline accumulation and K^+ uptake. Improved root biomass in MEF5%-treated can lead to improved nutrient uptake and subsequent retention in leaves, leading to reduced Na^+ toxicity, notably in plants grown at 80 mM.

Glycophytes, such as tomato, are plants adapted to low-Na^+ environments and do not tolerate salinity greater than 100 mM NaCl (Assaha et al. 2017). The main contributing factor to ionic stress and salt stress sensitivity is almost exclusively attributable to excess Na^+ accumulation and K^+ deficiency, especially in the aerial parts of plants. K^+ activate more than 50 enzymes in the plant, and cannot be substituted by Na^+ (Tester 2003). Therefore, mechanisms of Na^+ and K^+ uptake and translocation are key to the survival of glycophytes in saline environments (Wu 2018). This ion homeostasis is mainly mediated by HKT and non-selective cation channels, and salt overly sensitive pathway (SOS1), a Na^+/H^+ antiporter (Ji et al. 2013; Assaha et al. 2017; Zhao et al. 2020). In this study, the investigation of improved ion homeostasis was based on the leaf Na^+ to K^+ ratios. The results showed that decreasing Na^+/K^+ ratios in the leaf biomass of treated plants was due to enhanced K^+ uptake in plants grown at 80 mM NaCl. Lower Na^+/K^+ ratio in MEF-treated plants grown at 120 mM was due to both K^+ accumulation and Na^+ decrease (Fig. 4a and Supplementary information S3). Enhanced K^+ and reduced Na^+ content in the leaf biomass of MEF5%-treated plants imply that MEF5% improved both salt tolerance and growth through improved ion homeostasis, in plants subjected to 80 mM and 120 mM NaCl. Partial characterization of the microalgae

Table 3 Selected microalgae and cyanobacteria crude extracts and their composition

	Polysaccharides (mg mL^{-1})	Neutral Sugars (mg mL^{-1})	Protein content (mg mL^{-1})	N (mg L^{-1})	NO^{3-} (mg L^{-1})	P (mg L^{-1})	K (mg L^{-1})
Cyanobacteria							
Aphanothece sp.	0.0096	0.049	0.054	2.43	0.74	2.55	6.04
Arthrospira maxima	0.0284	0.048	0.078	3.94	0.87	5.11	46.35
Chlorophyta							
Chlorella ellipsoideae	0.0116	0.855	0.535	17.2	2.0	25.8	169.8
Dunaliella salina	0.007	0.029	0.046	2.13	1.09	3.04	18.32

and cyanobacteria species indicate that MEF extracts were composed of neutral sugars, polysaccharides, proteins, and nutrients (Table 3).

It can be suggested that this organic content of MEF may have similar effects as root exudates. Root exudates are a mix of a wide variety of compounds including carbohydrates, amino acids, and organic acids and intervene in salt stress tolerance (Vives-Peris et al. 2020). For example, salt-stressed Arabidopsis seedlings inoculated with *Trichoderma* spp. showed enhanced elimination of Na^+ through root exudates (Contreras-Cornejo et al. 2014). Thus, the organic composition of MEF5% could have exhibited positive effects on Na^+ toxicity in the soil and on nutrient uptake by mimicking root exudates.

NPK uptake parallels vegetative plant growth and is crucial for plant growth and development (Alcantara and Gonzaga 2020). Many crops take up and store the majority of the nutrients during the vegetative growth. These stored nutrients are translocated to developing fruit during reproductive growth (Mengel 1995). In this study, uptake of NPK significantly increased in MEF5%-treated, with the exception of plants grown at 150 mM NaCl conditions. Improved N absorption in salt-stressed plants progressively increases the accumulation of soluble sugar, soluble protein, and free amino acids and activity of the antioxidant defense system (Sikder et al. 2020). On the other hand, improved P absorption and use efficiency in salt stressed plants improve tolerance through increased chlorophyll, carotenoid, proline, soluble sugar, and free amino acid content (Bargaz et al. 2016). It can be suggested that improved root biomass in MEF5% increased the roots' contact surface with the root substrate and favored nutrient absorption. However, PCA analysis indicated that root growth was not closely associated with NPK uptake. This indicates that other factors such as the organic composition of MEF may have improved nutrient uptake in plants under saline conditions.

MEF5% extracts also enhanced ROS scavenging enzyme activities of SOD, POD, and CAT (Fig. 3d, e, and f). ROS act as signaling molecules that regulate biological processes and plant responses to a variety of biotic and abiotic stresses (Turkan 2018). Excessive ROS accumulation under salt stress conditions is damaging to cellular structures (Huang et al. 2019; Zhao et al. 2020; Hasanuzzaman et al. 2020). To reduce the oxidative stress caused by excessive ROS accumulation, plants activate ROS-scavenging systems (Hanin et al. 2016). Enhancement of these antioxidant systems increases salt stress tolerance in plants due to their capacity to maintain ROS homeostasis (Bose et al. 2014). Enhancing SOD, POD, and CAT activities in salt-stressed plants alleviate lipid peroxidation by neutralizing ROS effects. SOD catalyzes the dismutation of O_2^- to H_2O_2 and O_2 (Ahanger et al. 2018). H_2O_2, produced by SOD activity, is then decomposed into H_2O and O_2 by CAT in the cytoplasm or scavenged by ascorbate peroxidase in the chloroplast and the cytosol (Ahanger et al. 2018; Hasanuzzaman et al. 2020). In the present study, SOD and CAT activities in MEF-treated plants negatively correlated with MDA accumulation (Fig. 4c), indicating that enhanced CAT and SOD activities lowered MDA accumulation, a product of lipid peroxidation. Previous studies have demonstrated that antioxidant enzyme activities including CAT, SOD, and POD attenuate oxidative damage via the detoxification of ROS (Caverzan et al. 2019; Tahjib-Ul-Arif et al. 2019; Khan et al. 2020), and alleviate salt stress in the wild salt-tolerant tomato species *Lycopersicon pennellii* (Mittova et al. 2003). The present study indicated that MEF5% can promote salt stress tolerance by reducing lipid peroxidation through enhanced ROS scavenging enzyme activities but have no effect on plants subjected to excessive saline conditions (150 mM). MEF5% can also improve plant growth under normal conditions, and reduce ROS damage resulting from natural cellular processes (photosynthesis and respiration).

Other parameters investigated in this study include comparative lipid profiling of MEF-treated and non-treated tomato plants. Soil supplementation with MEF5% induced a significant decline in leaf SFA, UFA, and VLCFA contents in all plant groups, with the exception of plants grown at 150 mM NaCl (Table 1). Modifications in membrane phospholipids act as signaling components during salt stress. Exposure of plants to both salt and osmotic stress induce several phospholipid signals such as polyphosphoinositides and phosphatidic acid (PA) within 5 min (Van Zelm et al.

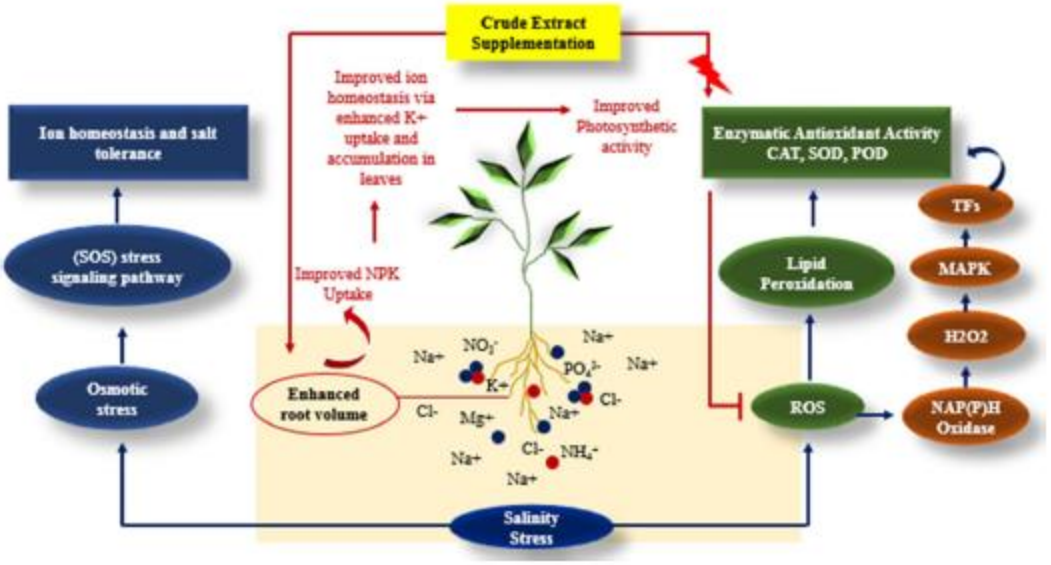

Fig. 6 The possible biostimulant effects of MEF5% on tomato plants under saline conditions. Bioactive organic compounds in extracts may directly stimulate the antioxidative enzyme system, thereby mitigating lipid peroxidation in plant cells. Additionally, MEF5% favorizes root growth and consequently improve the plants' nutrient uptake, ion homeostasis, and, subsequently, photosynthetic activity and plant growth under saline conditions

2020). The formation of PA is another putative downstream response of Ca^+ signaling during salt stress (Galvan-Ampudia et al. 2013).

Future studies should investigate the differential phytohormone expression profiles and accumulation of Na^+ and K^+ in the leaf, stem, and root biomass of treated and untreated tomato plants (salt-sensitive and salt-tolerant variants) subjected to salt stress. Comparative analysis of gene expression profiles of high affinity N, P, and K^+ transporter proteins is also needed to elucidate MEF mode of action and effect on nutrient uptake. Studying the effects of microalgae and cyanobacteria extracts on tomato yield will be a major contribution to understanding biostimulant effects on salt stress tolerance. Figure 6 highlights the major mechanisms attributed to improved salinity tolerance in this study.

Conclusion

Combined microalgae-cyanobacteria formulation can be a stimulator of salt tolerance responses, nutrient uptake, and plant growth of *Solanum lycopersicum*, notably in plants grown at lower salinity levels. In the present study, salt tolerance in treated tomato plants was mainly due to improved ion homeostasis through enhanced NPK uptake and leaf accumulation, which resulted in lower Na^+/K^+ ratios. Based on the partial characterization, the organic composition of MEF5% could have exhibited positive effects on Na^+ toxicity in the soil and nutrient uptake by mimicking root exudates. In addition, MEF5% treatment induced ROS scavenging enzyme activities which lowered lipid peroxidation in plants grown under 80 mM and 120 mM NaCl, with the exception of plants subjected to excessive saline conditions (150 mM). MEF5%-supplementation also lowered the total fatty acid content of tomato plants across all NaCl levels. Enhanced root mass subsequently increases the roots' surface for nutrient absorption. These results indicate that MEF5% could be a plant growth promoter under normal conditions by improving plant chlorophyll content, biomass production, and lowering ROS damage resulting from natural cellular processes (photosynthesis and respiration).

Supplementation of crops with MEF5% in saline conditions could also be another important research tool to boost tolerance, nutrient uptake, and vegetative growth in tomato crops under saline soils. Such product innovation will contribute to the development of sustainable agriculture products.

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Author contribution HA = project conception, funding acquisition, project supervision, design of methodology and revision of the manuscript. CMJ = writing (original draft preparation, review and editing), the study of physiological parameters, analysis of results. FR = experimental work, the study of physiological and biochemical parameters and manuscript revision. HAM = experimental work and analysis of results. NM = Study of nutrient uptake using Skalar nutrient auto analyser. AA = study of metabolomics using GC–MS and manuscript revision. MB = project supervision. DM = statistical analysis, visualization of results and generation of graphs using GraphPad Prism software. LS = thesis director.

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Data availability statement Besides the data provided in supplementary information, the data generated during and/or analyzed during the current study is available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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III-3. SYNTHÈSE

Le stress salin exerce des effets négatifs sur la croissance, le développement et la machinerie métabolique des plantes. Dans cette étude, la taille des plantes et les concentrations foliaires de pigments photosynthétiques ont considérablement diminué avec l'augmentation des concentrations de NaCl (**Fig. 2a**). Des concentrations élevées de NaCl déclenchent la dégradation de la chlorophylle par des réactions oxydatives (Mitsuya et al. 2003) et un faible potentiel.

Un faible potentiel osmotique déclenche la fermeture des stomates chez les plantes, entraînant une réduction de la fixation du CO_2 et une augmentation de la photorespiration et de la production d'H_2O_2 dans les peroxysomes (Noctor 2002; Huang et al. 2015; Zhao et al. 2020). Dans la présente étude, le traitement des plantes avec le MEF 5% a amélioré la teneur en chlorophylle des plantes non stressées et des plantes cultivées à 80 mm de NaCl. Selon l'analyse en composantes principales (ACP), le traitement avec le MEF 5% était étroitement associé à une meilleure absorption des nutriments chez les plantes cultivées à 80 mm et 120 mm de NaCl. Une meilleure absorption des nutriments, notamment du K^+, chez les plantes stressées par le sel peut jouer un rôle majeur dans l'amélioration de la tolérance à la salinité (Acosta-Motos et al. 2017). Ces résultats montrent que le MEF 5% peut être un promoteur de la croissance des plantes et un stimulateur de la tolérance au stress dans des conditions non salines ou de salinité modérée. L'augmentation significative de la croissance et de la teneur en chlorophylle chez les plantes non-stressées traitées avec le MEF5% et celles cultivées à 80 mM de NaCl peut s'expliquer par un meilleur ajustement osmotique.

Dans des conditions normales, l'ajustement osmotique ou la turgescence cellulaire est maintenu par l'accumulation du K^+ vacuolaires. Cependant, l'influx excessif du Na^+ dans le cytoplasme cellulaire induit une dépolarisation membranaire résultant en un efflux de K+ cytosolique. Ce phénomène induit alors la libération du K^+ vacuolaire vers le cytosol, pour composer le K^+ cytosolique perdu (Latz et al. 2013). L'épuisement des pools du K^+ vacuolaires diminue la turgescence cellulaire due à l'absence d'équilibre osmotique (Barragán et al., 2012). Dans des conditions de stress salin, les plantes maintiennent l'ajustement osmotique de deux manières principales : i) par la synthèse de novo des osmolytes compatibles et ii) par l'absorption élevé des ions inorganiques (Na^+, Cl^- et K^+) (Zhao et al. 2020). Les osmolytes compatibles, dont la glycine-bétaïne et la proline, jouent un rôle important dans l'ajustement osmotique en compensant la pression osmotique de Na^+ (Yan et al. 2013; Acosta-Motos et al. 2017; Chun et al. 2018). L'ajustement osmotique par absorption d'ions inorganiques tels que Na^+ et K^+ nécessite un coût en carbone très faible par rapport à la production d'osmolytes organiques (Munns et al. 2020). L'accumulation de Na^+ est généralement préférée chez les halophytes qui surmontent la toxicité de Na^+ par séquestration vacuolaire de Na^+ dans des cellules foliaires spécialisées (Zhao et al. 2020). L'augmentation de la rétention intracellulaire du K^+ et l'accumulation cytosolique de la proline et de sucres a également amélioré la tolérance de la tomate transgénique contre le stress salin (Leidi et al. 2010). Dans la présente étude,

l'accumulation de la proline a été déclenchée de manière significative par le MEF5% chez les plantes soumises à des conditions élevées de NaCl (NaCl de 120 mm et 150 mm). Des effets légèrement significatifs ont également été enregistrés chez les plantes témoins non stressées (**Fig. 3 a**). Ces résultats indiquent que l'amélioration de la tolérance au sel chez les plantes traitées avec le MEF5% résulte à la fois d'une accumulation élevée de la proline et de l'absorption de K^+. L'amélioration de la biomasse racinaire dans les plantes traitées avec le MEF à 5% peut entraîner une meilleure absorption des nutriments et leurs rétentions dans les feuilles, entraînant une réduction de la toxicité du Na^+, notamment chez les plantes cultivées à 80 mM NaCl.

Les glycophytes comme la tomate, sont des plantes adaptées aux environnements à faible teneur en Na^+ et ne tolèrent pas une salinité supérieure à 100 mM NaCl (Assaha et al. 2017). Le principal facteur contribuant au stress ionique et à la sensibilité au stress salin est presque exclusivement attribuable à l'accumulation excessive de Na^+ et à la carence en K^+, en particulier dans les parties aériennes des plantes. Le K^+ déclenche l'activation de plus de 50 enzymes dans la plante et ne peut pas être substitué par le Na^+ (Tester 2003). Par conséquent, les mécanismes d'absorption et de translocation de Na^+ et de K^+ sont essentiels à la survie des glycophytes dans des environnements salins (WU 2018). L'expression différentielle des transporteurs de potassium à haute affinité (HKT) est un trait déterminant important de la tolérance au stress salin chez les glycophytes et peut déterminer la tolérance différentielle chez les plantes par l'exclusion du Na^+ des feuilles (Assaha et al., 2015). En outre, la voie SOS comprenant SOS1, SOS2 et SOS3 est suggérée pour médier la signalisation cellulaire pendant le stress salin afin de réguler la concentration de Na^+ dans le cytosol (Ji et al., 2013 ; Zhao et al., 2020 ; Martínez-Atienza et al., 2007).

Dans cette étude, l'amélioration de l'homéostasie ionique était basée sur les rapports Na^+ / K^+ dans les feuilles. Les résultats ont montré que la diminution du rapport Na^+/ K^+ dans la biomasse foliaire des plantes traitées était due à une absorption améliorée de K^+ chez les plantes cultivées à 80 mM NaCl. Le rapport Na^+/ K^+ inférieur chez les plantes traitées avec le MEF5% et cultivées à 120 mM était dû à la fois à l'accumulation de K^+ et à la diminution de Na^+. La caractérisation partielle des espèces de microalgues et de cyanobactéries indique que les extraits de MEF étaient composés de sucres neutres, de polysaccharides, de protéines et de nutriments (**tableau 3**). On peut suggérer que cette composition organique de MEF peut avoir des effets similaires à ceux des exsudats de racines. Les exsudats racinaires sont un mélange d'une grande variété de composés comprenant des glucides, des acides aminés et des acides organiques qui interviennent dans la tolérance au stress salin (Vives-Peris et al. 2020). Par exemple, la soumission des semis d'Arabidopsis au stress salin et inoculés avec *Trichoderma* spp. a montré une augmentation de l'élimination du Na^+ par les exsudats racinaires (Contreras-Cornejo et al. 2014). Ainsi, la composition organique du MEF 5% pourrait avoir des effets positifs sur la toxicité du Na^+ dans le sol et sur l'absorption des nutriments en imitant les exsudats de racines.

L'absorption de NPK est parallèle à la croissance végétative des plantes et est cruciale pour la croissance et le développement des plantes (Alcantara et Gonzaga 2020). De nombreuses cultures absorbent et stockent la majorité des nutriments pendant la croissance végétative. Ces nutriments stockés sont transférés aux fruits en développement pendant la croissance reproductive (Mengel, 1995). Dans cette étude, l'absorption de NPK a augmenté de manière significative chez les plantes traitées avec le MEF 5%, à l'exception des plantes cultivées dans des conditions de NaCl élevé (150 mM). Une meilleure absorption de l'azote dans les plantes stressées par le sel augmente progressivement l'accumulation des sucres solubles, de protéines solubles et des acides aminés libres ainsi que l'activité du système de défense antioxydant (Sikder et al. 2020). D'autre part, l'amélioration de l'absorption et de l'efficacité d'utilisation du P dans les plantes stressées améliore la tolérance grâce à l'augmentation de la teneur en chlorophylle, en caroténoïdes, en proline, en sucres solubles et en acides aminés libres (Bargaz et al. 2016). On peut suggérer que l'amélioration de la biomasse racinaire chez les plantes traitées avec le MEF5% a augmenté la surface de contact des racines avec le substrat racinaire et favorisé l'absorption des nutriments. Cependant, l'analyse ACP a montré que la croissance des racines n'était pas étroitement associée à l'absorption de NPK. Cela indique que d'autres facteurs tels que la composition organique du MEF peuvent avoir amélioré l'absorption des nutriments chez les plantes dans des conditions salines.

Les MEFs ont également amélioré les activités enzymatiques antioxydantes de la Superoxyde dismutase (SOD), de la Peroxydase (POD) et la Catalase (CAT) (**Fig. 3d, e et f**). Les ROS agissent comme des molécules de signalisation qui régulent les processus biologiques et les réponses des plantes à une variété de stress biotiques et abiotiques (Turkan, 2018). Cependant, l'accumulation excessive de ROS dans des conditions de stress salin nuit aux structures cellulaires (Huang et al., 2019; Zhao et al., 2020; Hasanuzzaman et al., 2020). Ainsi, pour réduire le stress oxydatif causé par une accumulation excessive de ROS, les plantes activent des systèmes antioxydants qui peuvent être non enzymatiques (métabolites antioxydants, y compris l'ascorbate, le glutathion et les tocophérols) ou enzymatiques (CAT, SOD, POD et glutathion réductase (GR)) (Hanin et al., 2016). L'augmentation de la tolérance au stress salin chez les halophytes est d'ailleurs en partie attribuée à leur capacité élevée à maintenir l'homéostasie du ROS (Bose et al. 2014). Le SOD catalyse la dismutation de O_2- en H_2O_2 et O_2 (Ahanger et al., 2018). Le H_2O_2 produit par l'activité SOD est ensuite décomposé en H_2O et O_2 par CAT dans le cytoplasme ou récupéré par l'Ascorbate peroxydase dans le chloroplaste et le cytosol (Ahanger et al., 2018; Hasanuzzaman et al., 2020). Dans la présente étude, les activités de SOD et de CAT chez les plantes traitées avec le MEF5% étaient négativement corrélées à l'accumulation de MDA **Fig. 4c**, indiquant que l'augmentation des activités CAT et SOD a réduit l'accumulation de MDA, un produit de la peroxydation lipidique causé par les ROS.

D'autres paramètres évalués dans cette étude comprennent le profilage lipidique comparatif des plantes de tomates traitées et non traitées par le MEF. Les modifications des phospholipides membranaires agissent

comme des composants de signalisation lors du stress salin. L'exposition des plantes au stress salin et osmotique induit plusieurs signaux phospholipidiques tels que les polyphosphoinositides et l'acide phosphatidique (PA) (Van Zelm et al. 2020). La formation de PA est une autre réponse en aval présumée de la signalisation Ca^{+} pendant le stress salin (Galvan-Ampudia et al. 2013). Dans la présente étude, la supplémentation du sol en MEF à 5 % a entraîné la diminution significative des teneurs foliaires en acides gras saturés (SFA), acides gras insaturés (UFA) et en acides gras à longues chaînes (VLCFA), à l'exception des plantes cultivées à une teneur en NaCl de 150 mm (**tableau 1**).

III-4. CONCLUSION

La formulation combinée de microalgues et de cyanobactéries peut être un stimulateur des réponses de l'absorption des nutriments, de tolérance à la salinité et de la croissance des plantes de *Solanum lycopersicum* L., notamment chez les plantes cultivées à des niveaux de salinité plus faibles. Dans la présente étude, la tolérance à la salinité chez les plantes de tomates traitées était principalement due à une homéostasie ionique améliorée grâce à une absorption amélioré de NPK et à une accumulation foliaire de K^{+}, ce qui a entraîné des rapports Na^{+}/ K^{+} plus faibles. D'après la caractérisation partielle, la composition organique du MEF 5% aurait pu avoir des effets positifs sur la toxicité du Na^{+} dans le sol et l'absorption des nutriments en imitant les exsudats racinaires. De plus, le traitement MEF5% a induit la stimulation de l'activité des enzymes anti-ROS réduisant la peroxydation lipidique chez les plantes cultivées à 80 mM et 120 mM NaCl, à l'exception des plantes soumises à des conditions salines élevés (150 mM). Le traitement des plantes avec le MEF5% a également réduit la teneur totale en acides gras chez les plants de tomates à tous les niveaux de NaCl. L'augmentation de la masse racinaire a également augmenté la surface racinaire pour l'absorption des nutriments. Ces résultats indiquent que le MEF5% pourrait être un promoteur de la croissance des plantes dans des conditions normales en améliorant la teneur en chlorophylle des plantes, la production de biomasse et en réduisant les dommages causés par les ROS résultant des processus cellulaires naturels (photosynthèse et respiration).

Le traitement des cultures agricoles avec le MEF5% dans des conditions salines pourrait également être un autre outil important pour l'amélioration de la tolérance, l'absorption des nutriments et la croissance végétative des cultures de tomates dans des sols salins. Une telle innovation de produits contribuera au développement de produits agricoles durables.

CHAPITRE IV

CHAPITRE IV

L'étude métabolique et transcriptomique des effets d'extraits d'*Aphanothece* sp. sur la croissance et les traits d'acquisition du phosphore chez la tomate (*Solanum lycopersicum* L.)

IV-1. INTRODUCTION

Le phosphore (P) est l'un des nutriments les plus importants et nécessaires à la croissance et au développement des plantes. La concentration du phosphate inorganique (Pi) dans le sol ne dépasse généralement pas 10 µM, ce qui est nettement inférieur à la concentration intracellulaire millimolaire (5-20 mM) requise pour une croissance optimale des plantes (Shen et al. 2011; Hasan et al. 2016).

Afin d'améliorer la fertilité du sol et la production végétale, plus de 40 millions de tonnes métriques d'engrais phosphatés (engrais-P) sont appliquées aux terres agricoles de manière globale chaque année (Oloo et Asbon 2020). Moins de 20 à 25% de l'engrais-P appliqué au sol est absorbé par les plantes cultivées et une grande partie est perdue par le lessivage (Daverede et al. 2004; Hart et al. 2004; Wang et al. 2012b) ou fixée aux particules du sol, et non disponible pour l'absorption par les plantes (Shen et al. 2011; van Dijk et al. 2016; Dixon et al. 2020; Bindraban et al. 2020). Le développement de nouvelles variétés de plantes plus efficiente dans l'utilisation de P contribuera à l'augmentation de l'absorption du P et à la diminution des pertes d'engrais P. Ces études incluent le développement de plantes transgéniques efficientes dans l'utilisation de P, qui fonctionnent mieux que d'autres génotypes soumis à des concentrations équivalentes du P (Dawson et Hilton 2011; Aziz et al. 2014; Abbas et al. 2018; Irfan et al. 2019).

De nombreuses plantes cultivées ont développé plusieurs stratégies pour augmenter l'acquisition de P peu disponible dans le sol (Bucher 2007). Ces systèmes adaptatifs morphologiques, physiologiques, biochimiques et moléculaires comprennent une production élevée d'exsudats racinaires (Pantigoso et al. 2020), la modification de l'architecture racinaire (Dixon et al. 2020) et la régulation des protéines de transport de P à haute affinité (Clark et Zeto, 2000; Poirier et Bucher, 2002; Gu et al. 2016). Les transporteurs de P à haute affinité sont constitués de symporteurs Pi/H^+ et font partie de la famille de TRANSPORTEUR DE PHOSPHATE 1 (PHT1). Ils sont plus largement répartis dans les tissus végétaux et sont impliqués à la fois dans l'absorption du P par les racines et sa mobilisation interne (Nussaume et al. 2011; Chen et al. 2014; Młodzińska et Zboińska 2016). L'amélioration des mécanismes d'adaptation chez les plantes pourrait améliorer l'absorption et l'efficacité de l'utilisation du P. Par exemple, une croissance élevée des racines augmente la surface racinaire pour l'absorption des minéraux et de l'eau et la capacité des racines à explorer plus de volume du sol (Aziz et al. 2014; Irfan et al. 2020). Les plantes transgéniques,

produisant plus d'acides organiques, amélioraient également l'efficacité l'acquisition du P insoluble par rapport aux plantes témoins (López-Bucio et al. 2000).

D'autres part, l'application exogène des produits bioactifs naturels, y compris les biostimulants de plantes, améliore l'absorption des nutriments par les racines (De Pascale et al. 2017). Un nombre croissant d'études ont été menées pour mettre en évidence les propriétés biostimulantes des extraits de microalgues et de cyanobactéries sur différentes activités biologiques chez les plantes supérieures notamment l'augmentation de la croissance racinaire, l'absorption des nutriments, la performance des cultures, l'état physiologique et la tolérance au stress abiotique (De Morais et al. 2015; Garcia-Gonzalez et Sommerfeld 2016; El Arroussi et al. 2016; Barone et al. 2018; EL Arroussi et al. 2018; Rachidi et al. 2020).

La présente étude met en évidence les effets des extraits totaux d'*Aphanothece* sp. sur la croissance des racines, l'assimilation des nutriments et les profils d'expression comparatifs des phytohormones et des gènes de la famille Pht1 dans les plants de tomates soumis à différentes concentrations d'engrais TSP (Triple Super Phosphate). Cette étude valorise l'utilisation directe de produits bioactifs organiques pour moduler les traits adaptatifs des plantes dans des conditions de suffisance en P, afin d'améliorer l'acquisition du P dans les plantes cultivées, une stratégie durable pour la gestion des engrais-P et l'efficacité de l'utilisation du P dans les sols agricoles.

Metabolic and transcriptomic study of *Aphanothece sp biostimulant* effects on plant growth and phosphorus acquisition traits in Tomato plants (*Solanum lycopersicum* L.)

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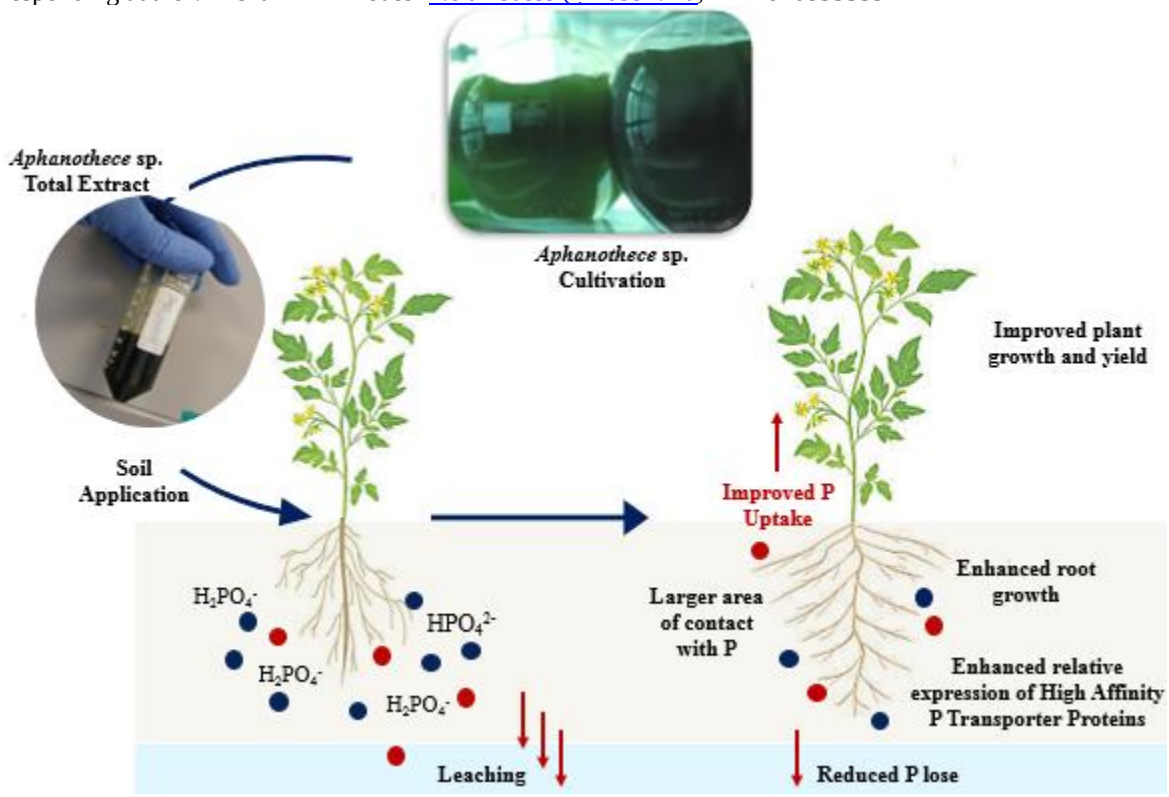


ABSTRACT
Excessive inputs of chemical Phosphorus (P) fertilizers to agricultural soils and low P-absorption efficiency by crops increases P loss to the environment, leading to environmental damage. This study investigates the biostimulant properties of *Aphanothece* sp Extracts (ApE) on P absorption efficiency traits of tomato plants supplied with triple superphosphate (TSP) fertilizer (0.3mM, 0.6mM and 1.2mM). ApE extracts had significant effects on the root growth, P uptake, root biomass production and chlorophyll and lipid contents. ApE-treated plants exhibited higher root dry weight (DW) and P concentration in the leaf and root biomass, compared with control plants. The relative transcription of high affinity P transporter genes (*Le*PT3, *Le*PT4 and *Le*PT5) in roots was enhanced in ApE-treated plants supplied with 0.3mM P, and (*Le*PT2, *Le*PT4 and *Le*PT5) in ApE-treated plants supplied with 0.6mM P, but significantly decreased in high P conditions. GC-MS analysis also revealed that ApE-treated plants supplied with 0.6mM P accumulated higher levels of metabolites, including unsaturated fatty acids and sterols. According to the Principal Component Analysis (PCA), P concentration in roots was closely associated with root DW in plants supplied with 0.3mM and 0.6mM P. Improved P-uptake in ApE-treated plants was largely due to significant root growth increase, resulting in higher P absorption by roots.

Key words: *Aphanothece* sp, cyanobacteria, biostimulant, phosphorus absorption, tomato

1. INTRODUCTION

Phosphorus (P) is one of the most important nutrients required for plant growth and development. It is well documented that inorganic phosphate (Pi) concentration in the soil solution is usually not more than 10 μM, which is significantly lower than the millimolar intracellular concentration (5–20 mM) required for optimal plant growth (Shen et al. 2011; Hasan et al. 2016).

In order to improve soil P fertility and crop production, over 40 million metric tons of P-fertilizer are applied to agricultural land worldwide every year (Oloo and Asbon 2020). Less than 20 - 25% of the applied P fertilizer is absorbed by crops and most of it is lost through runoffs (Daverede et al. 2004; Hart et al. 2004; Wang et al. 2012b) or fixed to soil particles and unavailable for plant uptake (Shen et al. 2011; van Dijk et al. 2016; Dixon et al. 2020; Bindraban et al. 2020). The development of novel P-use efficient crop varieties will help increase P-absorption by crops and reduce P-fertilizer loss to the environment. These studies include the development of P-efficient transgenic crops that perform better than other genotypes with equivalent P inputs (Dawson and Hilton 2011; Aziz et al. 2014; Abbas et al. 2018; Irfan et al. 2019).

Many crop plants have evolved several strategies to increase the acquisition of poorly available P from the soil (Bucher 2007). These morphological, physiological, biochemical and molecular adaptive systems include increased production of root exudates (Pantigoso et al. 2020), modification of the root system architecture for topsoil foraging (Dixon et al. 2020) and the regulation of high-affinity P-transporter proteins for the uptake and subsequent redistribution of P (Clark and Zeto 2000; Poirier and Bucher 2002; Gu et al. 2016). High affinity P-transporters consist of Pi/H+ symporters and are members of the PHOSPHATE TRANSPORTER 1 (PHT1) gene family. They are more widely distributed throughout plant tissues and are implicated in both root uptake the internal mobilization of P (Nussaume et al. 2011; Chen et al. 2014; Młodzińska and Zboińska 2016). Improving such adaptive mechanisms in crops could enhance P absorption and use efficiency. Enhanced root growth increases the roots surface for mineral and water absorption, and the roots' capacity to explore more soil volume (Aziz et al. 2014; Irfan et al. 2020). Studies also demonstrated that transgenic plants, producing more organic acids, significantly enhanced the absorption efficiency of insoluble P compounds compared with control plants (López-Bucio et al. 2000).

Exogenous application of natural bioactive products, including plant biostimulants also enhance nutrient uptake by roots (De Pascale et al. 2017). biostimulants are defined as "*fertilizing products the function of which is to stimulate plant nutrition processes independently of the product's nutrient content with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere: i) nutrient use efficiency, ii) tolerance to abiotic stress, iii) quality traits, or iv) availability of confined nutrients in the soil or rhizosphere*" (Ricci 2020). Microalgae and cyanobacteria are gaining popularity as renewable resources for the development of innovative bioproducts in agriculture, due to their ability to produce a wide range of biologically active molecules such as sulfated polysaccharides, osmolytes, phytohormones, amino acids and phenolics (Cuellar-Bermudez et al. 2015; de Morais et al. 2015; Renuka et al. 2018). An increasing number of studies have been conducted to highlight the biostimulating properties of microalgae and cyanobacteria extracts on

different biological activities in higher plants; including increased root growth, nutrient uptake, crop performance, physiological status and abiotic stress tolerance (De Morais et al. 2015; Garcia-Gonzalez and Sommerfeld 2016; El Arroussi et al. 2016; Barone et al. 2018; EL Arroussi et al. 2018; Chanda et al. 2020; Rachidi et al. 2020).

The present study investigated the effects of *Aphanothece* sp. extract supplementation on root growth, nutrient assimilation and comparative expression profiles of phytohormones and Pht1 family genes in tomato plants supplied with different concentrations of (Triple Super Phosphate) TSP fertilizers. This study highlights the direct use of organic bioactive products to modulate P-adaptive traits that enhance P-acquisition in crop plants, a sustainable strategy for P-fertilizer management and use efficiency in agriculture soils.

3. RESULTS

3.1 Effects of *Aphanothece* sp. extracts on root and shoot biomass and chlorophyll content

3.1.1 Shoot and Root Dry Weights

P and ApE supply significantly affected tomato growth and biomass accumulation. As shown in **Fig. 1**, all growth indices including plant biomass production, root/shoot ratios and SPAD values significantly increased in ApE-treated plants supplied with 0.3mM and 0.6mM P. Treatment of tomato plants with ApE increased the shoot DW by 10% and 22% at 0.3mM and 0.6mM P respectively. The root biomass accumulation measured as root DW also significantly increased (26% and 36%) in plants subjected to 0.3mM and 0.6 mM P conditions respectively. No significant effects were recorded in plants supplied with 1.2 mM of P.

3.1.2 The Shoot to root ratios

Plant shoot/root (S:R ratio) biomass partitioning across all treatments was affected by both P concentration and ApE treatment. Relative allocation of biomass to roots (measured as S:R ratio) was highest in plants subjected to 0.3mM and 0.6mM mM P and significantly decreased in plants subjected to 1.2mM P. The mean S:R ratios decreased by -12% and -10% at 0.3mM and 0.6mM P respectively.

3.1.3 Chlorophyll content

The chlorophyll content in leaves indicated by the SPAD value significantly increased in plants supplied with 0.6mM P (**Fig. 1**). The comparative SPAD value of plants treated with ApE and untreated plants, was highest (10%) in plants subjected to the 1.2mM P. Plants subjected to high P exhibited the highest mean SPAD values despite their lower biomass production measured as shoot and root DWs. There weren't any significant differences between the SPAD mean values of treated and untreated plants supplied with 0.6mM P.

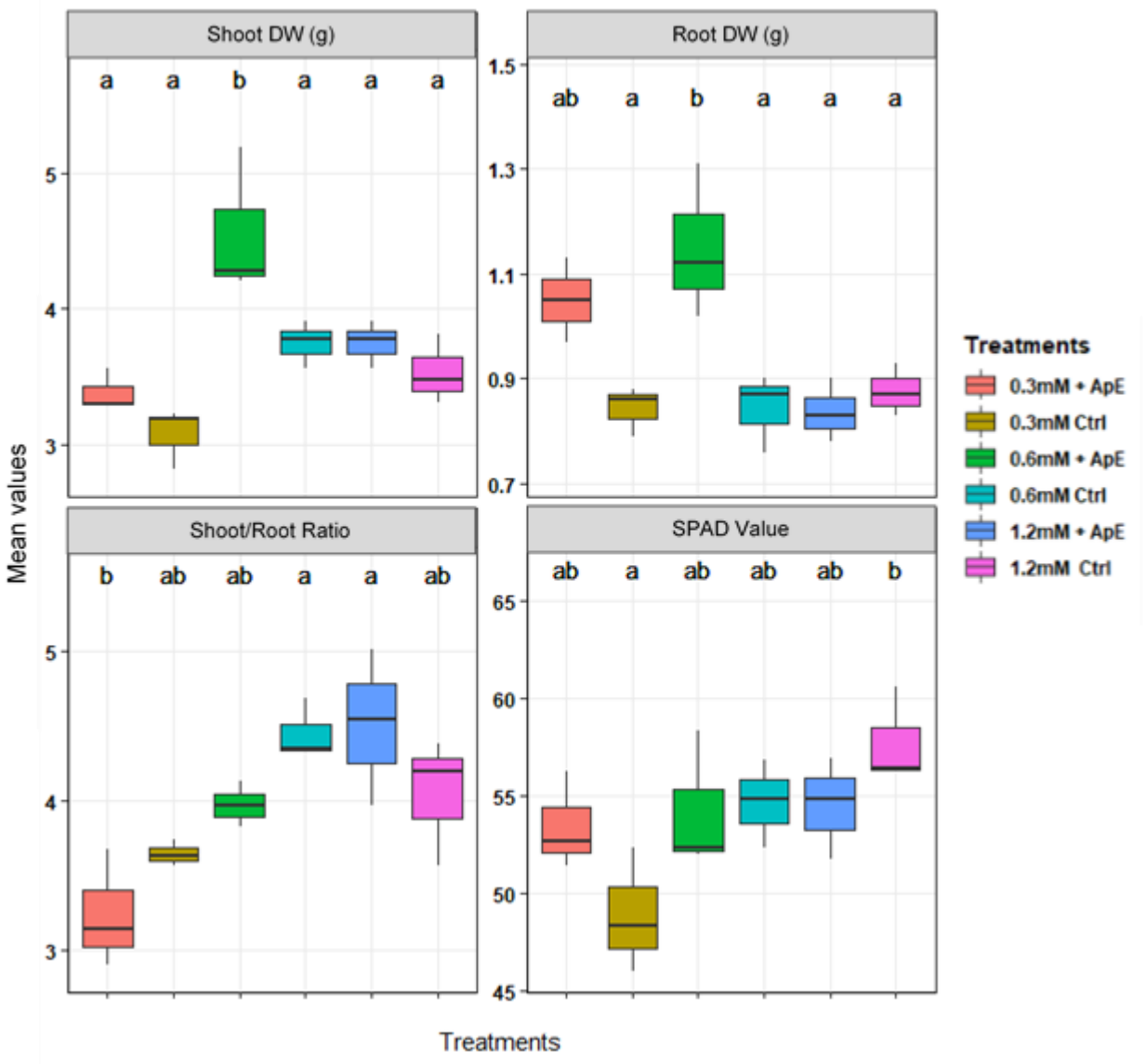


Figure 1. *Shoot and Root DW, shoot to root ratios and SPAD values of plants subjected to increasing P supply (0.3mM, 0.6mM and 1.2 mM of P). Different small letters indicate significant differences between ApE-treated and untreated plants across different P levels (Two-way ANOVA_Tukey's HSD test, P < 0.05). Each value is the mean ± SD of three replicate measurements.*

3.2 Effects of ApE on phosphorus concentration in the leaf and root biomass

ApE treatment enhanced P concentration in the leaf biomass of plants supplied with 0.3mM P but had no effect on the leaf P concentration of plants irrigated with 0.6mM and 1.2mM P (**Fig. 2**). ApE treatment significantly increased (78.35% and 50%) P concentration in the root biomass of tomato plants under 0.3mM and 0.6mM P conditions. There was no significant difference in the root P concentration between ApE-treated and untreated plants under 1.2 mM P conditions.

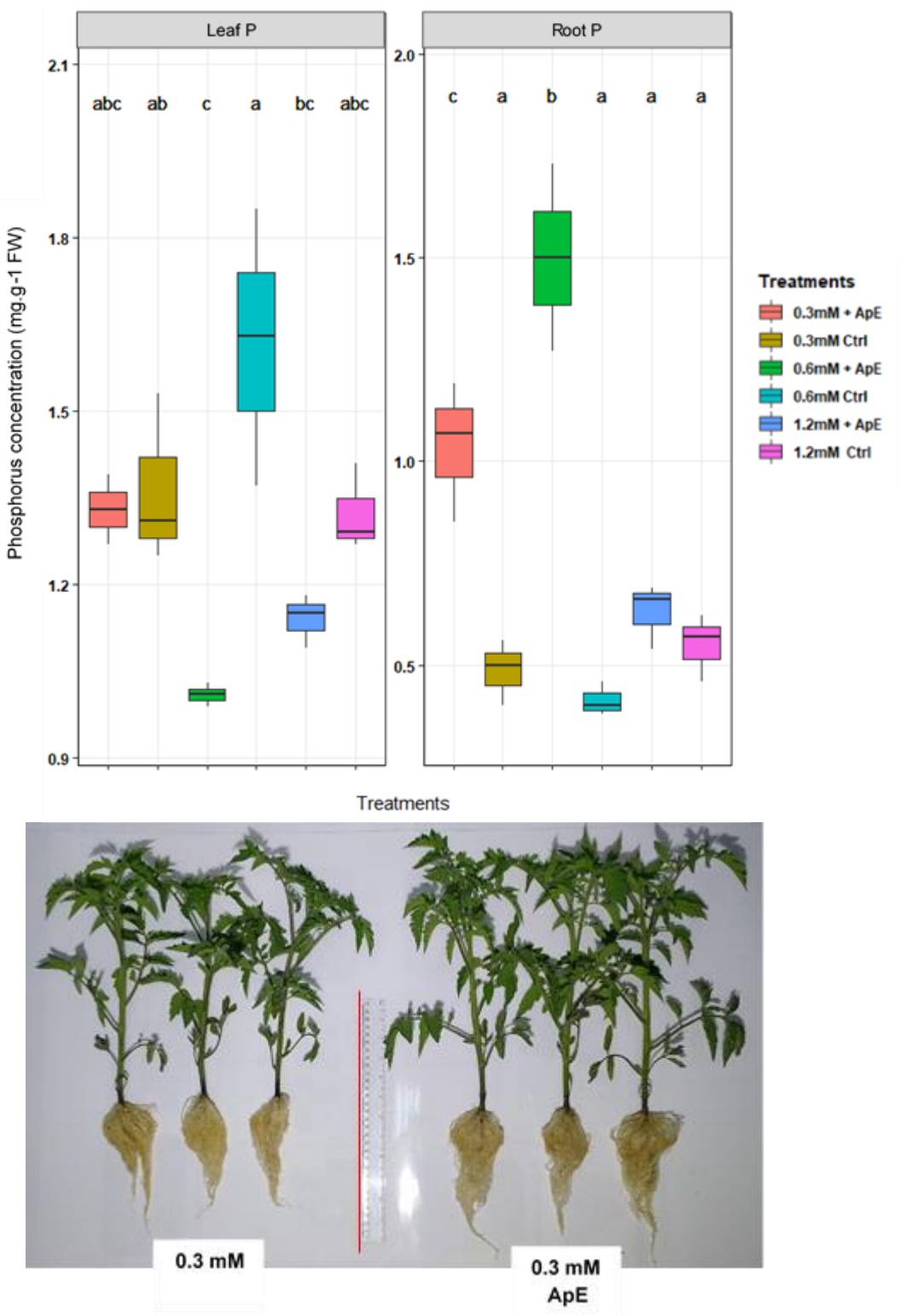
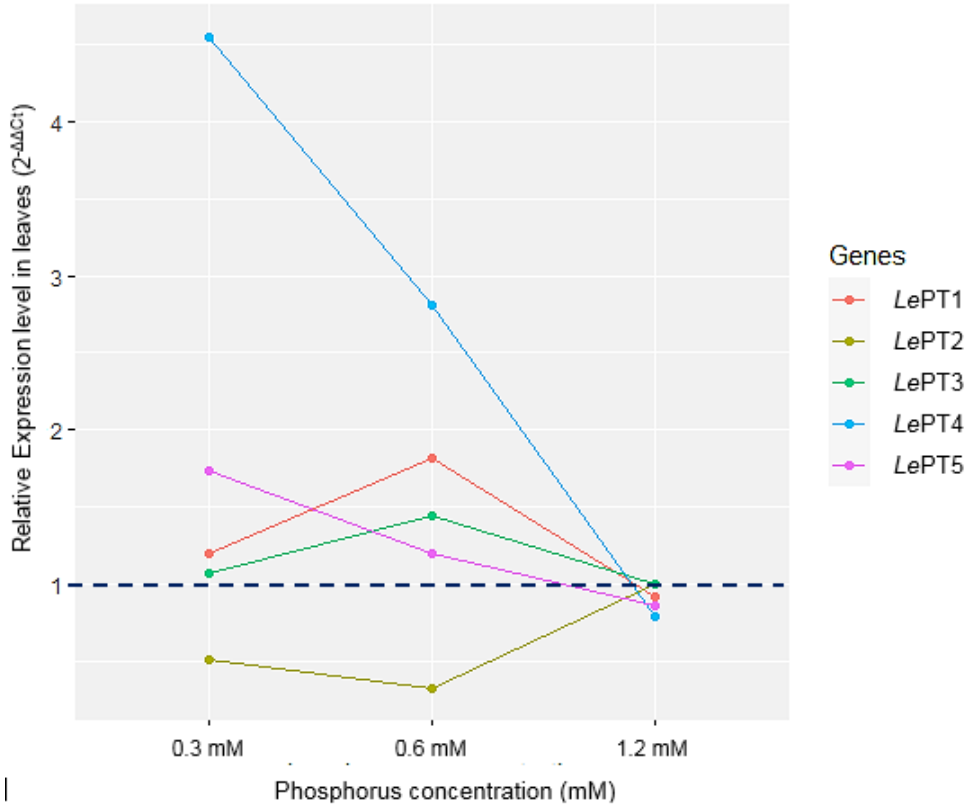


Figure 2: *The effect of* ApE *treatment on Phosphorus concentration of in tomato plants subjected to increasing P supply (0.3mM, 0.6mM and 1.2mM P). Different small letters indicate significant differences between the ApE treated and untreated plants across different P levels (Two-way ANOVA_Tukey's HSD test, P < 0.05). Each value is the mean ± SD of three replicate measurements.*

3.3 Real-time RT-PCR analysis of Pht1 genes in tomato roots and leaves in response to *Aphanothece* sp. extract supplementation under increasing P levels.

The relative quantification of LePT1 – LePT5 genes indicated that these genes are differentially induced (in both leaves and roots) in response to ApE treatment and P concentrations (**Fig. 3**). Except for *Le*PT2 and *Le*PT3, the relative expression of *Le*PT1, *Le*PT4 and *Le*PT5 in leaves was slightly enhanced (1.2, 4.5 and 1.7-fold respectively) in plants under 0.3mM P conditions and by 1.8, 2.8 and 1.2-fold in plants under 0.6mM P conditions. *Le*PT2 showed relatively distinct tissue-specific profile under 0.6mM P conditions, with 5.2-fold increase in the roots but significantly low expression in the leaves. The relative expression of *Le*PT5 also increased by 3.0-fold in the roots of ApE-treated plants under 0.6mM P conditions. *Le*PT5 transcription was upregulated in both leaves and roots of plants grown at 0.3mM P. All genes were repressed in both the leaves and roots of tomato plants subjected to 1.2mM P supply conditions. ApE supply influenced P absorption, via enhanced expression of root transporter genes under 0.3mM and 0.6mM P. Conversely, correlation between the root's internal P concentration and root transporter gene expression in ApE-treated plants indicated that P absorption was more closely associated with increased root surface than enhanced expression of root transporter genes.



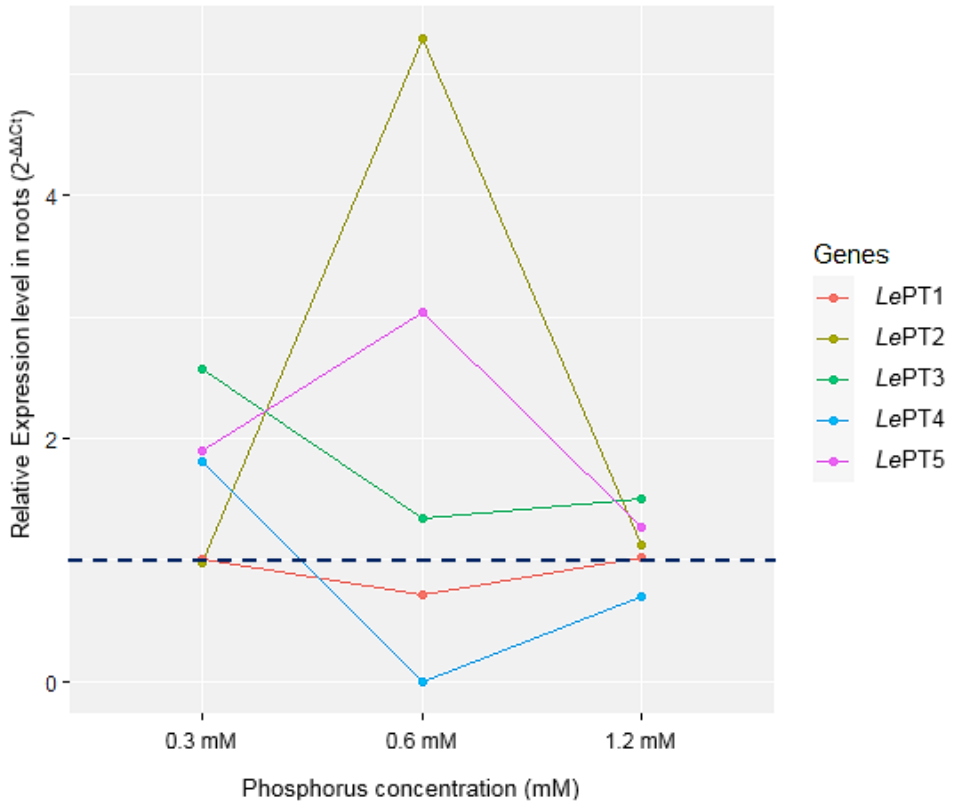


Figure 3. *Relative expression levels of LePT1 - LePT5 transcripts in the leaves and roots of treated and untreated plants subjected to three P levels. 35-days old treated and untreated tomato plants were grown in vermiculite supplied with different P (0.3mM, 0.6mM and 1.2mM) P concentrations.*

3.4 Metabolite profile analysis of *Aphanothece* sp. biostimulant effects on tomato plants under different phosphate regimes.

3.4.1 Comparative leaf profiles of fatty acids in treated and untreated tomato plants

Palmitic acid (C16:0), Roughanic acid (C16:3), Stearic acid (C18:0) and α-Linolenic acid (C18:3) were the dominant fatty acids across all treatments. The highest plant growth and fatty acid accumulation was recorded in tomato plants supplied was 0.6 mM P. Plants supplied with 0.3 mM P exhibited lower fatty acid accumulation whereas high supply P supply (1.2mM) significantly reduced fatty acid content in leaves (**Fig. 4**). C18:3 and C16:0 were the highest accumulated fatty acids across all P conditions. The concentration of C16:0, C16:3, C18:0 and C18:3 fatty acids in the leaf biomass increased by 46.66%, 102.89%, 52.99% and 55.24% respectively, in ApE-treated plants supplied with 0.6mM P. Lower (0.3mM) or high (1.2mM) P concentrations resulted in reduced ApE effects on fatty acid accumulation.

Other minor fatty acids detected in small amounts included myristic acid (C14:0), Margaric acid (C17:0),Oleic acid (C18:1), Nonadecylic acid (C19:0), Behenic acid (C22:0), Tricosylic acid (C23:0), lignoceric acid (C24:0) and Cerotic acid (C26:0). High P concentration significantly reduced the quantity and variety of fatty acids. All minor fatty acids were significantly higher in plants supplied with 0.3mM and 0.6mM P. The fatty acid concentrations were significantly enhanced after treatment with ApE. C14:0, C19:0, C22:0, C24:0 and C26:0 increased by 49.46%, 81.38%, 64.23%, 72.20% and 123.39% respectively in plants supplied with 0.6mM P.

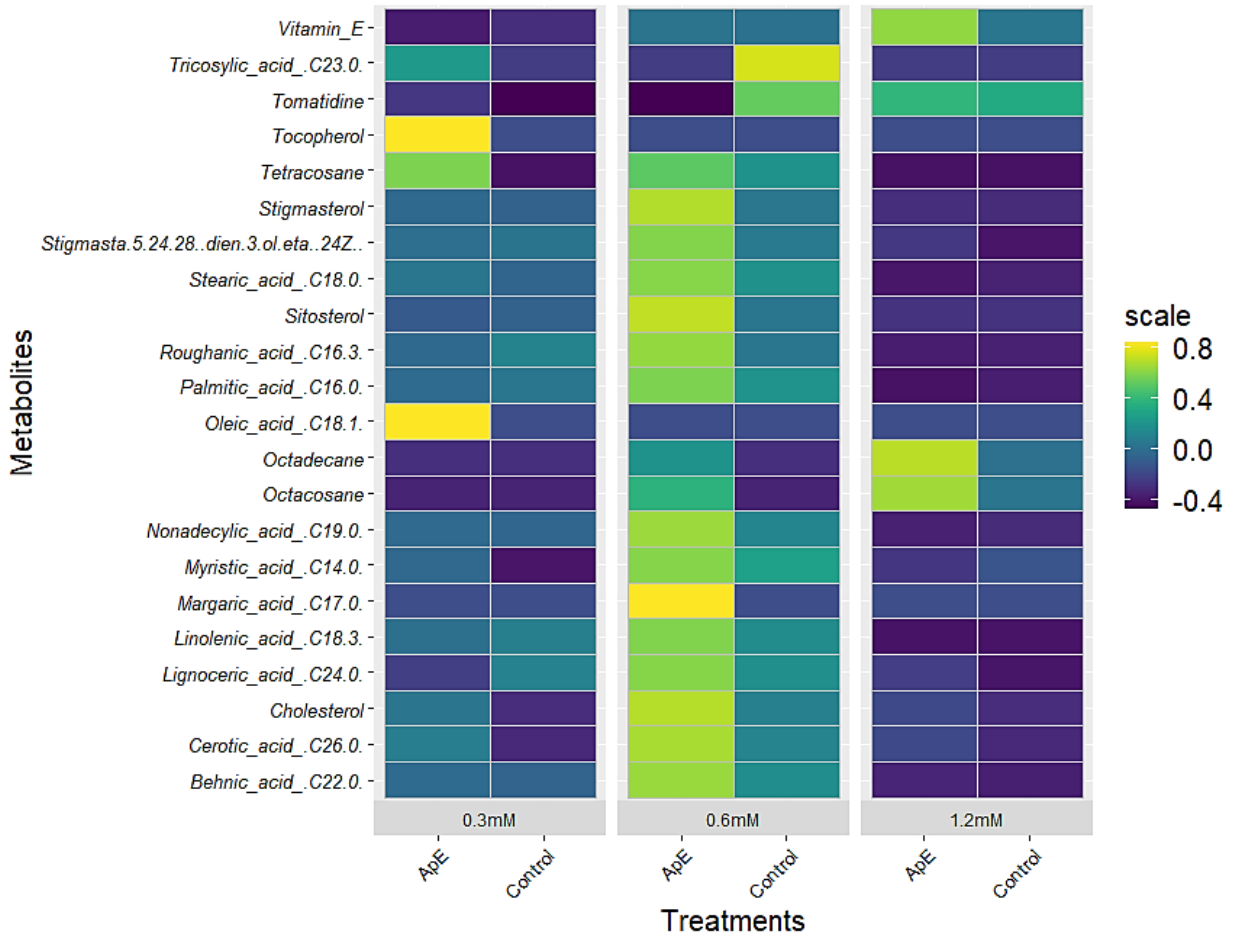


Figure 4. *The Profile of the most abundant Fatty Acids in 35-days old tomato leaves of treated and untreated plants subjected to three P regimes. Values were normalized with an internal standard (Dodecane). Fatty acids are abbreviated with the number before the colon indicating the number of carbon atoms, and the number after the colon indicating the number of double bonds.*

3.4.2 Comparative leaf profiles of sterols in treated and untreated tomato plants

To investigate the effect of ApE on sterol biosynthesis, sterol profile modifications were examined in response to changes in P concentration. The leaf sterol content was lower in plants supplied with 1.2mM P compared with plants supplied with 0.6mM and 0.3mM P (**Fig. 4**). Sufficient (0.6mM) P supply significantly enhanced the accumulation of all detected sterols (notably cholesterol and stigmasterol). The application of ApE to plants under 0.6mM P conditions significantly enhanced sterol contents, which increased by 2.4 and 2.5-fold for γ-Sitosterol and cholesterol respectively and by 2-fold for stigmasterol and its derivative. ApE exhibited no effects on sterol contents for plants supplied with 0.3mM or 1.2mM P but stimulated the synthesis of cholesterol under both P regimes. These results indicate a relationship between ApE treatment and sterol metabolism. The sterol biosynthesis was downregulated in response to high P concentration (1.2mM), but was optimum under sufficient P conditions (0.6mM). ApE application to plants significantly enhanced lipid synthesis under sufficient P conditions (0.6mM).

3.5 Comparative leaf profiles of endogenous phytohormones in ApE-treated and untreated plant

Plants supplied with 0.6mM P exhibited the highest ApE biostimulant effects. Therefore, treated and untreated tomato plants subjected to 0.6mM P conditions were selected for the evaluation of ApE effects on the leaf profiles of endogenous phytohormones.

Phytohormone levels of Gibberellins (GA1 pathway and GA4 pathway), Cytokinins (CKs) including DHZ, iP and tZ and other acid hormones (IAA, ABA, JA and SA) in leaves were analyzed. ApE treatment only exhibited ameliorative effects on GA4 concentration. The concentration levels of GA1, DHZ, iP, tZ, IAA, ABA and JA were significantly reduced in tomato plant leaves following treatment with ApE. ApE-treatment may have affected the synthesis or redistribution of phytohormones in the plant. However, this study is the first step towards understanding biostimulant effects of phytohormone profiles. The next step requires a complete study on the comparative phytohormone profile study in both leaves and roots, to understand the whole phytohormone distribution in treated and untreated plants.

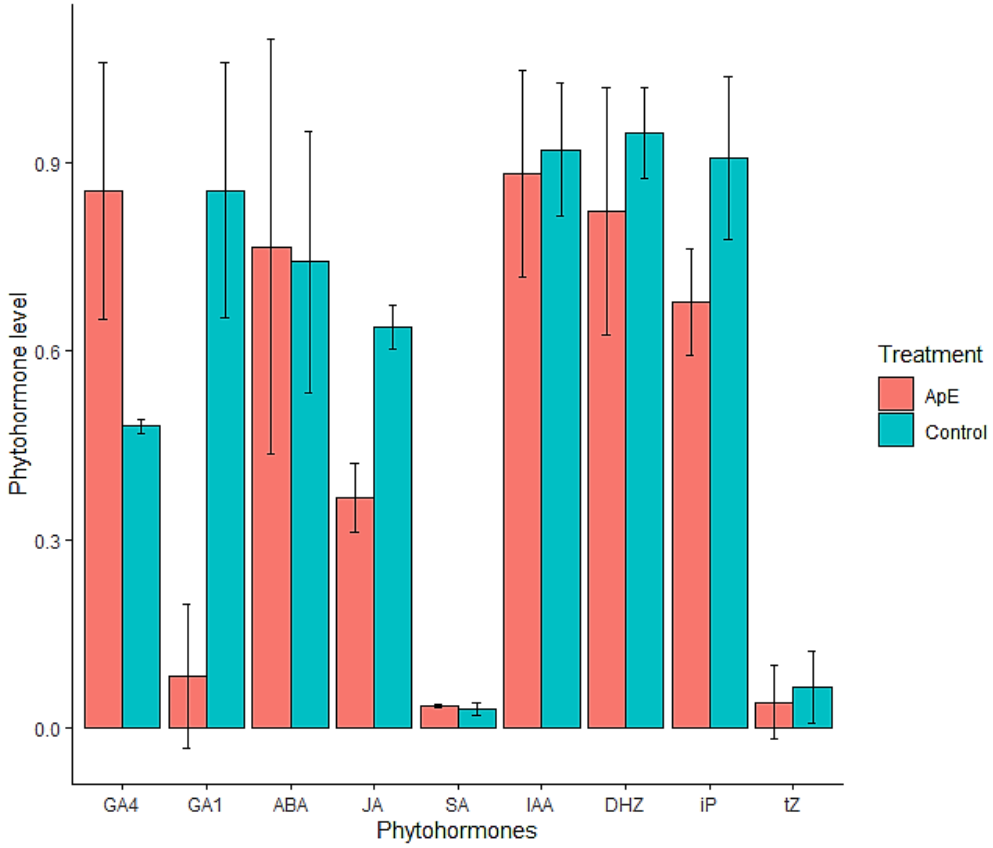


Figure 5: *Effect of ApE on endogenous phytohormones in the leaves of plants subjected to 0.6mM P conditions. gibberellic acid 4 (GA4), gibberellic acid 1 (GA1), abscisic acid (ABA), jasmonic acid (JA), salicylic acid (SA), indole-3-acetic acid (IAA), isopentenyladenine (iP), trans- zeatin (tZ), cis-zeatin (cZ), and dihydrozeatin (DHZ). Values are expressed as means ± standard error of the mean (n=3).*

4. DISCUSSION

Nutrient uptake, plant performance and crop productivity is determined by a number of growth characteristics, including root growth and architecture, transporter protein synthesis and photosynthesis efficiency (Lonhienne et al. 2015; Hussain et al. 2019).

In this study, we investigated the effects of ApE on plant growth and P absorption traits in tomato plants. ApE enhanced the root growth (root DW) of plants irrigated with 0.3mM and 0.6mM P, compared with untreated plants (**Fig. 1**). In agricultural soils, root growth and activity are affected by diverse physical and chemical soil properties including nutrient concentration, organic matter content, carbon-to-nitrogen ratio and pH. Nutrient supply also affect the shoot /root ratio, with lower ratios usually observed under low nutrient conditions (Chapin et al. 1993; Jong-Goo and Marc 2004; Lynch et al. 2011; Lynch 2011). In many P deprived environments, plants increase the lateral branching of the root (Lynch 2011). Increase in lateral roots and length in the upper parts of the soil lead to enhanced phosphorus-uptake capacity, as the top soil is often richer in phosphorus

(Wang et al. 2004; Yan et al. 2004; Lynch 2011; Bhattacharya 2019). Such morphological adaptations favor P-uptake efficiency. In P-fertilized soils, increased root growth improves P-uptake efficiency by increasing the roots' surface contact with nutrients and water (Lynch 2011). In the present study, P concentration in the root biomass significantly correlated with the root DW and shoot/root ratio. The root expression of *Le*PT5 phosphorus transporter gene was also closely associated with root P concentration in the root DW, indicating that P absorption was slightly due to enhanced transcription of PhT1 genes and largely due to improved root growth in ApE-treated plants.

The upregulation of high affinity P transporters is an important component in increasing P-absorption. Pht1 genes are expressed dominantly in the root epidermis and root hairs, in response to P deprivation, suggesting their role in P capture and uptake (Chen et al. 2014). Previous data obtained by Chen et al. (2014), provided direct evidence for strong expression of most tomato Pht1 genes in the roots under low P supply conditions. In the present study, low level expression of *Le*PT1 and *Le*PT3-*Le*PT5 was detected in the leaves of plants subjected to 0.3mM and 0.6mM P conditions. All genes were not influenced by ApE-treatment under high P conditions, indicating that ApE-treatment exhibit the highest biostimulant effects under low or sufficient P concentrations. ApE application to plants in P rich soils does not largely influence P uptake and translocation within the plant through the upregulation of Pht1 genes. Enhanced root expression of PhT1 genes (notably LePT5) in ApE-treated plants under sufficient P conditions is evidence that P uptake by roots is not solely influenced by P availability (Mudge et al. 2002; González et al. 2005). For instance, exogenous sugars stimulate the accumulation high-affinity transporter transcripts in dark-grown, P-sufficient *Lupinus albus* seedlings (Liu et al. 2005). In this study, exogenous application of ApE extracts in P-sufficient conditions slightly enhanced the accumulation of leaf *Le*PT4 and *root Le*PT2, *Le*PT3 and *Le*PT5 transcripts. *Aphanothece* sp extracts contain high levels of neutral sugars and carbohydrates (Chanda et al. 2020) and may also contain organic compounds that help stimulate rooting, resulting in the production of greater root mass, which improves nutrient uptake (Panda et al. 2012; Gomes et al. 2018).

ApE significantly improved the root DW of plants subjected to 0.3mM or 0.6mM P conditions. The root P concentration ($mg.g^{-1}$) significantly correlated with root biomass improvement (**Fig 6**) and the expression of *Le*PT5 genes in roots. Root *Le*PT3 also slightly correlated with P concentration in leaves. Tomato plants supplied with 1.2mM P accumulated the least P concentration in roots and exhibited no differences in the shoot and root DWs between treated and untreated plants. SPAD values also positively correlated with the shoot DW and the shoot/root ratio, indicating that biomass production was influenced by chlorophyll content in leaves (**Fig 6**).

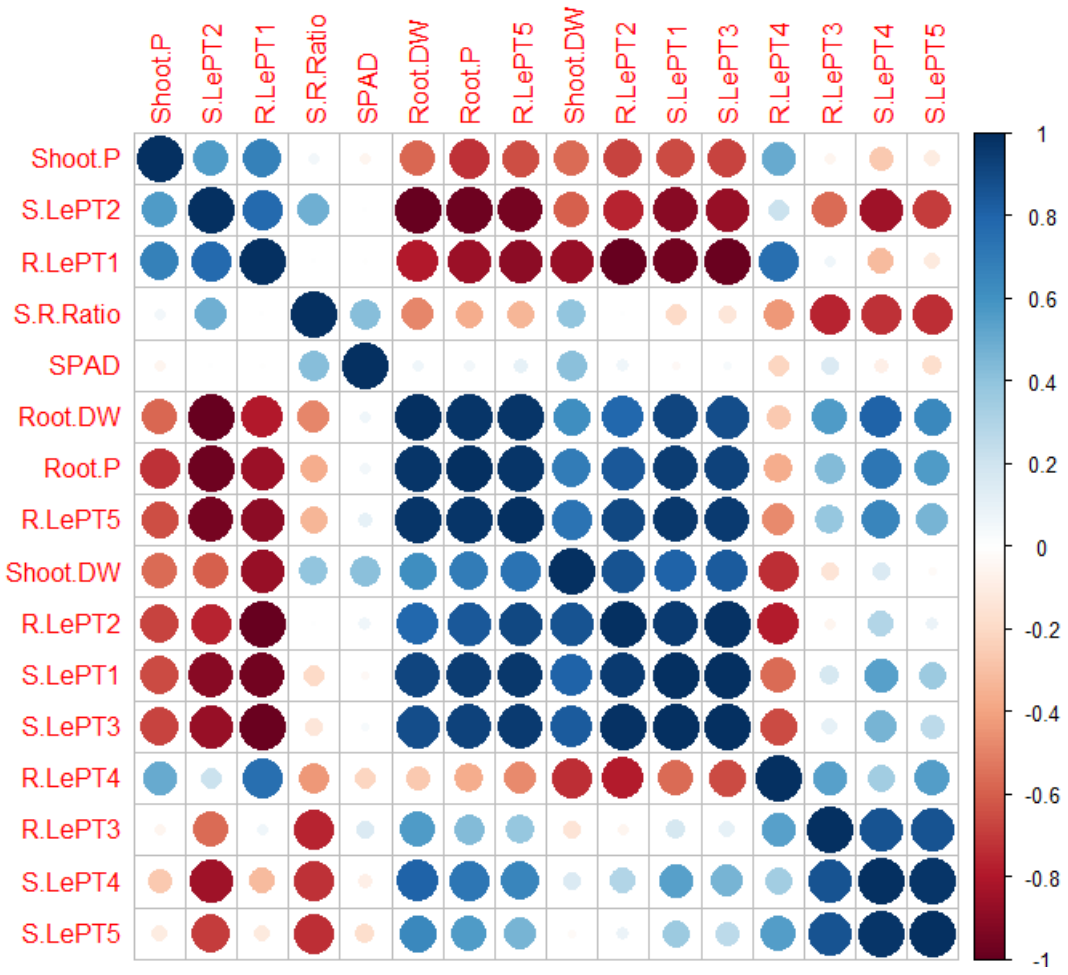


Figure 6: *Pearson correlation matrix of variables under different P regimes (P < 0.05). Large circles represent strong correlations and smaller circles represent weaker correlations. The dark blue color (1) indicate completely positive correlation and dark red (−1) indicates completely negative correlation between two traits (n=3).*

According to Principal Component Analysis (PCA), Root*Le*PT3, shoot*Le*PT4 and *shoot*LePT5 were closely associated with ApE-teated plants subjected to 0.3mM P conditions. Other traits such as root*Le*PT5, root P concentration and root DW were also closely related with ApE-treated plants in 0.3mM P conditions (**Fig 7**). On the other hand, shoot DW, chlorophyll content, shoot*Le*PT1, shoot*Le*PT2 and root*Le*PT2 were closely associated with ApE-treated plants subjected to 0.6mM P conditions (**Fig 7**). In the present study, 11/16 of the recorded traits were significantly influenced by ApE treatment in 0.3mM and 0.6mM P conditions. ApE application to plants exposed to high P conditions (1.2mM) had no significant effects on all recorded traits.

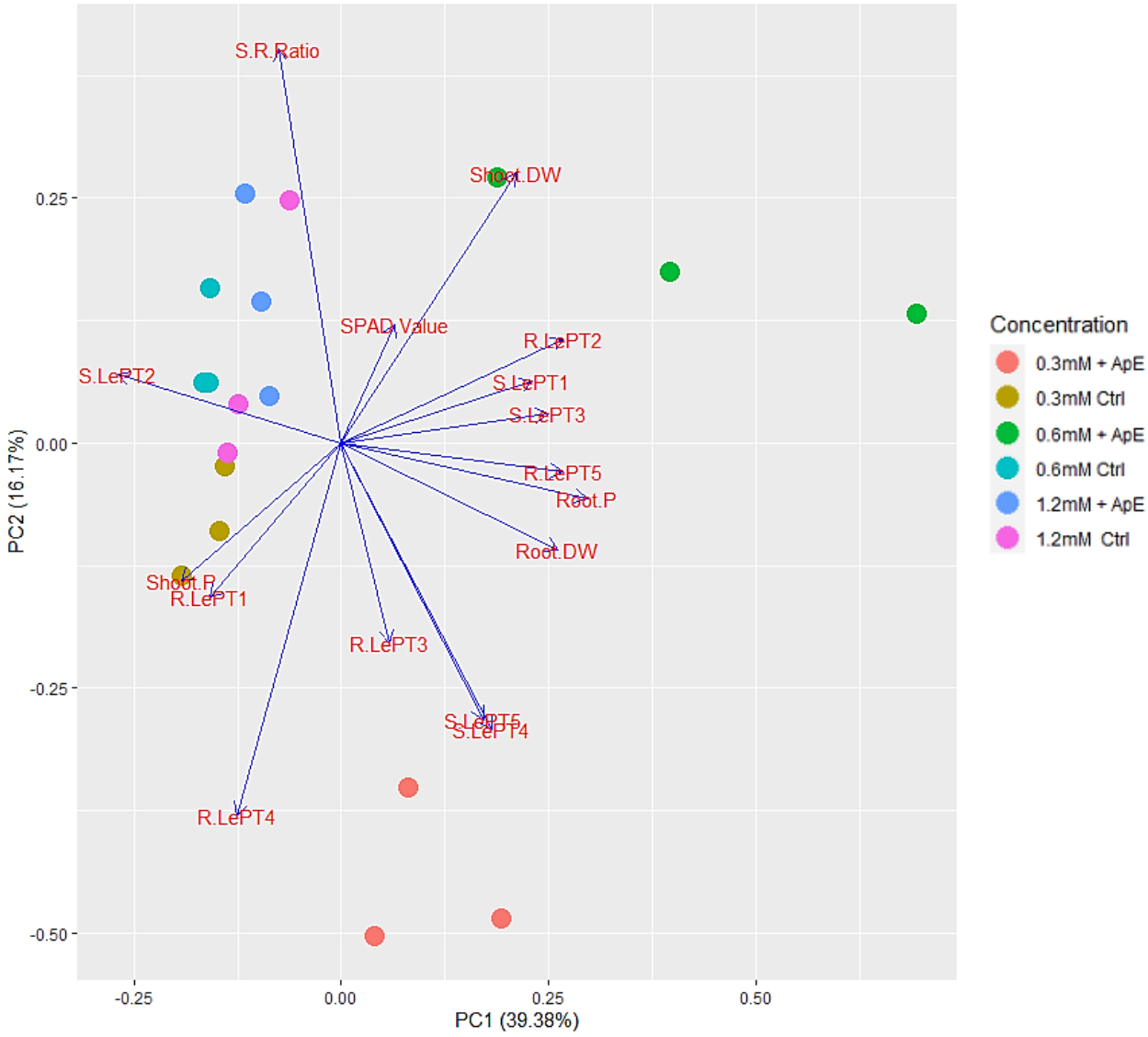


Figure 7*: principal component analysis (PCA) to understand treatment-variable associations. Concentration and treatment conditions were dispersed in different ordinates based on their dissimilarity. The vectors originating from the middle point of the biplot indicate positive or negative interactions of the recorded traits. The length of a vector in the biplot indicate the contribution of the traits on the principal components. Circles of the same color indicate the three replicates of the corresponding cluster. S or R at the beginning of the gene names indicate Shoot or Root expression respectively, S.R ratio (Shoot to root ratio), Shoot DW (Shoot Dry Weight) and Root DW (Root Dry Weight).*

A range of metabolic modifications is thought to be involved in internal P use efficiency (Veneklaas et al. 2012). Although P is not a constituent of chlorophyll (Marschner 1995). Higher P absorption and assimilation by plants improve photosynthetic capacity and biomass production. P-containing molecules such as ATP, NADPH and ribulose 1,5-bisphosphate (RuBP) have key roles in CO_2 fixation (Hammond and White 2011; Veneklaas et al. 2012). Thus, insufficient P influence leaf net CO_2 assimilation rate and other components of photosynthesis (Ellsworth et al. 2015). On the other hand, higher P levels in the plant does not equal higher phosphorus use efficiency (PUE) and high yield (Balemi and Schenk 2009). At high P levels, up to 75 % of P in leaves can be present as orthophosphate,

whereas 85–95 % of cellular P is mostly stored in the vacuoles (Akhtar et al. 2008). In the present study, increased leaf P concentration in treated plants supplied with 0.3mM and 0.6mM P did not correlate with the shoot DW, although the root DW correlated with P levels in roots. Plants subjected to 0.3mM and 0.6mM conditions also recorded the highest biomass increase and fatty acid contents.

Fatty acids are building-blocks for the majority of cellular lipids essential for membrane function. Plant growth and responses to environmental changes such as phosphate starvation, require lipid remodeling (Moellering and Benning 2011; Troncoso-Ponce et al. 2013; Li et al. 2016). In plants, *de novo* lipid synthesis from acetyl-CoA up to C16 - C18 chain lengths occurs in the plastid stroma (Lim et al. 2017). A fraction of C16–18 fatty acids is integrated into lipids inside plastids (the 'prokaryotic' pathway), whereas the majority is exported to the Endoplasmic Reticulum (ER) for further elongation, acyl editing, and lipid assembly (the "eukaryotic' pathway"). In some plants, both pathways are involved in lipid metabolism so that their leaf tissues contain both C16:3 and C18:3 fatty acids in large amounts (Mongrand et al. 1998). However, most vascular plants, mainly use the "eukaryotic pathway" for synthesis of plastid lipids and only use the 'prokaryotic pathway' for the sole synthesis of phosphatidylglycerols (PG) which do not contain triunsaturated C16:3 fatty acids (Mongrand et al. 1998; Li et al. 2016). In the present study, all plants contained larger amounts of C16:0 and C18:3 fatty acids, with lower amounts of C16:3 and C18:0 fatty acids. Major fatty acids in this study contained 18 carbon atoms, notably C18:3. Plants also contained high levels of C16:0 but not triunsaturated C16:3 fatty acids. Lipid synthesis was enhanced upon treatment with ApE, notably in plants supplied with 0.6mM P, indicating that ApE improved plant performance through enhanced lipid synthesis. Other lipid forms such as sterols, are equally essential components of eukaryotic cell membranes and vital for plant growth and development (Malinsky et al. 2013). Campesterols act as precursors for the biosynthesis of brassinosteroids (Schaller 2004; Boutté and Grebe 2009), a group of hormones that are essential for the regulation of plant development and morphogenesis. β-Sitosterol and stigmasterol also play a role in maintaining the structure and function of cell membranes in plant responses to biotic and abiotic stresses (Griebel and Zeier 2010; Wang et al. 2012a; Aboobucker and Suza 2019; Rogowska and Szakiel 2020). Treatment of plants with ApE significantly enhanced sterol content under 0.6mM P conditions, which increased by 2.4 and 2.5-fold for γ-Sitosterol and cholesterol respectively and by 2-fold for stigmasterol and its derivative, indicating the effective role of ApE in improving crop performance and development.

Further investigation of ApE effects on plant growth and phosphorus traits was evaluated on phytohormone profiles in leaves of tomato plants supplied with 0.6mM P. Phytohormones are low-molecular-weight natural products that essentially regulate physiological and developmental processes, and plant responses to environmental stresses. Phytohormones such Auxin are known to intervene in root architecture changes. Under P-starvation, Auxin affects primary roots, lateral roots and root hairs in responses. Thus, increased Auxin accumulation in root tips can enhance P absorption by inhibiting primary root growth and stimulating lateral root formation for P-foraging (Lambers et al. 2006). On the other hand, Gibberellins (GAs) are the key regulators of the source-sink metabolism process. In this study, the effect of ApE on phytohormones was evaluated in the leaf biomass of ApE-treated plants. Only GA4 significantly increased after treatment with ApE in tomato leaves. This study is the first step towards understanding biostimulant effects of phytohormone profiles. Future studies should include whole plant hormone profiling for comparative hormone redistribution patterns in treated and untreated plant groups, notably roots.

5. CONCLUSION

The sustainable management of P overuse in agriculture requires the exploitation of P-adaptive traits that enhance P-acquisition and P-use efficiency in crop plants. Such traits include root growth, P transporter protein synthesis and proficiency of P recycling within the plant itself. In this study, ApE exhibited positive effects on plant growth parameters including plant size, P uptake, chlorophyll content and lipid content. ApE effects were also recorded on the expression of PHT1 gene members, indicating that ApE biostimulant effects on P uptake were attributed to improved root growth and slight expression of high affinity P transporter genes in roots, notably *Le*PT5. *Aphanothece* sp extracts contain organic bioactive compounds that may stimulate rooting, resulting in the production of greater root mass and higher mineral uptake. Increased P absorption efficiency by crops through soil ApE application will improve crop yield and reduce P loss to the environment through leaching, which cause significant environmental damage.

6. MATERIALS AND METHODS

6.1 Plant material and growth conditions

Solanum lycopersicum L. (JANA F1, provided by BAYER Nunhems Netherlands BV) seeds were germinated on MS-Agar medium in magenta boxes. After germination (5 days after sowing), the seedlings were transplanted to 12cm diameter pots filled with vermiculite (The bottom of the pots were covered with aluminum foil). The pots (one plant per pot) were arranged in a completely randomized block design in a phytotron growth chamber, at 25 °C with 16/8 h light/dark cycles and 60–70% relative humidity. The experimental design consisted of three culture groups defined by P concentration (plants supplied with; 0.3 mM, 0.6 mM and 1.2 mM P). Each culture group consisted of ApE-treated and untreated plants.

6.2 Plant Phosphorus treatments

Based on the recommended P dose by (SMITH et al. 1983; Khan et al. 2012), and the concentrations previously established when screening microalgae extracts (Chanda et al. 2020; Mutale-joan et al. 2021), three P conditions were determined. The P sources in the nutrient solution, $KHPO_4$ (2.67 gL^{-1}) and KH_2PO_4 (1.64 gL^{-1}), were substituted with 2.232g of grinded TSP fertilizer for 0.3mM P, 4.465g for 0.6mM P and 8.926g for 1.2 mM P. The plant nutrient solutions were prepared by mixing 100 mL of the macronutrient stock (solutions A and B), with 50 mL of the micronutrient supplement (solution C), filled to 5 L with distilled water (**Table 1**). Plants were supplied with 100 mL of the nutrient solution every four days, and with distilled water every two days. At seven days-old, Tomato plants were supplied with 20mL ApE 5% every week for four weeks.

Table 1: *The salts used to make up the nutrient solution. The applied dilute solution was prepared by mixing 100 mL of solutions A and B of the macronutrient stock, with 50 mL of the micronutrient supplement (solution C), filled to 5 L with distilled water.*

Solution _A		**Solution_B**		**Solution_C**	
Element	**g/L**	**Element**	**g/L**	**Element**	**mg/L**
$Mg\ (NO_3)26H_2O$	4.94	TSP	2.232	H_3BO_3	128.80
NH_4NO	8.48	KCl	1.134	$CuCl_2\_2H_2O$	4.84
KNO_3	2.28	Na_2SO_4	0.940	$MnCl_2\_4H_2O$	81.10
				$(NH_4)6\ Mo_7O_{24}\_4H_2O$	0.83
				$ZnCl_2$	23.45
				Ferric citrate pentahydrate	809.84

At 35 days old, Tomato plants were harvested. The roots were washed under running tap water. Six replications were sampled in each treatment. Three replicates were selected for morphological studies. The leaves and roots of the three most homogenous replicates were individually ground in liquid nitrogen and stored at –80 °C. The biomass was utilized to study metabolomics, phytohormone and gene expression profiles of treated and untreated plant groups.

Nondestructive Chlorophyll measurement: The chlorophyll content was measured by a chlorophyll meter (SPAD-502, Minolta, Japan) before harvest. The SPAD-502 meter was calibrated using the reading checker supplied by the manufacturer before measurement. Each leaf SPAD value obtained was the average of 4 readings (2 on each side of the leaf midrib).

6.3 Culture of microalgae

Aphanothece sp was collected from the AlgoBioTech of the Moroccan Foundation for Advanced Science, Innovation and Research (MAScIR). The cyanobacteria species was cultivated in 10 L round bottom flasks containing BG11 medium at pH = 7.2 and agitated by air bubbling. The cultures were placed in a controlled culture room at 25°C, and 135 µmol photons m^{-2} s^{-1} of continuous white fluorescent light. After 30 days, the biomass was harvested by sedimentation, then centrifugation at 5000 rpm for 5 minutes at 4°C. The collected biomasses were dried at 50 °C for 7 days.

6.4 Preparation of the microalgae extract formulation:

The dry biomass of *Aphanothece* sp was grinded in liquid nitrogen. The ground biomass (300mg) was hydrolysed in 20 mL of 2% sulfuric acid. The resultant slurry was heated for 3 h at 95 °C with constant stirring (interrupted every 30 min by 1 min vortexing and 15 min sonification). The mixture was then autoclaved at 121°C and 106 kPa for 30 minutes. The total *Aphanothece* sp. crude extract (ApE) was cooled to room temperature, and stored at -20°C. The pH of the extract was adjusted to 5.8 before application to plants by soil drenching. The applied ApE concentration was 5% (v/v of ApE in distilled water).

6.5 Determination of P concentration in the shoot and root biomass

Treated and untreated plants for each P-concentration level were supplied with equal recommended doses of nutrients. Phosphorus concentrations in the root and leaf biomass were subsequently analysed using a skalar nutrient auto analyser (SAN++ Continuous Flow Analyzer) at the green biotechnology laboratory of MAScIR, the resulting differences observed in the nutrient concentrations between the treated and untreated plants indicated the effect of ApE supplementation on nutrient absorption by roots.

6.6 Quantitative reverse transcription PCR (RT-qPCR)

6.6 1 RNA extraction and quantification using Qubit

Total RNA was extracted according to the manufacturers' instructions using the RNeasy Plant Mini Kit (QIAGEN, USA). The leaf or root biomass (100mg) ground in liquid nitrogen was lysed in 450µL of the RLT lysis buffer and vigorously vortexed. The lysate was transferred to a QIAshredder homogenizer placed in a 2mL collection tube and centrifuged at full speed. Ethanol (200µL) was added to the cleared lysate to provide ideal binding conditions. The lysate was then loaded onto the RNeasy Mini spin column. Total RNA was bound to the RNeasy membrane and contaminants were washed away using the buffers according to the manufacturers' instructions. Pure, concentrated RNA was eluted in free RNase water and stored at -80°C.

Extracted RNA was quantified using Qubit according to the manufacturers' instructions Invitrogen™ Qubit™ RNA HS Assay Kit (ThermoFisher SCIENTIFIC).

6.6.1 cDNA Reverse Transcription

The Reverse Transcription step was performed on total RNA according to the manufacturers' instructions (QuantiTect Reverse Transcription Kit). All reactions were set up on ice to minimize the risk of RNA degradation. Diluted RNA (14.2 µL) was added to 5.8 µL reaction mix; containing 2 µL 10XRT Buffer, 0.8 µL 25XdNTP Mix (100 mM), 2µL 10XRT Random Primers and 1 µL MultiScribe™ Reverse Transcriptase. The RNA concentration in the final volume (20µL/tube) was 50ng. The tubes were centrifuged and put in the Thermocycler. The following thermal profile was utilized for the Reverse Transcription: 25 °C for 10 min, 37 °C for 120 min, 85 °C for 5 min and 4°C to end the reaction.

6.6.3 qPCR Analysis

cDNA (2 µL) was added to the reaction mix containing 5µL SYBR Green master mix, 1 µL free RNase H_2O, 1µL forward primer and 1µL reverse primer. The final volume was 10 µL per reaction well. The 96 well plate was centrifuged and placed in the qPCR system (ABI 7500 Applied Biosystems). The following thermal profile was utilized for the qPCR: 50 °C for 2 min and one cycle of 95 °C for 15 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min and 72°C for 0.30 s. The annealing temperature was modified according to the melting temperature of the primers utilized for each reaction plate (**Table 2**). The analysis was performed in triplicate. The relative gene expression changes between the experimental (ApE-treated plants) and calibrator sample (untreated plants) were calculated using Le α-tubulin as the reference gene. The Comparative Ct ($2^{-\Delta\Delta Ct}$) method was applied and the relative quantitative value was expressed as the relative expression level of the target gene in comparison with the refence gene.

Table 2*: The forward and reverse primer sequences of the Phosphate Transporter genes (PT1 - PT5). Gene-specific primers for the qRT-PCR analysis were designed using NCBI.*

Accession	Primer	Melting Temperature	Primer sequence
LePT1 AF022873	Forward	61.30	GTATGCTGTTCACATTCTTGGTTCC
	Reverse	58.10	TCTCTTTCTAATCCCAAATACCACA
LePT2 AF022874	Forward	62.10	CATTGGACACTGGAGGCTAACC
	Reverse	57.87	ATAAGAACCCATACGCTCCCA
LePT3 AY804011	Forward	59.35	GCAGTATTCGCGATGCAAGG
	Reverse	61.40	GAAATCAGCCTCAGGAGGGG
LePT4 AY885651	Forward	61.78	CCGCCTTGAGGGAGATGTTTG
	Reverse	61.00	CAGAGTAGCGAATGTCCATTTGTG
LePT5 AY885653	Forward	60.30	GGCGAATGAAGATGCCTGAAAC
	Reverse	56.50	TACCAATTAAGTGATGTCCGTG
Le α-tubulin TC115716	Forward	57.10	TGAACAACTCATAAGTGGCAAAG
	Reverse	62.10	TCCAGCAGAAGTGACCCAAGAC

6.7 GC-MS Lipidomic Analysis of tomato plant

6.7.1 Extraction

Leaf biomass (400mg FW) ground in liquid nitrogen was added to a glass vial. Then 10µL internal standard Dodecane and 4mL of Chloroform ($CHCl_3$) (pre-cooled at -20 °C) were added to the vial. The vials were thoroughly vortexed for 1min and heated at 85°C for 30 min on a heat block (Labet International, Edison, USA). The vials were then vortexed for 1 min and sonicated for 15 min at 60°C in an ultrasound bath (Branson ultrasonic Sonifier 450, Danbury, USA). This process was repeated four times. Then 2mL methanol was added to the vials, thoroughly vortexed and transferred back into the ultrasound bath for 2 h at 60°C. (The 2h was interrupted by 1min vortexing every 30min). For the separation phase, 1mL of distilled water was added to the vials, and the bottom organic phase was transferred to clean vials with the help of a separating funnel. The $CHCl_3$ solvent was then evaporated completely under nitrogen flow.

6.7.2 Transesterification

In this step, 500 µl of 6% methanolic HCl (v/v) was added to the dried residue. The mixture was heated for 30min at 85^0C, then vortexed and sonicated for 15 min at 60°C. This process was repeated four times. The mixture was then dried under nitrogen flow, and 250 µL distilled H_2O and 750 µL $CHCl_3$ were added to the dried residue and vortexed for 1min. The bottom organic phase was transferred to clean vials with the help of a separating funnel and conserved at -20 for GC-MS analysis.

Metabolomics analysis was carried out using gas chromatography (GC) (Agilent 7890A Series GC,

USA) coupled to mass spectrometry (MS) equipped with multimode injector and BD-ASTMD6584 column (15m x 0.320mm x 0.1µm) and electron impact ionization. 4 µL of the soluble extract was injected into the column by 1: 5 split mode using helium as the carrier gas at 3 $mLmin^{-1}$. The Detection was done using full scan mode between 30–1000 m / z, with gain factor of 5. The temperatures of the ion source and the quadrupoles were 230 and 150 ° C, respectively. The oven temperature was maintained at 30 ° C for 1 min and then increased at 10 ° C min − 1 to 250 ° C then 20°C until 340°C. The identification was carried out using NIST 2017 MS Library. The amount of each compound was estimated by comparing the peak area with that of the internal standard (dodecane).

6.8 Plant hormone analysis

Quantification of plant hormones was conducted by the Institute of Molecular and cellular plant Biology in Spain (IBMCP) by Ultra Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS) using a Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap Mass Spectrometer.

Analyzed Phytohormones included:

- Gibberellins (GA1 pathway and GA4 pathway),
- Other acid hormones (IAA, ABA, JA and SA)
- Cytokinins (CKs) (DHZ, iP and tZ)

Abbreviations_ gibberellic acid 4 (GA4), gibberellic acid 1 (GA1), abscisic acid (ABA), jasmonic acid (JA), salicylic acid (SA), indole-3-acetic acid (IAA), isopentenyladenine (iP), trans- zeatin (tZ), cis-zeatin (cZ), and dihydrozeatin (DHZ),

6.9 Statistical analysis

Statistical analyses were performed using RStudio. Results represent the descriptive statistics and statistically significant differences between the mean values of the control and treated plant samples. The statistically significant differences between the mean values was determined using Tukey's HSD test. Results are expressed as the mean ± SE of three replicates. Significance levels ($p < 0.05$) are represented by different letters. In order to perform the analysis, the data (mean values) was normalized into a standard range of -1 to +1 using the equation $x' = (x_{mean} - x_i) / (x_{max} - x_{min})$. The first two components explained the maximum variance in the datasets.

DECLARATIONS

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Authors contribution

HEA= Project conception, Funding acquisition, project supervision, design of methodology and revision of the manuscript. CMJ = Writing (original draft preparation, review and editing), the study of physiological parameters, analysis of results. FR = Experimental work (RT-qPCR). NM = Study of nutrient uptake using Skalar nutrient auto analyser. AA = Study of Metabolomics using GC-MS and manuscript revision. HEH Experimental work (RT-qPCR) and revision of the manuscript. KL = Project supervision and revision of the manuscript. LS= Thesis director.

Competing interests

The authors declare no competing interests.

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IV-3. SYNTHÈSE

L'absorption des nutriments, la performance des plantes et la productivité des plantes agricoles sont déterminées par un certain nombre de caractéristiques de croissance, y compris la croissance et l'architecture des racines, la synthèse des protéines de transport et l'efficacité de la photosynthèse (Lonhienne et al. 2015; Hussain et coll. 2019).

Dans la présente étude, les effets de l'extrait total d'*Aphanothece* sp. (ApE) sur la croissance des plantes et les traits d'absorption du P chez les plantes de tomates ont étés étudiés. L'ApE a amélioré la croissance racinaire (poids sec racinaire) des plantes irriguées avec 0,3 mM et 0,6 mM de P, par rapport aux plantes non traitées (**Fig. 2**). Une croissance élevée des racines améliore l'efficacité de l'absorption du P en augmentant le contact de surface des racines avec les nutriments et l'eau (Lynch 2011). Par exemple, l'augmentation des racines latérales dans les parties supérieures du sol améliore la capacité d'absorption du phosphore, car la couche supérieure du sol est souvent plus riche en phosphore (Wang et al. 2004; Yan et coll. 2004; Lynch 2011; Bhattacharya 2019). La stimulation de telles adaptations morphologiques dans les sols fertilisés (riche en P) favoriserait l'efficacité de l'absorption de P. Dans la présente étude, la concentration du P dans la biomasse racinaire était significativement corrélée avec le poids sec racinaire et le rapport tige/racine. L'expression racinaire du gène *Le*PT5 codant les protéines transporteur du P était également étroitement associée à la concentration de P racinaire et le poids racinaire, ce qui indique que l'absorption de P était légèrement due à une transcription améliorée des gènes PhT1 et en grande partie à une amélioration de la croissance racinaire chez les plantes traitées avec l'ApE.

La surexpression des gènes codant les protéines transporteurs de P à haute affinité est un élément important dans l'augmentation de l'absorption de P chez les plantes cultivées. Les gènes Pht1 sont exprimés de manière prédominante dans l'épiderme et les poils racinaires, en réponse à un manque en P, suggérant leur rôle dans la capture et l'absorption de P (Chen et al. 2014). La plupart des gènes Pht1 de la tomate dans les racines sont fortement exprimés dans des conditions de faible apport en P (Chen et al. 2014). Dans la présente étude, une expression faible des gènes *Le*PT1 et *Le*PT3-*Le*PT5 a été détectée dans les feuilles des plantes soumises à 0,3 mM et 0,6 mM.P. Le gène foliaire *Le*PT2, n'a pas été exprimé dans toutes les conditions de P, ce qui prouve que *Le*PT2 est exclusivement exprimé dans les racines appauvries en P comme indiqué précédemment par Chen et al. (2007, 2014). Ces résultats montrent également que l'application des ApE aux plantes dans des sols riches en P influence l'expression racinaire des gènes PhT1 (notamment *Le*PT5) chez les plantes traitées avec l'ApE dans des conditions de suffisance en P. Ceci montre que l'absorption de P par les racines n'est pas uniquement influencée par la disponibilité de P (Mudge et al. 2002) et (González et coll. 2005). Les extraits d'*Aphanothece* sp. contiennent des niveaux élevés de sucres et de

glucides neutres (Chanda et al. 2020). Les sucres exogènes stimulent l'accumulation de transcriptions de transporteurs à haute affinité chez les plantules de *Lupinus albus* cultivées à l'obscurité et dans des concentrations suffisantes de P (Liu et al. 2005). Dans cette étude, l'application exogène des extraits ApE dans des conditions P-suffisantes a légèrement augmenté l'accumulation de transcrits racinaires des gènes *Le*PT2, *Le*PT3 et *Le*PT5.

Les extraits d'*Aphanothece* sp peuvent contenir des composés organiques qui aident à stimuler l'enracinement, entraînant le développement d'une plus grande masse racinaire, ce qui améliore l'absorption des nutriments (Panda et al. 2012; Gomes et al. 2018). Le traitement des plantes avec l'ApE a amélioré le poids sec racinaire chez les plantes soumises à 0,3 mM ou 0,6 mM de P. La concentration de P ($mg.g^{-1}$) dans la biomasse racinaire était corrélée avec l'amélioration de la biomasse racinaire (**Fig. 5**) et l'expression des gènes LePT5 dans les racines. L'expression du *Le*PT3 racinaire était également légèrement corrélée avec la concentration en P dans les feuilles. Les valeurs de SPAD étaient également positivement corrélées avec le rapport poids sec tige/racine, ce qui indique que la production en biomasse était influencée par la teneur en chlorophylle dans les feuilles.

Selon l'analyse en composantes principales (APC), le poids sec des tiges, la teneur en chlorophylle, et l'expression des gènes *Le*PT1 foliaire, et *Le*PT2 et *Le*PT2 racinaires ont été étroitement associées aux plantes traitées avec l'ApE soumises à 0,6 mM de P. Dans la présente étude, 11/16 des traits étudiés étaient significativement influencés par le traitement ApE chez les plantes soumises à 0,3 mM et de 0,6 mM de P. L'application d'ApE aux plantes exposées à des concentrations élevées de P (1,2 mm) n'a eu aucun effet significatif sur tous les traits étudiés.

Une gamme de modifications métaboliques sont impliquées dans l'efficacité interne de l'utilisation de P (Veneklaas et al. 2012). Bien que le P ne soit pas un constituant de la chlorophylle (Marschner 1995). Une absorption et une assimilation plus élevées du P par les plantes améliorent la capacité photosynthétique et la production de biomasse. Les molécules contenant du P telles que l'ATP, le NADPH et le ribulose 1,5-bisphosphate (RUBP) jouent un rôle clé dans la fixation du CO_2 (Hammond et White 2011; Veneklaas et al. 2012). Ainsi, un P insuffisant influence le taux d'assimilation nette du CO_2 par les feuilles et d'autres composants de la photosynthèse (Ellsworth et al. 2015). Dans la présente étude, l'augmentation de la concentration de P foliaire chez les plantes traitées avec l'ApE et soumise à 0,3 mM et 0,6 mM de P n'était pas corrélée avec le poids sec des tiges, bien que le poids sec des racines soit corrélé avec la concentration racinaire du P.

Les plantes soumises à 0,3 mM et 0,6 mM de P ont également montré des teneurs élevées en acides gras. Les acides gras sont les éléments constitutifs de la majorité des lipides cellulaires essentiels au

fonctionnement membranaire. Chez les plantes, la synthèse lipidique *de novo* à partir de l'acétyl-Coa jusqu'à des longueurs (C16-C18) de chaines de carbone, se produit dans le stroma du plastide (Lim et al. 2017). Une fraction des acides gras C16-18 est intégrée dans les lipides à l'intérieur des plastes (voie “procaryote”), tandis que la majorité est exportée vers le réticulum endoplasmique (ER) pour un allongement ultérieur, une modification des acyles et un assemblage lipidique (voie "eucaryote"). Chez certaines plantes, les deux voies sont impliquées dans le métabolisme des lipides, de sorte que leurs tissus foliaires contiennent à la fois des acides gras C16:3 et C18:3 en grandes quantités (Mongrand et al. 1998). Cependant, la plupart des plantes vasculaires utilisent principalement la "voie eucaryote" pour la synthèse des lipides plastidiens et n'utilisent la "voie procaryote" que pour la synthèse des phosphatidylglycérols (PG) qui ne contiennent pas d'acides gras triinsaturés C16:3 (Mongrand et al. 1998; Li et coll. 2016). Dans la présente étude, les plantes traitées et soumises à 0.3 et 0.6 de P, contenaient des quantités plus importantes d'acides gras C16:0 et C18:3, avec des quantités plus faibles d'acides gras C16:3 et en C18:0. Les plantes contenaient également des niveaux élevés d'acides gras C16:0, mais pas d'acides gras C16:3 triinsaturés. La synthèse lipidique a été améliorée par le traitement avec l’ApE, notamment dans des plantes soumis à 0,6 mM de P.

D'autres formes lipidiques telles que les stérols sont également des composants essentiels des membranes cellulaires eucaryotes et vitales pour la croissance et le développement des plantes (Malinsky et al. 2013). Les campestérols agissent comme des précurseurs de la biosynthèse des brassinostéroïdes (Schaller 2004; Boutté et Grèbe 2009), un groupe d'hormones essentielles à la régulation du développement et de la morphogenèse des plantes. Le β-sitostérol et le stigmastérol jouent également un rôle dans le maintien de la structure et de la fonction des membranes cellulaires des plantes en réponse aux stress biotiques et abiotiques (Griebel et Zeier 2010; Wang et al. 2012a; Aboobucker et Suza 2019; Rogowska et Szakiel 2020). Le traitement de plantes avec l’ApE a considérablement amélioré la teneur en stérols chez les plantes soumises à 0,6 mM de P, ce qui indique le rôle efficace de l' ApE dans l'amélioration des performances et du développement des plantes.

IV-4. CONCLUSION

La gestion durable de la surutilisation du P en agriculture nécessite l'exploitation de traits adaptatifs des plantes en réponse à une déficience de P dans le sol. La stimulation de ces traits a amélioré l'efficacité de l'acquisition et de l'utilisation du P dans les plantes agricoles. Ces traits comprennent la croissance des racines, la synthèse des protéines transporteur du P et le recyclage de P au sein de la plante elle-même. Dans cette étude, ApE a montré des effets positifs sur les paramètres de croissance des plantes notamment au niveau de la taille de la plante, l'absorption de P, et la teneur en chlorophylle et en lipides. L’ApE avait également des effets positifs sur l'expression des gènes PHT1, indiquant que les effets des biostimulants

des ApE sur l'absorption de P ont été attribués à une amélioration de la croissance des racines et à une légère expression des gènes transporteurs de P à haute affinité dans les racines, notamment *Le*PT5. Les extraits d'*Aphanothece* sp contiennent des composés bioactifs organiques qui peuvent stimuler l'enracinement, entraînant l'augmentation de la biomasse racinaire et une plus grande absorption des minéraux. L'augmentation de l'efficience de l'absorption du P dans les cultures agricoles via l'application de l'ApE au sol pourrait améliorer le rendement des cultures et réduire les pertes de P dans l'environnement qui causent des dommages environnementaux importants.

CHAPITRE V

CHAPITRE V

Les microalgues et les cyanobactéries : Comment l'exploitation de ces ressources microbiennes peut relever les défis sous-jacents liés aux sources alimentaires et à l'agriculture durable

V- INTRODUCTION

Depuis des milliers d'années, la production alimentaire dans la plupart des régions du monde dépend presque entièrement de l'agriculture conventionnelle (Wilhelm 1972; Spengler 2015; Styring et al. 2017; Worley et al. 2019; Angelakis et al. 2020). Néanmoins, la production alimentaire est gravement affectée par la croissance exponentielle de la population, les maladies des plantes, la qualité des sols et le changement climatique mondial (Coll et al., 2012; Du et al., 2004; Oldfield et Dearing, 2003; Vlek et Steg, 2007). Ces quatre facteurs majeurs ont mis en péril la garantie de l'approvisionnement alimentaire mondial.

Comment produire et fournir suffisamment de nourriture à une population de 9.8 milliards de personnes, tout en préservant l'environnement et l'écosystème pour les générations futures ? La réponse est plus complexe que nous ne pouvons l'imaginer. Tout d'abord, l'agriculture devrait produire 60% plus de nourriture qu'aujourd'hui (Fróna et al., 2019). Mais, l'expansion des terres arables pour la production alimentaire ne sera pas une solution durable due à divers facteurs comme la population croissante et la dégradation des sols (Bruinsma, 2017; Gibbs et Salmon, 2015; Josephson et al., 2014; Taiz, 2013).

Les maladies des plantes causent déjà des pertes de 10% à 16% du rendement des cultures chaque année (Chakraborty et Newton, 2011; Strange et Scott, 2005; Waddington et al., 2010), et l'usage des produits chimiques est la méthode la plus pratique pour éradiquer les maladies et assurer un bon rendement. Cependant, l'agriculture moderne est mise au défi de réduire l'utilisation de produits chimiques tels que les engrais et les pesticides et de rechercher des méthodes écologiques pour améliorer la résilience et la productivité des cultures.

Au cours des dernières années, l'agriculture durable est devenue la cible de la productivité alimentaire. Les matières organiques telles que les biostimulants de plantes ont été décrites comme des alternatives durables pour améliorer la croissance des plantes, l'absorption des nutriments, l'efficacité de l'utilisation des nutriments, la tolérance au stress abiotique et/ou la qualité des cultures (Du Jardin 2015; Yakhin et al. 2017). Il existe plusieurs catégories de biostimulants de plantes : les acides humiques et fulviques, les acides aminés et les hydrolysats de protéines, les bactéries promotrices de la croissance des plantes (PGPB) et les extraits d'algues (Calvo et al., 2014; du Jardin, 2015). Le développement émergent de produits agricoles

innovants a conduit à l'exploitation des microalgues et les cyanobactéries comme bioressources potentielles pour les biostimulants de plantes.

Les microalgues (eucaryotes) et les cyanobactéries (procaryotes) sont des organismes photosynthétiques microscopiques unicellulaires qui poussent dans divers habitats aquatiques et même dans des sols humides. Les microalgues et les cyanobactéries sont non seulement des bioressources durables mais économiques qui sont exploitées pour diverses applications en tant qu'ingrédients alimentaires, produits bioactifs et matières premières pour la production du biodiesel (Khan et al., 2018). Plusieurs espèces de microalgues et de cyanobactéries présentent des qualités pharmacologiques et biologiques due à leur capacité à synthétiser des produits utiles tels que les acides gras polyinsaturés, lipides, protéines, polysaccharides et autres métabolites bioactifs, provenant du CO_2 atmosphérique (Khan et al., 2018). Certaines études ont aussi signalé la présence de phytohormones (cytokinine, auxine, gibbérellines et brassinostéroïdes) dans les extraits cellulaires et le milieu de croissance de plusieurs espèces de microalgues (Bajguz, 2009).

La présente revue se concentre sur les applications biotechnologiques des microalgues et des cyanobactéries en agriculture en tant que bioressources pour le développement de produits (stimulateurs de croissance, biostimulants, bioélecteurs) et leur exploitation prometteuse en tant que ressources alimentaires.

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Microalgae and Cyanobacteria: How Exploiting These Microbial Resources Can Address the Underlying Challenges Related to Food Sources and Sustainable Agriculture: A Review

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ABSTRACT

For thousands of years, crop production has almost entirely depended on conventional agriculture. However, the reality is changing. The ever-growing population, global climate change, soil degradation and biotic/abiotic stresses are a growing threat to food production and security. Thus, sustainable alternatives to increase crop production for a population projected to reach 9.8 billion by 2050 are a major priority. In addition to vertical and soilless farming, innovative products based on bioresources, including plant growth stimulants, have been a target for sustainable food production. Such solutions have led to the exploitation of microorganisms, including microalgae and cyanobacteria as potential bioresources for food and plant biostimulant products. Microalgae (eukaryotic) and cyanobacteria (prokaryotic) are photosynthetic microorganisms with the capacity to synthesize a vast array of bioactive metabolites from atmospheric CO_2 and inorganic nutrients. The present review outlines the nutritional value of microalgae and cyanobacteria as alternative food resources. The potential aspects of microalgae and cyanobacteria as stabilizers of the net change in soil organic carbon (C) levels for reduced farmland degradation are also highlighted. The applications of microalgae and cyanobacteria as remedies for improved soil structure and fertility, and as enhancers of crop productivity and abiotic stress tolerance in agricultural settings are outlined. This review also discusses the co-cultivation of crops with microalgae or cyanobacteria in hydroponic systems to favor optimum root CO_2/O_2 levels for optimized crop production.

Keywords: Microalgae, cyanobacteria, food sources, sustainable agriculture.

INTRODUCTION

Agricultural production is critically affected by plant diseases, soil quality, global climate change and the exponentially growing population. Global climate change leads to increased weather-related disasters, such as floods and droughts, causing food shortages and famine (Myers et al. 2017). Plant diseases and soil degradation also affect plant growth and development (Teng and Johnson 1988; Savary et al. 2012), leading to reduced crop yield and food production. This scenario threatens food security and is aggravated by the growing population projected to reach 9.8 billion by 2050.

Over the years, the development of chemical products such as synthetic fertilizers and pesticides significantly improved crop productivity. However, the extensive application of such chemical products cause negative impacts on the environment and the ecosystem (Bhandari 2014; Prashar et al. 2016; Zhang

et al. 2018). Thus, modern agriculture is challenged to search for sustainable food sources and ecofriendly methods of improving crop resilience and productivity. In this context, organic products such as plant biostimulants have been described as sustainable alternatives to improve plant growth, nutrient uptake, nutrient use efficiency, tolerance to abiotic stress and/or crop quality (Calvo et al. 2014; du Jardin 2015). The emerging development of innovative sustainable agricultural products has led to the exploitation of microorganisms including microalgae and cyanobacteria as potential bioresources for food and plant biostimulants (Gonçalves 2021; Alvarez et al. 2021). Microalgae (eukaryotic) and cyanobacteria (prokaryotic) are unicellular microscopic photosynthetic organisms that grow in diverse aquatic habitats and even humid soils (Khan et al. 2018). Microalgae and cyanobacteria have been described as high-nutrient food resources (García et al. 2017). Several species are presently exploited for the generation of protein food supplements and nutraceutical products such as omega-3, astaxanthin and β-carotene (Nethravathy et al. 2019; Rahman 2020), making them sustainable alternative food resources with health benefits. In agricultural settings, microalgal and cyanobacterial biomass application to farmlands can stabilize the net change in soil organic carbon (C) levels and reduce farmland degradation. Microalgae and cyanobacteria can also produce bioactive substances such as sulfated exopolysaccharides (EPS) and phytohormones that are beneficial for soil structure and plant growth (Gayathri et al. 2015; Abinandan et al. 2019). Microalgae and cyanobacteria extracts improve plant growth and nutrient uptake, contributing to crop growth and yield (Renuka et al. 2018; Alvarez et al. 2021). Microalgae and cyanobacteria can be grown in hydroponic system substrates, fixing carbon dioxide (CO_2) through photosynthesis and releasing bioactive compounds such as sulfated EPS and phytohormones into the nutrient substrate (Zhang et al. 2017; Barone et al. 2019).

The present review outlines the nutritional value of microalgae and cyanobacteria as sustainable alternative food resources. This review also discusses how the utilization of microalgae and cyanobacteria can be exploited to help restore degrading or abandoned farmlands. The applications of microalgae and cyanobacteria as remedies for improved soil structure and fertility, and as enhancers of crop productivity and abiotic stress tolerance in agricultural settings are outlined. The present review also highlights the use of microalgae and cyanobacteria for optimized crop production in hydroponic co-culture systems.

Microalgae and cyanobacteria

Microalgae and cyanobacteria are microorganisms that exist in various aquatic and terrestrial ecosystems (Rajvanshi and Sharma 2012), and carry out oxygenic photosynthesis, a high-energy demanding process of water oxidation to molecular oxygen (O_2) and reduction of CO_2 to organic compounds (Tamagnini et al. 2002). Oxygenic photosynthesis first appeared in the ancestors of present-day cyanobacteria more than 3.7 billion years ago (Björn and Govindjee 2014). Cyanobacteria are considered among the oldest life forms on Earth and are the original producers of the Earth's oxygenic atmosphere (Chauvat and Cassier-Chauvat 2012; Saad and Atia 2014). The close association of cyanobacteria with green algae, green plants and other organisms arose more than 1.2 billion years ago from an early endosymbiosis event where a cyanobacterium was taken up into a heterotrophic organism (Björn and Govindjee 2014). Cyanobacteria were originally classified as blue-green algae (Cyanophyta) under botanical codes (Oren 2014a; Demoulin et al. 2019), until their prokaryotic features were established in the 1960s and a proposal was made to include cyanobacteria within the bacteriological code (Stanier et al. 1978). Cyanobacteria in nature are all oxygenic photoautotrophs, with the possible exception of their capacity for facultative anoxygenic photosynthesis (Lau et al. 2015; Garcia-Pichel et al. 2020). Microalgae and cyanobacteria are capable of producing a broad variety of unique, potent substances (Chu and Phang 2019; Kini et al. 2020).
Microalgae and cyanobacteria are suitable bioresources for food and commodities production, and can address the underlying challenges related to food sources and sustainable agriculture (Stephens et al. 2013; Saifullah et al. 2014).

1. Microalgae and cyanobacteria are reliable food resources for human nutrition

Food security is a major priority worldwide, affecting both developing and developed countries (Rosegrant and Cline 2003; Schmidhuber and Tubiello 2007; Savary et al. 2012). The rapidly growing population will demand 60% increase in agricultural output by 2050 (Alexandratos and Bruinsma 2012), but climate change and soil degradation threaten the current and future agricultural production (Rosenzweig et al. 2014). Croplands and pastures are one of the largest terrestrial biomes on the planet, covering ~40% of the land surface and making agricultural production the planet’s single most extensive form of land use (Foley et al. 2005). The human population growth and agricultural intensification are major drivers of environmental degradation. In the tropics, new

farmlands are cleared to the detriment of rainforests, savannas and diverse ecosystems (Gibbs et al. 2010; Costantini 2015). Microalgae and cyanobacteria are sustainable resources for food and agricultural product innovation; they are primary producers that exist in various aquatic and terrestrial ecosystems, and require no arable land for production (Rajvanshi and Sharma 2012; Hopes and Mock 2015).

Microalgae and cyanobacteria can produce a broad variety of nutritional value compounds, including proteins, lipids, carbohydrates, different pigments, vitamins and anti-oxidants. Microalgae are a pivotal food source since their first use by the Chinese 2,000 years ago to survive during famine (Mobin et al. 2019). Microalgae species such as *Dunaliella tertiolecta* and *Euglena gracilis* are effective sources of healthy food in Japan (Nethravathy et al. 2019). Cyanobacterial species such as *Arthrospira spp.*, also known as Spirulina, have also been used as sources of food by many civilizations. The Aztecs were among the first people who harvested this cyanobacterium in lake Texcoco within the valley of Mexico (Barrios et al. 2017). Spirulina is rich in proteins (55% to 70% protein content per total dry weight), iron and essential unsaturated fatty acids such as omega-3 (Tokuşoglu and Ünal 2003), and are one of the richest natural green sources of vitamin B12 (Dochi et al. 2010). Spirulina also has various beneficial effects on human health, including antihypertensive effects, prevention of renal failure and the growth of beneficial intestinal *Lactobacillus* bacteria (Beheshtipour et al. 2013). *Arthrospira* ssp. are part of the diet of certain human populations where these cyanobacteria grow naturally, such as in the lakes of Chad in Africa (Spolaore et al. 2006; Mata et al. 2010; Hamed 2016), where spirulina (locally known as Dihé by the local people) is harvested for food (**Figure 1 a** and **b)** (Caterina et al. 2004).

Microalgal species such as *Dunaliella salina* produce β-carotene under stress conditions, which provide numerous benefits for human health (Hosseini and Shariati 2009; Oren 2014b; Wu et al. 2020). β-carotene is a major carotenoid present in the human diet and the main source of vitamin A in humans (Johnson 2002; Elvira-Torales et al. 2019), but research is still needed for dietary recommendations of algal sourced β-carotene, and caution should be taken for large doses (EFSA 2012).

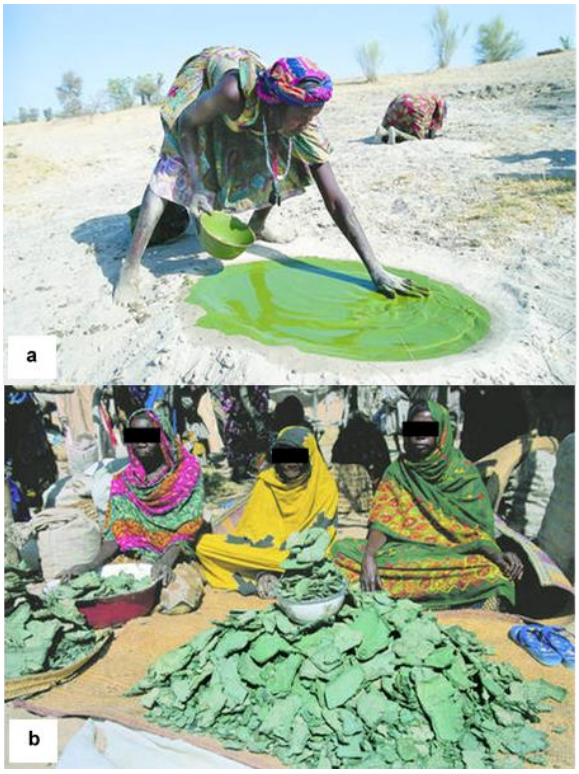

Fig. 1 *Women of Chad preparing **(a)** and selling **(b)** spirulina dried on sand, locally known as Dihé by the local people of Chad (Caterina et al. 2004).*

Microalgal species such as *Haematococcus pluvialis* produce astaxanthin, a carotenoid well known for its antioxidant activity as well as anticancer, photoprotection, and anti-inflammatory properties (Yuan et al. 2011). Astaxanthin was approved by the U.S. FDA as an effective nutritional supplement with potent antioxidant properties (Nethravathy et al. 2019). Microalgae and cyanobacteria also produce long-chain polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which are effective nutraceuticals with many health benefits (Sathasivam et al. 2019). **Table 1** illustrates examples of dietary ingredients produced by microalgal and cyanobacterial species, beneficial for human consumption.

Table 1: *Dietary ingredients produced by microalgae and cyanobacteria for healthy food production*.

Bioactive compounds	**Microalgae/ cyanobacteria species**	**References**
Asthaxanthin	*Haematococcus pluvialis*	(Shah et al. 2016; Li et al. 2020)
Carbohydrates	*Porphyridium cruentum* and *Spirogyra* sp	(Chen et al. 2013; Samiee-Zafarghandi et al. 2018)
Essential PUFAs	*Crypthecodinium* sp., *Schizochytrium* sp., and *Ulkenia* sp.	(Doughman et al. 2007; Nethravathy et al. 2019)
Exopolysaccharides and carotenoids (β-carotene)	*Dunaliella salina*	(Pignolet et al. 2013; Koller et al. 2014)
Lipids	*Schizochytrium* sp., *Pavlova lutheri, Chlorella* sp., *Scenedesmus* sp., and *Isochrisis* sp.	(Rodolfi et al. 2009)
Lutein	*Chlorella zofingiensis, Chlorella sorokiniana, Muriellopsis* sp., *Scenedesmus almeriensis, Coccomyxa acidophila*, and *Chlorella protothecoides*	(Nethravathy et al. 2019)
Phycocyanin and phycoerythrin	*Arthrospira* sp. and *Porphyridium cruentum*	(Hsieh-Lo et al. 2019)
Proteins	*Spirulina maxima* and *Chlorella* sp.	(Garrido-Cardenas et al. 2018; Amorim et al. 2020; Lafarga et al. 2021)
Sterols	*Isochrysis galbana* and *Pavlova lutheri*	(Ahmed and Schenk 2017; Randhir et al. 2020)
Vitamin C, K, B_{12}, A, and E	*Chlorella* sp., *Arthrospira* sp., *Isochrisis galbana, Porphyridium cruentum, Pavlova* sp., and *Tetraselmis* sp.	(Tarento et al. 2018; Jacob-Lopes et al. 2019)

2.1 Microalgal and cyanobacterial products market

Microalgal and cyanobacterial biomass production can be used to obtain different kinds of extracts economically important in commerce. Leading commercial players in microalgal and cyanobacterial products include Cyanotech Corporation (U.S.), DIC Lifetec Co. Ltd. (Japan), Cellana Inc. (U.S.), Alltech, Inc. (U.S.), Algaetech International Sdn Bhd (Malaysia), BlueBioTech GmbH (Germany) and Parry Nutraceuticals Limited (India) (Algae Products Market Information 2018). The Cyanotech Corporation sources *Arthrospira* sp. food supplements and astaxanthin from natural microalgae *Haematococcus pluvialis*. The current market value of astaxanthin is around $2,000/kg (Shah et al. 2016; Nethravathy et al. 2019). Although consumable by humans, microalgae-derived astaxanthin is principally consumed by the salmon feed industry (Nethravathy et al. 2019). *H. pluvialis* produced astaxanthin gives salmonids the typical salmon coloration, which is desired by the customers. Major microalgal astaxanthin producers in the market include: Cyanotech corporation (USA), Alga technologies (Israel), Jingzhou Natural Astaxanthin Inc (China), Fuji chemical industry Co. Ltd (Japan), and Parry Nutraceuticals (India) (Shah et al. 2016; Nethravathy et al. 2019).

Other beneficial ingredients such as lutein are commercially marketed in the form of powder, capsules, or oleoresins containing 3% to 80% of lutein (Nethravathy et al. 2019). Microalgae-based lutein production provides a promising alternative to marigold petals, the current main commercial supply of lutein (Fernández-Sevilla et al. 2010; Lin et al. 2015; Xie et al. 2021). Several microalgae species including *Chlorella* spp., *Scenedesmus* spp., *Muriellopsis* spp., and *Dunaliella* spp. produce significant lutein content. However, production of microalgal lutein at commercial scale is still at the laboratory stage and not yet implemented in large-scale commercial production (Lin et al. 2015). Microalgae and cyanobacteria can be dried as food or consumed in the form of capsules and tablets as food supplements (Spolaore et al. 2006; Mata et al. 2010), or incorporated into other food and dairy products, such as pasta, bread, soft drinks, yogurt or snacks (Gross 2004; Mohamed et al. 2013). High value molecules produced by microalgae and cyanobacteria such as DHA and lutein are found in human breast milk and can be incorporated in formulas (Eggersdorfer and Wyss 2018).

Microalgae and cyanobacteria are suitable alternatives for consumers who are increasingly concerned about their health and diet control (Hamed 2016). Illnesses such as high cholesterol and heart disease linked to poor eating habits could be reduced by the substantial consumption of healthy microalgal or cyanobacterial food products due to their high nutrition value (Hamed 2016). For example, β-1,3-glucan polysaccharides, the main component in *Chlorella* spp. are active immunostimulators, free-radical scavenger and reducers of blood lipids (de Jesus Raposo et al. 2015; Sathasivam et al. 2019).

Microalgae and cyanobacteria production and commercialization is often limited by the high cost of cultivation. The optimization of culture medium is an important strategy for increasing the cost-effectiveness of the productions (Ronga et al. 2019). Low-cost resources such as nutrient-rich wastewaters, agricultural by-products and inexpensive synthetic fertilizers, are effective strategies for increasing the cost-effectiveness of the production (Mata et al. 2010; Gong and Jiang 2011). However, such strategies could not be suitable for algal biomass production destined for human consumption. Microalgae and cyanobacteria cultivation for food production can be grown in controlled optimized conditions to maximally promote the growth rate and productivity (Andrianantoandro et al. 2006; Narala et al. 2016; Tan et al. 2020). Culture parameters in photobioreactors (PBRs) can also be controlled to trigger enhanced production of specific value-added products under artificial growth conditions for the industrialization of microalgae and cyanobacteria (Kothari et al. 2017). Cultivation parameters including pH, temperature, light intensity, nutrient composition and concentration and CO_2 supply are major growth factors of microalgae and cyanobacteria (Renhe et al. 2021; Figueroa-Torres et al. 2021).

2.2 Microalgae and cyanobacteria vs. crop plants

Microalgae and cyanobacteria are good sources of proteins, carbohydrates, lipids and several useful metabolites. Microalgae and cyanobacteria do not require arable land (they are cultivated in controlled culture systems) and they can grow in brackish or seawater, which is unusable for normal agriculture (Masojídek and Torzillo 2014). In addition, the microalgal yield of storable compounds can greatly exceed that of traditional crops**. Table 2** illustrates the advantages of microalgae and cyanobacteria cultivation over crop plants, including higher efficiency of CO_2-fixation and energy conversion, biomass productivity, and lower resources requirements.

Table 2: *Advantages of cultivation of microalgae and cyanobacteria in comparison with crop plants*.

	Microalgae/ cyanobacteria	**Crop plants**	**References**
CO_2-fixation efficiency	Up to 58% in PBRs and 46% in open ponds	Generally, 10 to 50 times lower in terrestrial plants compared to microalgae and cyanobacteria	(Costa et al. 2000; Chiu et al. 2007; Ramanan et al. 2010; Langley et al. 2012)
Photon conversion efficiency	Ranges from 8 to 10%	Approximately 4.6% for C3 plants and 6.0% for C4 plants at 30 °C. However, it drops to 2.9% and 4.2%, respectively, when measured in the field	(Zhu et al. 2010; Bhola et al. 2014; Benedetti et al. 2018)
Biomass production by CO_2 sequestration	High	Low	(Bhola et al. 2014)
Theoretical expected maximal productivity	280 ton of algal biomass $(ha)^{-1}$ $year^{-1}$ in PBRs and 100 tons ha^{-1} $year^{-1}$ in open ponds	An average of 10 tons ha^{-1} $year^{-1}$	(Rodolfi et al. 2009; Melis 2009)
Oil production per unit area	30 tons ha^{-1} $year^{-1}$	4–5 ton ha^{-1} $year^{-1}$	(Chisti 2007; Rodolfi et al. 2009; Mata et al. 2010)
Protein yield per unit area	4–15 ton ha^{-1} $year^{-1}$	0.6–1.2 ton ha^{-1} $year^{-1}$, 1–2 ton ha^{-1} $year^{-1}$, and 1.1 ton ha^{-1} $year^{-1}$ in soybean, pulse legumes, and wheat, respectively	(Van Krimpen et al. 2013)

Land use and water resources	Do not require arable land Grow in brackish and even seawater	Require arable land with a freshwater supply (consume approximately 70% of the freshwater withdrawn per year)	(Pimentel et al. 2004; Hüfner 2010; Masojídek and Torzillo 2014)
Resilience to harsh environments	High	Low	(Bhola et al. 2014)

Microalgae can be produced at a large scale in artificial open systems (circular and raceway ponds where the culture is directly exposed to open air) or closed systems, where the culture is fully enclosed in a PBR (Narala et al. 2016). Open systems can be divided into natural ponds (eutrophic lakes or small natural basins) and artificial raceway ponds (Hamed 2016). Raceway ponds are the most widely used systems for the commercial production of microalgae (Jerney and Spilling 2018). They are generally the cheapest to construct, and their function is quite simple (Borowitzka 2013; Enzing et al. 2014). They are generally constructed as oval-shaped recirculation channels in which the culture medium flow is guided around bends by baffles placed in the flow channel, and they are stirred with a paddlewheel to ensure culture homogenization, as shown in **Figure 2 a**. PBRs are reactors that offer a closed-culture environment in which phototrophs are grown or used to carry out photobiological reactions (Tredici 2004) (**Figure 2 b** and **c**). PBRs allow better control of culture growth conditions, and most importantly, the microalgae are protected and relatively safe from invasion by competing microorganisms (Vishwanath Patil1 et al. 2005; Narala et al. 2016).

PBRs are the best culture systems for optimized culture production and biomass quality, despite their high setup, maintenance and energy input costs. In comparison to PBRs, circular and raceway ponds have higher contamination risks, culture evaporation and exposure to weather components, making these culture systems highly dependent on geographical conditions (Kumar et al. 2015; Muhammad et al. 2020).

Open culture systems can be a great challenge for countries in the equatorial and monsoon climate regions of Africa but are highly suitable for the warm desert climate of Northern Africa.

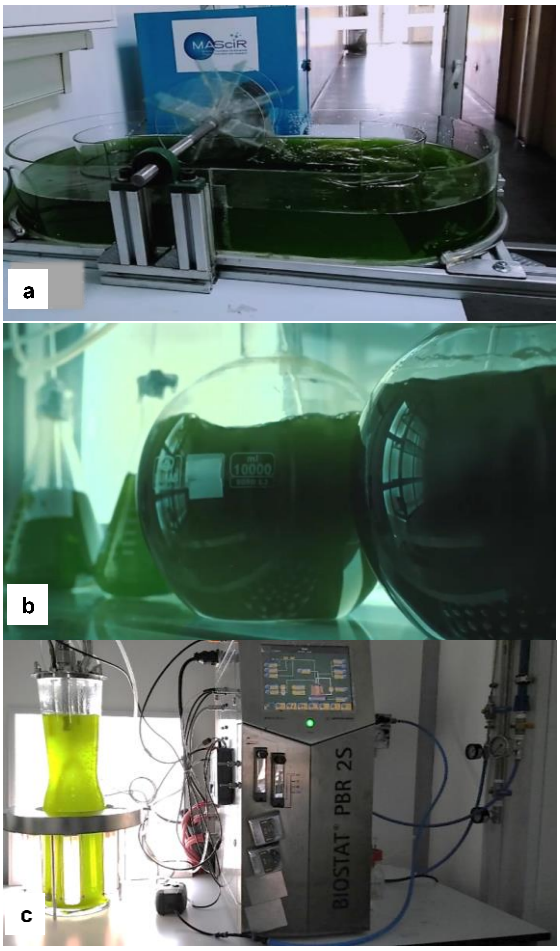

Fig. 2 *Raceway pond prototype (**a**), round flasks (**b**) and vertical column PBR (**c**) at the Microalgae Biotechnology Laboratory of the Moroccan Foundation for Advanced Science Innovation and Research (MAScIR).*

On the other hand, large-scale microalgal or cyanobacterial production using PBRs may not be practical in most parts of sub-Saharan Africa, considering that the larger population of these regions consists of smallholder farmers (Langyintuo 2020) with less financial capacity for sophisticated large-scale algal production.

1.3 Technological developments in microalgal and cyanobacterial cultivation to improve the algal production and industrialization

The latest technological developments in microalgal and cyanobacterial cultivation and harvesting, such as automation, phenotyping, and synthetic biology, were described by Fabris et al. (2020). A significant body of research has focused on optimizing conditions that maximally promote microalgae and cyanobacteria growth rates, or trigger enhanced production of specific value-added products under artificial growth conditions for the industrialization of microalgae and cyanobacteria (Andrianantoandro et al. 2006). **Figure 3** shows cyanobacterial culture optimization by manipulating different growth parameters for oriented production of EPS.

Fig. 3 *Exploitation of microalgae and cyanobacteria for research purposes by manipulating different growth parameters for oriented production of EPS. Photo taken at the Microalgae Biotechnology Laboratory of the Moroccan Foundation for Advanced Science Innovation and Research (MAScIR), 2020*.

The cultivation of microalgae and cyanobacteria has an advantage over heterotrophic systems due to their photosynthetic mechanisms and ability to fix C from inorganic sources (Fajardo et al. 2020). Despite the several advantages and inconveniences reported, there is no single "best practice" method for cultivating microalgae and cyanobacteria, especially at large scale (Fajardo et al. 2020). The final design of the system is dependent on the final product, the geographical location, and local resources available (e.g., accessibility to water, CO_2, and waste streams) (Fabris et al. 2020).

Advanced molecular techniques for research and development in microalgae are still underdeveloped compared to fungi, bacteria or even higher plants (Fajardo et al. 2020). Sophisticated studies including the overexpression or downregulation of several genes in a single organism, are only practically possible in *Chlamydomonas reinhardtii* (Wijffels and Barbosa 2010; Doron et al. 2016; Sanchez-Tarre and Kiparissides 2021). However, new advances in microalgae and cyanobacteria biotechnology are rapidly emerging, paving the way to the establishment of a sustainable, algae-based bioeconomy (Hamed 2016; Fu et al. 2019; Fabris et al. 2020; Fajardo et al. 2020). The available number of full or near-full genome sequences of diverse microalgal species has largely increased over the last decade (Kumar et al. 2020). Such species include *Chlamydomonas reinhardtii* (Colina et al.; Merchant et al. 2007; Nguyen et al. 2011; Schmollinger et al. 2014), *Chlorella pyrenoidosa* (Duan et al. 2019; Kumar et al. 2020), *Chlorella sorokiniana* (Tejano et al. 2019; Kumar et al. 2020), *Chlorella vulgaris*, *Dunaliella salina* (Yue et al. 2016; Wang et al. 2019; Kumar et al. 2020), *Phaeodactylum tricornutum* (Bowler et al. 2008; Yang et al. 2014; Longworth et al. 2016) and *Thalassiosira pseudonana* (Armbrust et al. 2004). Genome sequencing of microalgal species will provide a significant genetic resource for the study of their metabolic pathways, regulatory networks, and genetic potentials. Web-based resources such as pico-PLAZA (http://bioinformatics.psb.ugent.be/pico-plaza/), AlgaePath (http://algaepath.itps.ncku.edu.tw) and ALCOdb (http://alcodb.jp) are available databases for algal genomics (Kumar et al. 2020). These emerging technologies will provide tools for highly efficient algae-based solutions to a range of societal needs. In-depth understanding of algal biology, genetics and biochemical capacities, will improve both

their production and processing for consumption or product innovation in agriculture.

2. Microalgae and cyanobacteria for sustainable agriculture

3.1 Microalgal and cyanobacterial biomass application to degraded soils: a new approach to restoring organic matter on degraded farmlands

The conversion of natural ecosystems to agriculture results in the depletion of soil organic C levels, releasing 50 to 100 GT of C from the soil into the atmosphere (Lal 2009). This C loss is mainly caused by reduction in the amount of plant roots and residues returned to the soil, and higher soil erosion (Lemus et al. 2005; Ontl and Schulte 2012). The soil C deficit created by the depletion of soil organic C stocks presents an opportunity to store C in the soil through diverse land management approaches (Ontl and Schulte 2012). Naturally, soil organic C levels are based on the interactions between many ecosystem processes, of which photosynthesis, respiration, and decomposition are key. Carbonic matter in degraded soils may take years to restore (Ovsepyan et al. 2019), considering that soil organic C input rates are primarily determined by plant roots and litter deposited from plant shoots (Lemus et al. 2005; Ontl and Schulte 2012).

The microalgal and cyanobacterial biomass is rich in organic matter due to the organic C incorporated through photosynthesis, and is therefore a suitable target for the development of innovative products for soil quality recovery. The total carbohydrate and starch contents of microalgae are approximately 20% and 10% dry weight (DW), respectively (Laurens et al. 2012; Cheng et al. 2017). Cyanobacterial species also have high carbohydrate contents, up to 60% DW. The carbohydrate contents vary with microalgal or cyanobacterial species but can be significantly increased according to cultivation conditions and time (Cheng et al. 2017). The restoration of C storage on degraded land parcels can begin from the decay of microalgae and cyanobacteria organic compounds, that are highly enriched in C (Ontl and Schulte 2012). Other organic compounds produced by microalgae and cyanobacteria, including proteins and lipids, also contribute to soil organic matter (Ontl and Schulte 2012).

Microalgal and cyanobacterial biomass can also provide soil microbes with energy in the form of C. Although decomposition of this biomass by soil microbes will result in C loss from the soil as CO_2 from microbial respiration, a small proportion of the original C will be retained in the soil through the formation of humus, which is beneficial for soil water retention (Ontl and Schulte 2012; Bastida et al. 2012).

Microalgal and cyanobacterial biomass can boost the productivity of arable land parcels as soil amendments with the capacity to increase soil organic C and stabilize the balance between soil C inputs and outputs in farm soils (Alvarez et al. 2021). Various factors including climate change, historic land use patterns, land management strategies, and topographic heterogeneity influence soil C changes on farmlands, (Ontl and Schulte 2012; Todd 2012). Other ecosystem processes, such as soil erosion and leaching of dissolved C into groundwater, can also lead to C loss in soils (Todd 2012; Tiefenbacher et al. 2021; Thaler et al. 2021). C inputs from photosynthesis on agricultural farmlands are less than C losses from the soil; therefore, soil organic C levels decrease over time (Ontl and Schulte 2012). Thus, alimentation of the soil with C-rich products such as microalgal and cyanobacterial biomass could be an important method to stabilize the net change in soil organic C levels. When C inputs and outputs are in balance with each other, there is no net change in soil organic C levels.

3.2 Role of microalgae and cyanobacteria in soil fertility and structure

Microbes play a vital role in determining soil structure and fertility (Vaishampayan et al. 2001; Singh Jay Shankar 2014; Singh et al. 2016). Although most microalgal species can utilize both organic and inorganic nitrogen (N), they can only assimilate nitrite (NO_2^-), nitrate (NO_3^-), and ammonium (NH_4^+). Cyanobacteria, on the other hand, can convert atmospheric N_2 to ammonia by N_2-fixation (Cai et al. 2013; Ahmed et al. 2014). Cyanobacteria can also tolerate various stresses, such as drought, high or low temperatures, pH and salinity, giving them an advantage over various competitors in different ecological niches (Gröniger et al. 2000; Ibañez et al. 2012; Sand-Jensen and Jespersen 2012). Cyanobacteria in particular have been a specific target in agriculture due to their ability to fix atmospheric N_2 and CO_2 and produce a diversity of biologically active metabolites (Vaishampayan et al. 2001; Singh Jay Shankar 2014; Singh et al. 2016). Cyanobacteria establish symbiotic associations (Costa and Lindblad 2002; Subashchandrabose et al. 2011; Hamouda et al. 2016). Certain cyanobacterial species also adapt to local climatic conditions and survive in wet soils (Nisha et al. 2007). Up to 25% of the total biomass of the cyanobacteria surviving in wet soils is from EPSs (Nisha et al. 2007). EPS significantly contribute to the soil's nutritional status, structural stability and crop productivity. They act as a gluing agent on soil

particles, leading to soil aggregation, organic content accumulation, and an increase in the water holding capacity of the top layer of soil (Malam Issa et al. 2001; Nisha et al. 2007). An increase in water holding capacity and organic content favors the growth of plant-growth promoting rhizobacteria (PGPR) (Xiao and Zheng 2016). Therefore, microalgal or cyanobacterial growth in humid soils could subsequently ameliorate soil chemical, physical and biological properties that sustain soil fertility (Flaibani et al.; Zulpa et al. 2003; Paul and Nair 2008).

The aerobic N-fixing capacity of some cyanobacterial species makes them important players in the biogeochemical N cycle of both aquatic and terrestrial environments (Garcia-Pichel 2009). Cyanobacteria are a natural component of paddy fields and a beneficial contributor to rice production in tropical countries. Rice production in tropical countries is largely dependent on biological N_2 fixation by cyanobacteria (Vaishampayan et al. 2001), fixing approximately 20–30 kg N ha^{-1} (Ahmed et al. 2014; Singh et al. 2016). Cyanobacteria also improve the availability of phosphorus (P) to crops through the solubilization and mobilization of insoluble organic phosphates (Singh et al. 2016; Jhala et al. 2017; Gonçalves 2021).

Microalgal and cyanobacterial biofertilization enhanced the photosynthetic performance and growth of willow (*Salix viminalis* L.) (Grzesik et al. 2017) and rice plants (*oryza sativum*) (Jochum et al. 2018). The application of microalgal biomass (*Chlorella vulgaris* and *Scenedesmus dimorphus)* to rice plants resulted in significant plant height increase under greenhouse conditions (Jochum et al. 2018), indicating that microalgal biomass can provide a biological option for rice fertility programs. This approach could be especially important for organic rice production where synthetic fertilizers cannot be used (Jochum et al. 2018).

Microalgal and cyanobacterial biomass recovered from wastewater treatments, CO_2 sequestration or biodiesel generation could be valuable in agriculture as organic soil amendments (Ansari et al. 2019). The use of wastewater could provide inexpensive solutions to microalgae and cyanobacteria cultivation, which require significant nutrient input (Renuka et al. 2018). Conversely, agricultural commercialization of microalgae or cyanobacteria biomass generated from wastewater requires thorough quality evaluation and validation. Although wastewaters are rich in nutrients such as organic and inorganic forms of C, N and P, which can be effectively utilized by microalgae, they contain undesirable substances such as heavy metals, pesticides, pharmaceutical compounds, chemicals and pathogens (Rana et al. 2014; Arora et al. 2021). Microalgae and cyanobacteria accumulate heavy metals. Thus, direct application of wastewater Microalgal or cyanobacterial biomass to the soil may have potential risks on the environment, due to the presence of unwanted hazardous metals, toxins and pathogens (Renuka et al. 2018). Cyanotoxins produced by cyanobacteria can also be absorbed and accumulated by plants, including agricultural food crops, which can have direct negative effect on human and animals (Corbel et al. 2014).

3.3 Microalgal and cyanobacterial extracts as biostimulants of plant growth and productivity

Several experimental studies under open-field and greenhouse conditions have demonstrated the plant growth-enhancing capacity of microalgal and cyanobacterial extracts (Garcia-Gonzalez and Sommerfeld 2016; Puglisi et al. 2018; Chanda et al. 2019), indicating their potential use in agriculture as plant growth enhancers.

Crude bioextracts obtained from microalgae and cyanobacteria can ameliorate the growth and development of crops. Liquid extracts of microalgae and cyanobacteria enhanced tomato plant growth through increased chlorophyll content and uptake of N, P and potassium (K) (Chanda et al. 2020). Gas chromatography-mass spectrometry (GC-MS) analysis also showed that treatment with microalgal and cyanobacterial extracts enhanced the production of total lipids and pyridine-3-carboxamide, an amide active form of vitamin B3 (Surjana et al. 2010). The germination of lettuce in 2 and 3 g kg^{-1} soil with dry microalgal extracts derived from *C. vulgaris* promoted plant growth at the early stages of development (Faheed and Fattah 2008). In the same study, enhanced plant growth based on shoot and root dry weight and length was correlated with improved carotenoid and chlorophyll pigment biosynthesis. Sugar beet seedlings supplemented with extracts of the microalgae *C. vulgaris* or *Scenedesmus quadricauda* also exhibited higher total root length, surface area, and number of root tips (Barone et al. 2018). The different changes in root architecture/morphology induced by the microalgal extracts were attributed to the upregulation of several genes that may intervene in various metabolic pathways (Barone et al. 2018).

The biostimulant activity of microalgal and cyanobacterial extracts is associated with their complex biochemical composition. Biologically active molecules derived from microalgal and cyanobacterial extracts include key amino acids

(arginine and tryptophan), sulfated polysaccharides, polyunsaturated fatty acids, vitamins, osmolytes and phytohormones (Ronga et al. 2019; Colla and Rouphael 2020). This complex and multicomponent nature of extracts complicates the study of the mode of action and the production, registration and use of many biostimulants (Yakhin et al. 2017). Studies carried out in evaluating the effect of microalgae and cyanobacteria or their extracts on plant growth and tolerance mechanisms have been summarized in **Table 3**. Further studies such as hormone profiling, transcriptomics, proteomics, and metabolomics analysis of treated and untreated plants could also reveal biostimulant activated signaling pathways involved in the stimulation of plant responses (Yakhin et al. 2017).

Table 3: *The effect of microalgae and cyanobacteria (or their extracts) on soil fertility, and mechanisms of plant growth and salt stress tolerance*.

Microalgal and cyanobacterial species	**Crop**	**Observed effects**	**References**
Effects of microalgae and cyanobacteria on soil improvement			
Nostoc sp.		-EPSs act as a gluing agent on soil particles, leading to soil aggregation. -Increase in the organic content accumulation and water holding capacity of the top layer of soil.	(Maqubela et al. 2008; Parwani et al. 2021)
Calothrix ghosei, Hapalosiphon intricatus Nostoc sp.	*Triticum aestivum* L.	-Increase in organic carbon content in the soil.	(Karthikeyan et al. 2007)
Microalgae and cyanobacteria as biofertilizers			
Anabaena torulosa	*Triticum aestivum* L.	-Increase in the availability of N in the soil by N_2 fixation.	(Swarnalakshmi et al. 2013)
Nostoc entophytum and *Oscillatoria angustissima*	*Pisum sativum*	-Increase in N_2 fixation. -Increase in nutritional value of pea seeds. -Increase in growth parameters and photosynthetic pigments.	(Osman et al. 2010)
Cyanobacterial inoculum of: *Aulosira fertilissima, Anabaena sphaerica, Nostoc hatei, Cylindrospermum majus* and *Westiellopsis prolifica*	*Oryza Sativum*	-Increase in N availability in the soil. -Increase in grain and straw yields.	(Jha and Prasad 2006)
Cyanobacterial consortia of: *Anabaena doliolum, Cylindrospermum sphaerica,* and *Nostoc calcicola*	*Pennisetum glaucum Triticum aestivum*	-Increase in N and P availability in the soil. -Improvement of nutritional properties.	(Nisha et al. 2007)
Effects of microalgae and cyanobacteria (or their extracts) on nutrient uptake			

Microalgal-cyanobacterial consortia of: *Chlorella sp., Scenedesmus sp., Chlorococcum sp., Chroococcus sp., Phormidium sp., Anabaena sp., Fischerella* and *Spirogyra* sp.	*Triticum aestivum* L.	-Increase in N, P and K availability in the soil. -Increase in organic carbon content in the soil. -Increase in N, P and K contents in roots, shoots and grains.	(Renuka et al. 2018)
Chlorella ellipsoidea, chlorella pyrenoidos and Arthrospira maxima	*Solanum lycopersicum* L.	-Enhanced nutrient uptake via increased root length	(Chanda et al. 2020)
Effects of microalgae and cyanobacteria (or their extracts) on plant growth			
Chlorella vulgaris and *Arthrospira platensis*	*Zea mays*	-Increase in germination rate and plant yield. -Increase in fresh and dry weights of shoot, root and whole plant. -Increase in shoot length and in the number of leaves.	(Dineshkumar et al. 2017)
Calothrix ghosei, *Hapalosiphon intricatus* and *Nostoc* sp.	*Oryza sativum*	-Enhanced seedling growth by root-promoting hormones (auxins, cytokinins and gibberellic acid).	(Karthikeyan et al. 2007)
Chlorella vulgaris and *Scenedesmus quadricauda*	*Beta vulgaris* L.	-Higher total root length, surface area, and number of root tips.	(Barone et al. 2018)
Chlorella vulgaris	*Lactuca sativa* L.	-Enhancement of soluble carbohydrate, soluble protein and total free amino acids.	(Faheed and Fattah 2008)
Chlorella vulgaris and *Scenedesmus dimorphus*	*Salix viminalis* L., *Oryza sativum*	-Enhanced photosynthetic performance and growth.	(Grzesik et al. 2017; Jochum et al. 2018).
Effects of microalgae and cyanobacteria (or their extracts) on abiotic stress			
Extracts from *Dunaliella* spp. and *Phaeodactylum* spp.	*Capsicum annuum* L.	-Reduced production of superoxide radicals. -Low lipid peroxidation.	(Guzmán-Murillo et al. 2013)
Spirulina spp. and *Chlorella spp.*	*Triticum aestivum* L.	-Enhanced antioxidant capacity and protein content of the whole grains.	(Abd El-Baky et al. 2010)
Nannochloris sp.	*Solanum lycopersicum* L.	-Alleviated effects of water stress through	(Oancea et al. 2013)

		enhanced plant growth and nutrient uptake.	
Exopolysaccharides from *Dunaliella salina*	*Triticum aestivum, Solanum lycopersicum* L.	-Improved seed germination and seedling growth. -Enhanced plant size and K uptake.	(El Arroussi et al. 2016; EL Arroussi et al. 2018)
Effects of microalgae and cyanobacteria (or their extracts) on biotic stress			
A. platensis, D. salina and *Porphorydium* sp.	*Solanum lycopersicum* L.	-Improved carotenoid, chlorophyll and proteins content. -Improved Nitrate Reductase (NR), NAD-Glutamate Dehydrogenase (NAD-GDH) activities in plant leaves	(Farid et al. 2019; Rachidi et al. 2021)
Anabaena laxa and Calothrix elenkinii	*Coriandrum sativum, Cuminum cyminum, Foeniculum vulgare*	-Increase in the activity of β-1,3 endoglucanase in shoots and roots. -Increase in fungicidal activity. -Increase in germination rates. -Increase in shoot and root length. -Increase in plant dry weight.	(Senthil-Kumar et al. 2013)
Effects of microalgae and cyanobacteria in hydroponics			
Chlorella vulgaris	*Eruca vesicaria, Lactuca sativa, purple kohlrabi*	-Improved removal rate of total dissolved solids, total N, and total P of the nutrient solution.	(Huo et al. 2020)
Chlorella infusionum Scenedesmus quadricauda Chlorella vulgari	*Solanum lycopersicum* L.	-Increased supply of O_2 into the nutrient solutions by photosynthesis. -Improved crop root respiration and growth.	(Zhang et al. 2017; Barone et al. 2019)

3.4 Cyanobacteria and microalgae as biostimulants of crop abiotic stress tolerance

Crop productivity is often affected by diverse abiotic stresses usually provoked by drought, soil salinization and low or high temperatures. Soil salinization is a major agricultural constraint (Davidson 2000; Shrivastava and Kumar 2015; Ronga et al. 2019) affecting ~800 million ha globally (Munns and Tester 2008) and is exacerbated by inappropriate chemical fertilization management practices that lead to osmotic imbalance in the soil, affecting crop growth and productivity (Goykovic Cortés and Saavedra del Real 2007). Many studies have highlighted the biostimulant

properties of extracts from different microalgal and cyanobacterial species for improving abiotic stress tolerance in higher plants, including salt stress (Rodríguez et al. 2006; Shrivastava and Kumar 2015; EL Arroussi et al. 2018; Chanda et al. 2021).

Dunaliella sp. and *Phaeodactylum* sp. mitigated salt stress in bell pepper (*Capsicum annuum* L.) seedlings by reducing superoxide O_2^- production and lipid peroxidation (Guzmán-Murillo et al. 2013). Crude extracts of the cyanobacteria *Arthrospira* sp. and the microalga *Chlorella* sp. improved the salt stress tolerance of wheat (*Triticum aestivum* L.) and enhanced the antioxidant capacity and protein content of grains from treated plants (Abd El-Baky et al. 2010). Extracts from *Nannochloris* sp. also alleviated hydric stress in tomato (*Solanum lycopersicum L.*) as determined by improved root length and increased leaf area and plant height (Oancea et al. 2013). The application of EPS from halophilic microalgae (*Dunaliella salina*) also alleviated the effect of salt stress on the growth of tomato (*Solanum lycopersicum L.)* subjected to high salinity concentrations (3 and 6 g L^{-1} NaCl) by mitigating the decrease in length and dry weight of the plant's shoot and root systems, and increasing K uptake and the K^+/Na^+ ratio (EL Arroussi et al. 2018). In the same study, the accumulation of proline, phenolic compounds and Na^+, as well as catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) activities triggered by salt stress, were attenuated after treatment of plants with EPSs. Metabolic pathways involved in the plant's tolerance to stress, such as jasmonic acid-dependent pathways, were also enhanced.

Microalgal or cyanobacterial biostimulants are usually applied as total crude extracts containing diverse organic compounds, including hormones. This mixture of diverse organic compounds could contain molecules mimicking compatible solutes, signaling molecules or elicitors; hence, they could have multiple direct or indirect effects on plant growth and stress tolerance (Renuka et al. 2018; Colla and Rouphael 2020). **Figure 4** illustrates the possible biostimulant effects of Microalgae-cyanobacteria Extract Formulation (MEF) on salt-stressed tomato plants. Bioactive organic compounds in MEF can stimulate the antioxidative enzyme system, and consequently attenuate lipid peroxidation in plant cells. MEF can also enhance root growth, which favors nutrient uptake and consequent ion homeostasis in leaves and photosynthetic activity.

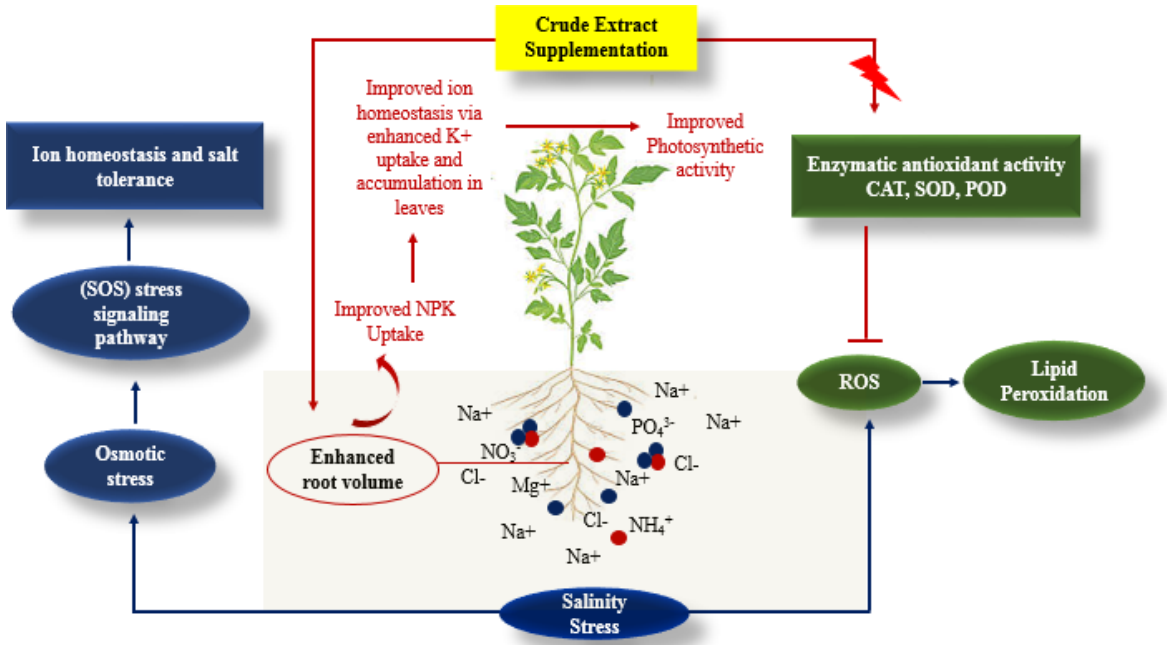

Fig. 4 *Possible biostimulant effects of Microalgae-cyanobacteria Extract Formulation (MEF) on salt-stressed tomato plants. MEF enhances root growth, which favors nutrient uptake and consequent ion homeostasis in leaves and photosynthetic activity. MEF stimulate antioxidative enzymes, which consequently attenuate lipid peroxidation in plant cells. Reactive Oxygen Species (ROS), Hydrogen Peroxidase (H_2O_2), Catalase (CAT), Peroxidases (POD), Superoxide Dismutase (SOD), and Salt Overly Sensitive pathway (SOS).*

3.5 Co-cultivation of microalgae/cyanobacteria and plants in hydroponic systems: an emerging innovative method for biologically stimulated crop growth and productivity

Soilless cultivation (hydroponics) is the future for agricultural production sectors. Research studies such as those on NO_3^- management or crop quality increases by managing the electrical conductivity of the solution are commonly exploited in hydroponics. Emerging new and innovative methods, such as the use of nanoparticles and beneficial microorganisms, including PGPRs, have also been described. Crop co-cultivation with microorganisms such as microalgae or cyanobacteria in hydroponics can ameliorate plant growth. Microalgae and cyanobacteria produce and release a wide range of bioactive compounds in the culture solution.

In hydroponics, microalgae and cyanobacteria can be grown in the nutrient solution. The release of bioactive compounds such as EPS and other metabolites in the substrate solution may trigger mechanisms related to plant tolerance, productivity and vigor (**Figure 5**). Soil application of EPSs obtained from *D. salina* mitigated the effect of salinity stress by enhancing reactive oxygen species (ROS)-scavenging enzyme activities, phenolic compounds and key metabolites involved in antioxidative stress mechanisms (EL Arroussi et al. 2018). Hydroponic co-cultivation of tomato plants with microalgae also stimulated crop performance and growth in terms of fresh and dry plant weight by ameliorating the substrate solution (Zhang et al. 2017; Barone et al. 2019). The plant growth stimulation was attributed to the constant use of CO_2 and delivery of O_2 to the hydroponic nutrient solution, through microalgal photosynthesis (Barone et al. 2019). The lower CO_2 levels, resulting from CO_2 fixation by microalgae or cyanobacteria, and the liberation of O_2 in the root zone of hydroponically grown plants can positively regulate plant growth. CO_2 accumulation in an O_2-deficient root zone can be a detrimental factor to certain crop plants in flooded fields (Boru et al. 2003b, a).

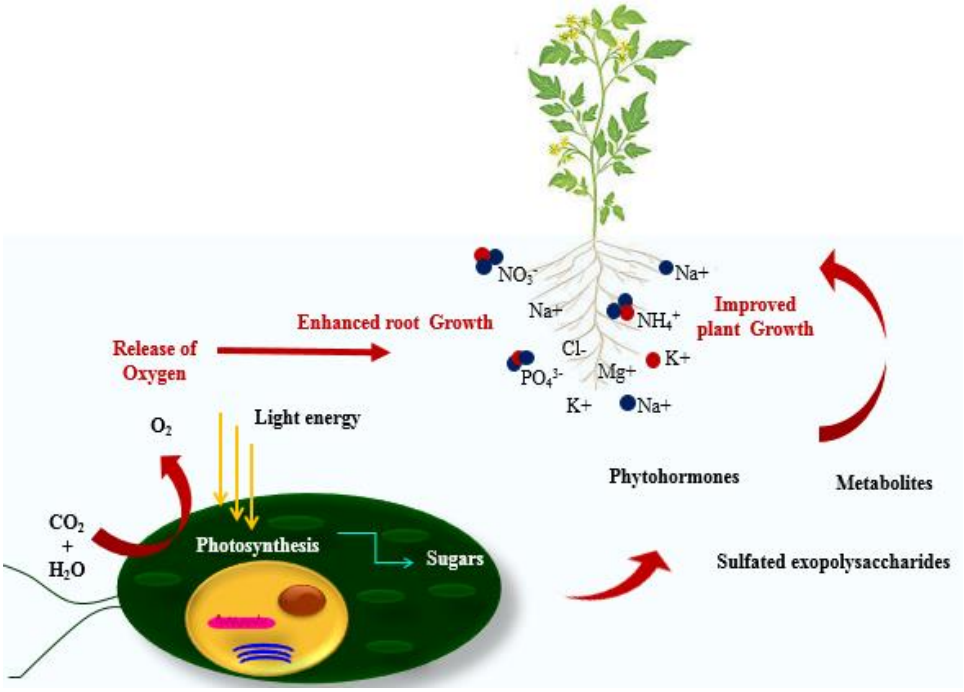

Fig. 5 *Possible biostimulant effects of hydroponics co-cultivation with microalgae or cyanobacteria on crops. Bioactive metabolites such as sulfated exopolysaccharides and phytohormones released in the substrate solution enhance plant growth. CO_2 fixation and O_2 release from photosynthesis by microalgae or cyanobacteria in the substrate solution ameliorate root respiration*.

Crop co-culture with photosynthetic microorganisms in hydroponics releases more O_2 in the nutrient solution to favor optimum root CO_2/O_2 levels for plant growth. The sole accumulation of CO_2 in the root zone does not negatively affect plant growth unless O_2 is deficient. Plant roots are naturally colonized by microorganisms and evolve more CO_2 (CO_2 concentrations in the rhizosphere is >10-fold higher

than in the atmosphere) (He et al. 2010). Exposure to no O_2 combined with elevated CO_2 levels attaining 30% (v/v) of total dissolved gases caused severe necrosis and stunted growth in soybean plants (Boru et al. 2003b). These results indicate that the co-culture technique could especially benefit crop plants with lower tolerance to higher dissolved CO_2 in O_2-deficient media.

The co-cultivation of crops with microalgae or cyanobacteria in hydroponic systems has been studied for several years (Hultberg et al. 2013; Ronga et al. 2019; Huo et al. 2020; Ergun et al. 2020; Supraja et al. 2020) as a new emerging innovative method for biologically stimulated crop growth and productivity. A plant-microalgae consortia relatively increased the pH and dissolved O_2 from microalgae photosynthesis, and significantly improved the removal rate of total dissolved solids, total nitrogen (TN), and total phosphorus (TP) of the nutrient solution (Huo et al. 2020). Aqueous extracts prepared from microalgae collected from a greenhouse hydroponics solution significantly inhibited the growth of *Fusarium oxysporum* on slow nutrient agar (Schwarz and Gross 2015). Conversely, Lettuce fresh weight, shoot/root ratio, water and N uptake were significantly reduced in the presence of the microalgae *Chlamydomonas* spp. and *Scenedesmus* spp., compared to treatments which excluded microalgae (Schwarz and Gross 2015).

Commercial crop co-cultivation with photosynthetic microorganisms may have limiting factors. Co-cultivation will require optimized nutrient solution for the growth of both crops and microalgal or cyanobacterial species. The source and concentration of N can affect the growth and biochemical composition of microalgal and cyanobacterial species (Ronga et al. 2019). Several factors such as nutrient composition and concentration, light intensity, pH and electroconductivity (EC) affect the growth and the chemical composition of microalgae or cyanobacteria in hydroponic systems (Danesi et al. 2002; Colla et al. 2007; Ogbonda et al. 2007). Crop co-cultivation with photosynthetic microorganisms is limited to soft water microalgal or cyanobacterial species, as many crops poorly develop in highly saline sea water (Shrivastava and Kumar 2015). Hydroponic containers made of opaque material can also influence the growth of microalgae or cyanobacteria (Tocquin et al. 2003; Bawiec et al. 2019). In industrial setups, the hydroponic system could be mounted with a compartmentalized PBR containing the photosynthetic microorganism and nutrient solution, and connected to the plant growth tray made of opaque material for optimized root growth, as illustrated in the drip system and nutrient film technique by Lee and Lee (2015). Challenges resulting from pH changes and growth of mold in the reservoir or tubing system can also be overcome by the use of non-recovery drip system. In this system, the hydroponic nutrient solution in the reservoir is delivered to each plant or pot using a pump, with the amount of nutrient solution for each plant adjusted by an electronic timer (Lee and Lee 2015). The nutrient solution is not collected and returned to the reservoir for recirculation through the system, thereby preventing fungal growth and pH changes (Lee and Lee 2015).

The biostimulant effects of microalgae or cyanobacteria in hydroponics may depend on a wide range of factors, including microalgae-excreted metabolites and phytohormones and O_2/CO_2 ratio changes in the root zone (nutrient solution). Analyzing all these factors will allow better exploitation of these microorganisms in developing microalgal and cyanobacterial biotechnology. Furthermore, hydroponic waste solutions contain high nutrients (Lee and Lee 2015). Thus, microalgae and cyanobacteria can be an effective method of removing nutrients from hydroponic waste solution, before they are discharged. The use of microalgae or cyanobacteria will prevent the generation of waste materials and hydroponic waste solution. The resulting biomass can be valorized in agriculture as soil conditioners, biostimulants or biofertilizers.

CONCLUSION

The rapidly growing population and climate change require further innovation to address the practical limitations and serious environmental concerns associated with current agricultural practices. Microalgae and cyanobacteria are targets for next-generation sustainable bioresources. They can be utilized to produce value-added bioproducts, and their rapid growth rates and higher biomass productivity offer several advantages over terrestrial crops. Microalgae and cyanobacteria can also be utilized in agricultural settings as soil conditioners and biostimulants of plant growth and abiotic stress tolerance.

Mass cultivation and commercial production of microalgae and cyanobacteria are highly dependent on the economics of their biomass production. The utilization of waste-substrates for the production of microalgal and cyanobacterial biomass is an economically viable strategy. Conversely, challenges associated with waste generated biomass, such as the presence of unwanted hazardous metals, toxins and pathogens still require thorough studies and field scale evaluation before their commercialization. In-depth studies of the biostimulatory effects of microalgal and

cyanobacterial extracts on plant growth-related parameters will also facilitate their production and processing for product innovation in agriculture. Technological developments in microalgal and cyanobacterial cultivation and harvesting, such as automation, phenotyping, and synthetic biology will provide tools for highly efficient algae-based solutions to meet a range of societal needs. Genetic manipulation, such as the overexpression or downregulation of several genes in a single organism, is also possible in the microalga *Chlamydomonas reinhardtii*, and is a practical method to improve product development of microalgae and cyanobacteria for large scale production.

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V-3. SYNTHÈSE

Les microalgues et les cyanobactéries sont la meilleure cible pour l'innovation des produits agricoles. Ce sont des producteurs primaires qui n'ont pas besoin de terres arables pour leur production. De plus, les microalgues poussent dans des eaux saumâtres et de mer, ce qui est inutilisable pour l'agriculture conventionnelle (Masojídek et Torzillo, 2014). Aussi, le rendement des microalgues en composés de stockage par unité de surface peut largement dépasser celui des cultures agricoles. D'autres méthodes innovantes comme l'agriculture verticale et l'agriculture hors-sol réduisent également la dépendance sur l'agriculture conventionnelle pour la production alimentaire (Healy et Rosenberg, 2013; Muller et al., 2017; Touliatos et al., 2016).

Les microalgues et les cyanobactéries comme ressources alimentaires

Les microalgues et les cyanobactéries sont de bonnes sources de protéines, de glucides, de lipides et de plusieurs métabolites utiles, et pourraient être des alternatives idéales pour les consommateurs modernes qui sont de plus en plus préoccupés par leur santé et le contrôle de leur alimentation. De nombreuses maladies telles que les maladies cardiaques liées à de mauvaises habitudes alimentaires pourraient être réduites par la consommation de microalgues ou de cyanobactéries en raison de leur valeur nutritive car elles sont riches en acides gras polyinsaturé comme l'Oméga 3, en Polysaccharides et en Protéines, vitamines et antioxydants.

Microalgues et cyanobactéries pour une agriculture durable

Les microalgues et les cyanobactéries sont riches en matière organique et leur application sur les sols dégradés pourrait constituer une nouvelle approche pour restaurer la matière organique dans les terres agricoles dégradées. La conversion des écosystèmes naturels pour l'agriculture entraîne l'épuisement des niveaux de carbone organique du sol, libérant 50 à 100 GT de carbone du sol dans l'atmosphère (Lal, 2009, Lemus et al., 2005). La restauration de la matière carbonique dans les sols dégradés peut prendre des années étant donné que les taux d'apport de carbone organique dans le sol sont principalement déterminés par les racines et la litière déposées par les pousses de plantes. Les apports de carbone provenant de la photosynthèse dans les terres agricoles sont plus faibles que le carbone perdu par le sol, par conséquent, les niveaux de carbone organique du sol diminuent avec le temps. Ainsi, l'alimentation du sol avec des produits riches en carbone tels que la biomasse de microalgues et de cyanobactéries pourrait être une méthode importante pour stabiliser la variation nette des niveaux de carbone organique du sol. Les microalgues et les cyanobactéries sont des microorganismes riches en glucides. La teneur totale en glucides et en amidon des microalgues est d'environ 20% et 10% en poids sec respectivement (Cheng et al., 2017; Laurens et al.,

2012). Les espèces de cyanobactéries présentent également des teneurs élevées en glucides, jusqu'à 60% du poids sec (Cheng et al., 2017). D'autres composés organiques produits par les microalgues et les cyanobactéries, y compris les protéines et les lipides, pourraient également contribuer à la matière organique du sol (Ontl et Schulte, 2012), augmentant ainsi les avantages de la biomasse des microalgues et des cyanobactéries pour le sol.

De plus, les microorganismes jouent un rôle vital dans la détermination de la structure et de la fertilité du sol (Singh et al., 2016; Singh Jay Shankar, 2014; Vaishampayan et al., 2001). Les exo polysaccharides libérés pars les microalgues et cyanobactéries agissent comme un agent de collage sur les particules du sol, conduisant à l'agrégation du sol, à l'accumulation de contenu organique et à l'augmentation de la capacité de rétention d'eau de la couche supérieure du sol (Malam Issa et al., 2001). Ce phénomène améliore la santé du sol et par conséquent, la productivité agricole.

Les cyanobactéries et les microalgues peuvent également agir comme biostimulants de la tolérance des plantes aux tresses abiotiques : la productivité des cultures est souvent affectée par divers stress abiotiques généralement provoqués par la sécheresse, la salinisation du sol et les températures basses ou élevées. De nombreuses études ont mis en évidence les propriétés biostimulantes d'extraits de différentes espèces de microalgues et de cyanobactéries sur un large éventail d'activités biologiques chez les plantes supérieures, y compris l'amélioration de l'activité des enzymes anti-oxydants et l'absorption des nutriments comme le K^+, qui atténue les effets négatifs du Na^+ en cas du stress salin (Barone et al., 2018; de Morais et al., 2015; Farid et al., 2019; Garcia-Gonzalez et Sommerfeld, 2016; Renuka et al., 2018; Stadnik et de Freitas, 2014). Les extraits de microalgues et de cyanobactéries agissent aussi comme des biostimulants de la croissance et de la productivité des plantes. Les microalgues et les cyanobactéries produisent des composés actifs chez les plantes supérieures (Chiaiese et al., 2018; de Morais et al., 2015) et ont montré des propriétés biostimulantes sur la croissance et la productivité des cultures agricoles (Rachidi et al., 2020; Renuka et al., 2018). L'augmentation de la croissance des plantes basée sur le poids et la longueur secs des pousses et des racines est attribuée à l'amélioration de la biosynthèse des caroténoïdes et des pigments chlorophylliens. L'augmentation de la densité racinaire chez les plantes traitées par les extraits micros algaux favorise l'absorption des nutriments par une augmentation de la surface d'absorption.

La co-cultivation des plantes et des autotrophes dans les systèmes hydroponiques est une méthode innovante émergente pour la production des cultures. Les microalgues et les cyanobactéries peuvent libérer des exo polysaccharides ou d'autres métabolites qui peuvent déclencher des mécanismes liés à la tolérance, à la productivité et à la vigueur des plantes. La co-cultivation de plantes de tomates avec des microalgues dans les systèmes hydroponiques peut stimuler la performance et la croissance des cultures en améliorant

la solution de culture, attribuée à l'utilisation constante de CO_2 et à l'apport d'oxygène à la solution nutritive hydroponique, grâce à la photosynthèse (Barone et al., 2018 ; Zhang et al., 2017).

V-4. CONCLUSION

Les microalgues et les cyanobactéries sont la meilleure cible en tant que bioressources durables de nouvelle génération pour la production alimentaire et le développement de la production agricole. Il s'agit d'un groupe diversifié d'organismes photosynthétiques unicellulaires ayant le potentiel de relever les défis sous-jacents en matière de sources alimentaires, de croissance et de productivité des cultures. Les microalgues et les cyanobactéries peuvent être utilisées pour produire des bioproduits à forte valeur ajoutée, soit naturellement, soit par manipulation génétique, tandis que leur taux de croissance rapide et leur productivité accrue de la biomasse dans les systèmes ouverts et les photobioréacteurs leur donne plusieurs avantages par rapport à l'agriculture conventionnelle. L'application d'extraits de microalgues et de cyanobactéries peut également améliorer la croissance et le développement des plantes et améliorer la production végétale en raison de leur potentiel à même de : i) stabiliser la variation nette des niveaux de carbone organique du sol et réduire la dégradation des terres agricoles, ii) améliorer la productivité des cultures grâce à une croissance et une tolérance accrue des plantes, iii) favoriser les niveaux optimaux de CO_2/O_2 des racines pour la croissance de plantes spécifiques dans les systèmes de co-culture hydroponiques.

DISCUSSION GENERALE

DISCUSSION GENERALE

Du laboratoire au champs : la physiologie et la biochimie des plantes sont mieux connue que jamais. Ces connaissances accompagnées par diverse méthodes et techniques scientifiques pourrait être exploités pour caractériser les biostimulants et leurs effets sur un large éventail de plantes cultivées. Les biostimulants influencent positivement la croissance ainsi que les activités et l'expression génétique des enzymes opérant dans le métabolisme primaire et secondaire des plantes. De plus, l'application des biostimulants au niveau des plantes entraîne une augmentation de l'absorption des nutriments dans leurs tissus. Pour ces raisons, le développement de nouveaux biostimulants et la compréhension de leurs effets sur la croissance et le développement des plantes est devenu un centre d'intérêt scientifique de l'agriculture durable. Cette étude exploite un ensemble de paramètres physiologiques, biochimiques et moléculaires dans l'étude des extraits de microalgues et cyanobactéries comme biostimulants de la tomate.

Criblage d'extraits de certaines espèces de microalgues et de cyanobactéries

La première partie de ce travail a consistait de faire un criblage d'extraits de certaines espèces de microalgues et de cyanobactéries pour leurs propriétés biostimulantes sur la croissance, la nutrition et le profile métabolique de la tomate. Les extraits dérivés de trois espèces d'eau douce (*Aphanothece* sp., *A. maxima* et *C. pyrenoidosa*), et deux espèces d'eau marine (*Tetraselmis* sp. et *N. gaditana)* ont amélioré la teneur en chlorophylle dans les plantes traitées par rapport au témoin. Nos résultats sont cohérents avec d'autres études qui ont montré l'effet positif des extraits d'algues sur la teneur en chlorophylle chez les plantes traitées. Cette augmentation du chlorophylle a été attribuée à une dégradation réduite de la chlorophylle et à un retard de la sénescence des plantes (Blunden et al., 1996; Calvo et al., 2014). De plus, les extraits de microalgues et de cyanobactéries pourraient avoir plusieurs effets sur les voies photosynthétiques. Par exemple, une étude par Jannin et al., (2013) a montré que les gènes impliqués dans la fixation du carbone, y compris la rubisco et l'anhydrase carbonique ont été sur exprimés dans les plantes traitées avec les extraits d'algues. Apart la teneur en chlorophylle, l'amélioration de l'absorption des nutriments chez les plantes traitées influence fortement l'efficacité photosynthétique et, par conséquent, la production de la biomasse. Selon les études faites par Makino, (2003) et Makino et al., (2000), les niveaux d'azote dans les plantes sont fortement corrélés avec le taux de photosynthèse, tandis que l'amélioration de la photosynthèse est reconnue comme l'un des moyens les plus efficaces d'augmenter l'efficacité de l'utilisation de l'azote et le rendement des plantes agricoles (Guo et al., 2019). D'ailleurs, Zhu et al., (2010) a mentionné que l'augmentation du rendement des cultures dépendent en grande partie de l'amélioration de la photosynthèse. Dans notre étude, la concentration d'azote dans la biomasse racinaire était étroitement associée au poids sec des racines et des tiges et à la chlorophylle b, ce qui indique que l'azote absorbé par les racines était en partie investi dans la machinerie photosynthétique. L'analyse en composantes principales (ACP) a également confirmé que l'amélioration

de la concentration du phosphate et de potassium dans la biomasse racinaire était étroitement corrélée à l'augmentation de la longueur des racines (Chapitre 11-2), ce qui indique que l'amélioration de l'absorption des nutriments observée chez les plantes traitées pourrait également être attribuée à l'augmentation de la taille des racines. Cela s'explique par le fait que l'amélioration de la croissance des racines augmente la surface racinaire et améliore directement l'accessibilité et l'absorption des nutriments.

Le profilage des métabolites à l'aide de la GC–MS a montré divers métabolites lipophiles, notamment des acides gras, des alcanes, des alcènes, des alcools, des terpènes, des tocophérols et des stérols. Une augmentation de l'accumulation d'acide palmitique C16:0 et stéarique C18:0 dans les plantes traitées pourrait indiquer une amélioration de la biosynthèse lipidique. La littérature montre que la première étape de la synthèse lipidique *de novo* chez les plantes commence avec la génération d'acides gras C16 et C18. Ces acides gras sont ensuite allongés en acides gras à très longue chaîne (VLCFAs), et principalement incorporés dans les sphingolipides (éléments structuraux dans les bicouches lipidiques de la membrane cellulaire) et les couches de cire cuticulaires (Dove and Mayes, 2006; Li-Beisson et al., 2013), aidant ainsi les plantes à réduire la perte de l'eau et l'invasion d'agents pathogènes. Les extraits de microalgues et de cyanobactéries ont également amélioré l'accumulation de pyridine-3-carboxamide dans toutes les plantes traitées. La Pyridine-3-carboxamide, également connue sous le nom de Nicotinamide ou de niacinamide, est une forme active amide de vitamine B3 (Surjana et al., 2010). La Pyridine-3-carboxamide est le précurseur primaire d'une coenzyme essentielle dans la production d'ATP connue sous le nom de nicotinamide adénine dinucléotide (NAD^+), et le seul substrat de la poly-ADP-ribose polymérase-1 (PARP-1) (Surjana et al., 2010). Aujourd'hui, la Pyridine-3-carboxamide est exploitée commercialement dans les compléments alimentaires des vitamines et les produits cosmétiques. Dans notre étude, une accumulation plus élevée de métabolites tels que la pyridine-3-carboxamide dans les plantes de tomates traitées indique que les extraits peuvent améliorer la composition biochimique et, par la suite, la qualité nutritionnelle des produits des plantes.

L'effet d'extraits de microalgues et de cyanobactéries sur la croissance et la tolérance de la tomate à la salinité

Dans cet axe, nous avons étudié les effets combinés des extraits bruts de Microalgues-Cyanobactéries sélectionnés par criblage. Ces extraits étaient dérivés de la biomasse combinée de deux espèces de microalgues et de deux espèces de cyanobactéries en tant que formulation biostimulante de la croissance et de la tolérance de la tomate au stress salin (NaCl). De plus, les extraits de microalgues et de cyanobactéries ont montré des effets biostimulants importants sur les plantes soumises au stress salin. Le traitement de la tomate avec ces extraits a amélioré leur croissance végétative, caractérisée par une biomasse de tiges plus élevée et une plus grande surface foliaire totale. Selon l'analyse ACP, l'amélioration de la croissance des plantes était étroitement associée aux pigments photosynthétiques

des feuilles, ce qui était davantage attribuable à l'amélioration de l'ajustement osmotique et de la conductance des stomates grâce à une meilleure absorption et accumulation de K^+ dans les feuilles.

Des concentrations élevées de NaCl déclenchent la dégradation de la chlorophylle par des réactions oxydatives et un faible potentiel osmotique. Un potentiel osmotique plus faible déclenche la fermeture des stomates chez les plantes, entraînant une réduction de la fixation du CO_2 et une augmentation de la photorespiration. D'un côté, l'augmentation de la photorespiration entraîne des effets néfastes des intermédiaires photo-respiratoires tels que le glycolate-2-phosphate sur le fonctionnement des chloroplastes (Huang et al., 2015). Tandis que, la réduction du rapport CO_2/O_2 causé par le stress salin favorise également la production de H_2O_2 dans les peroxysomes due à l'augmentation de la photorespiration. En fait, une étude de Noctor, (2002) a montré que l'augmentation de la photorespiration due à la fermeture des stomates représente plus de 70% de l'H_2O_2 produit à la suite du stress dû à la sécheresse chez les plantes C3. Dans les conditions du stress salin, les plantes assurent l'ajustement osmotique de deux manières principales : i) par la synthèse *de novo* des osmolytes compatibles et par ii) une absorption accrue d'ions inorganiques (Na^+, Cl^- et K^+) (Zhao et al., 2020). Les osmolytes compatibles, y compris la glycine-bétaïne et la proline, jouent un rôle important dans l'ajustement osmotique en compensant la pression osmotique de Na^+ (Acosta-Motos et al., 2017; Chun et al., 2018; Van Zelm et al., 2020; Yan et al., 2013). Dans notre étude, l'accumulation de proline a été amélioré par le traitement avec le MEF. Les plantes traitées ont présenté également une meilleure absorption de l'azote, du phosphore et du potassium. De plus, la peroxydation des lipides foliaires dû au stress oxydatif par les ROS a diminué avec l'amélioration des activités CAT et SOD dans les plantes traitées. D'ailleurs, les plantes traitées ont montré une diminution significative de la teneur en acides gras, indiquant une transformation des acides gras en d'autres formes lipidiques telles que les alcanes, qui sont essentiels dans la synthèse de la cire cuticulaire des plantes soumises au stress hydrique. La cire cuticulaire est synthétisée à partir d'acides gras à très longue chaîne (VLCFAs), qui sont ensuite convertis en acyl-CoAs gras à longue chaîne pour produire de la cire par deux voies; i) la voie de réduction des acyles qui génère des alcools primaires et des esters de cire, et ii) la voie de décarbonatation, qui produit des alcanes, des aldéhydes, des alcools secondaires et des cétones (Xue et al., 2017). La cire cuticulaire est une couche hydrophobe anti-transpiration qui joue un rôle important dans la tolérance des plantes aux stress abiotiques et biotiques, y compris les températures élevées et la salinité (Lewandowska et al., 2020; Wang et al., 2020; Xue et al., 2017).

D'autre part, l'application d'extraits de microalgues-cyanobactéries a amélioré l'absorption de K^+ et a réduit le rapport Na^+/K^+ dans les feuilles, suggérant leur rôle actif dans le rétablissement de l'homéostasie ionique. Ces extraits auraient également pu améliorer la tolérance au sel en agissant comme des osmoprotecteurs sur la rhizosphère, et par conséquent améliorer l'absorption des nutriments par les plantes (en raison de leur composition organique riche en sucres et en acides aminés). Nos résultats ont indiqué que les extraits combinés de microalgues et de cyanobactéries peuvent stimuler la croissance et

la tolérance des plantes dans des conditions de stress de salinité modérée grâce à i) une activité enzymatique antioxydante améliorée et ii) une meilleure absorption des nutriments, notamment K^+.

L'amélioration de l'activité antioxydante peut être un effet direct des molécules de type "signal", tandis que l'augmentation de l'absorption des nutriments et de l'activité photosynthétique sous stress salin peut être causée par l'amélioration du potentiel osmotique et de l'état de l'eau de la plante (si les extraits organiques totaux des microalgues ou des cyanobactéries contiennent des solutés compatibles).

L'effet des polysaccharides microalgaux sur les voies biochimiques de la défense des plantes

Les propriétés biostimulantes des extraits totaux d'algues ont généralement été attribuées à la présence de nombreux ingrédients bioactifs tels que les phytohormones, les composés phénoliques, les molécules organiques d'acides aminés et les métabolites secondaires bioactifs (Di Stasio et al., 2018). Néanmoins, il est difficile de déterminer les composés actifs des extraits totaux et leur mode d'action en raison de leur diversité et de leur complexité. D'autre part, les polysaccharides sont des composés purifiés et beaucoup plus faciles à exploiter. Leurs propriétés biostimulantes étant généralement attribuées aux sucres neutres constitutifs à la teneur en sulfate et au poids moléculaire (Ponce et al., 2003; Qi et al., 2005; Sangha et al., 2010; Zha et al., 2016). Les polysaccharides sont l'un des principaux composants des extraits d'algues totaux qui contribuent aux effets bénéfiques observés des extraits totaux sur les plantes (Di Stasio et al., 2018).

Les plantes déploient un large éventail de mécanismes de défense induits par les éliciteurs des composants de la paroi cellulaire de l'agent pathogène, y compris la chitine, les lipo-polysaccharides, les protéines telles que la flagelline bactérienne et d'autres produits chimiques d'origine naturelle et synthétique. Les microalgues et cyanobactéries pourraient être une source prometteuse pour la production des polysaccharides éliciteurs. Ces polysaccharides perçus par les récepteurs membranaires des plantes peuvent déclencher une série de mécanismes de défense. Dans son étude, (Farid et al., 2019) a montré que les extraits bruts de polysaccharides de microalgues peuvent induire l'immunité innée des plantes, selon la souche de microalgues. Dans son étude, les polysaccharides extraits de *C. vulgaris* et de *C. sorokiniana* ont montré une augmentation significative de l'activité de la β-1,3-glucanase, qui est l'une des protéines liées à la pathogenèse (PR), regroupées dans la famille PR-2 qui décomposent les composants de la paroi cellulaire des agents pathogènes. L'induction des protéines PR est une conséquence de l'activation des voies de défense des plantes, qui limitent l'entrée, ou la propagation ultérieure de l'agent pathogène (Gupta et al., 2013). Dans la même étude, les polysaccharides bruts de *C. sorokiniana* ont montré un effet stimulant significatif sur l'activité de la phénylalanine ammoniac lyase (PAL), indiquant une régulation ascendante de la voie phénylpropanoïde.

Les produits de la voie phénylpropanoïde chez les plantes sont des milliers de composés, qui peuvent se retrouver dans la composition de substances phénoliques telles que les phytoalexines, qui sont des

molécules toxiques contre les agents pathogènes (Fesel and Zuccaro, 2016). Les polysaccharides sulfatés des algues ont également présenté des effets similaires sur l'accumulation de phytoalexines, l'expression de gènes impliqués dans les voies de signalisation de l'acide salicylique (SA) et de l'acide jasmonique (JA) qui induisent la résistance des plantes aux agents pathogènes (Ghannam et al., 2013; Klarzynski et al., 2003). L'activation de ces voies de signalisation conduit à une expression accrue des gènes codant pour les protéines PR, PAL, lipoxygénase (LOX), des enzymes anti-oxydants et d'autres enzymes impliquées dans la synthèse de terpénoïdes et/ou alcaloïdes ayant des activités antimicrobiennes (Vera et al., 2011).

Déchiffrer le mode d'action possible des extraits de microalgues ou de cyanobactéries pourrait être plus facile avec des composés purifiés tels que les exopolysaccharides. Néanmoins, les biostimulants de microalgues ou de cyanobactéries sont généralement appliqués sous forme d'extraits bruts totaux contenant divers composés organiques, y compris les hormones. Ce mélange de composés organiques divers pourrait contenir des molécules imitant des solutés compatibles, des molécules de signalisation ou des éliciteurs ; par conséquent, ils pourraient avoir de multiples effets directs ou indirects sur la croissance, la défense et la tolérance des plantes au stress.

CONCLUSION GENERALE

Cette étude fournit un aperçu général des connaissances actuelles concernant les effets des extraits de microalgues et de cyanobactéries sur la croissance des plantes, les mécanismes de défense et la tolérance au stress. Il donne également une revue de l'application du profilage des métabolites pour étudier les interactions entre les plantes, les biostimulants et les stress abiotiques. La croissance des plantes et la tolérance aux stress abiotiques sont depuis longtemps la quête des producteurs ; diverses méthodes, y compris le génie génétique, les techniques de sélection traditionnelles et modernes ont été développées et appliquées pour améliorer la productivité des cultures. En outre, des stratégies de gestion agronomique, telles que l'irrigation fréquente et le greffage, ont également été conçues et mises en œuvre pour aider les plantes à faire face aux contraintes environnementales défavorables. Cependant, ces approches et programmes agricoles ont des limites et certaines pratiques agricoles entraînent des coûts environnementaux élevés. Par conséquent, l'utilisation de biostimulants est inévitablement nécessaire. L'agriculture a besoin de nouvelles méthodes innovantes et durables pour stimuler la productivité des cultures et la sécurité alimentaire pour la population humaine croissante.

D'autres études sont nécessaires pour comprendre la complexité des relations plante–biostimulant en termes de croissance et de tolérance des plantes. Nos études peuvent contribuer aux efforts continus d'identification et de définition des processus biochimiques et moléculaires multiformes induits par les biostimulants dans les plantes cultivées, améliorant la croissance, la défense et la tolérance des plantes au stress abiotique. L'application d'extraits bruts de microalgues et cyanobactéries peut s'avérer plus

efficace pour stimuler la croissance des plantes comparé à leurs polysaccharides purifiés, car les extraits bruts totaux contiennent plusieurs autres molécules bioactives qui peuvent fonctionner en synergie pour favoriser la croissance des plantes. Par ailleurs, bien que plusieurs molécules bioactives pourraient présenter des effets plus importants, elles peuvent également présenter des interactions antagonistes, ce qui pourrait entraîner moins d'effet sur la croissance des plantes par rapport aux composés purifiés. Ainsi, une étude comparative approfondie des extraits de microalgues totaux et de leurs polysaccharides purifiés est nécessaire pour éclairer cette hypothèse.

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ANNEXES

Supplementary data: Microalgae-cyanobacteria based-biostimulant effect on salinity tolerance mechanisms and tomato plant growth under salt stress

The supplementary Information include Soil salinity dosage (**S1**), Nutrient Solution composition (**S2**), Percentage increase of nutrient uptake (**S3**), Statistical analyses performed using R scripts (**S4**), Some highly detected alkanes (**S5**), all detected fatty acids (**S6**) and all detected alkanes (**S7**).

S1: *Shows the Physical chemical Parameters of the NaCl solution applied to the soil (Peat moss).*

	NaCl (mM)	0	100	150	200
NaCl concentrations applied to the soil	Salinity (ppt)	0	6.1	9.1	12
	E.C (mS)	0.01	10.12	15	19
	TDS gL-1	0.0037	6.97	10.1	13.05
Salinity retained in the soil (Peat moss)	NaCl (mM)	10±1.96	80±3.38	120±1.59	150±4.81
	E.C (mS)	1.39	8.74	12.42	15.64
	TDS gL-1	0.995	5.81	8.26	10.38
	Salinity (ppt)	0.7	5	7.3	9.3

S2: *Plant nutrient solution composition. The nutrient solution was prepared by mixing 100 mL of each of the macronutrient stock solution (A and B) with 50 mL of the micronutrient supplement (solution C) into 4.25 L of distilled water. The pH was adjusted to 6.0 by adding 1M of HCl or KOH.*

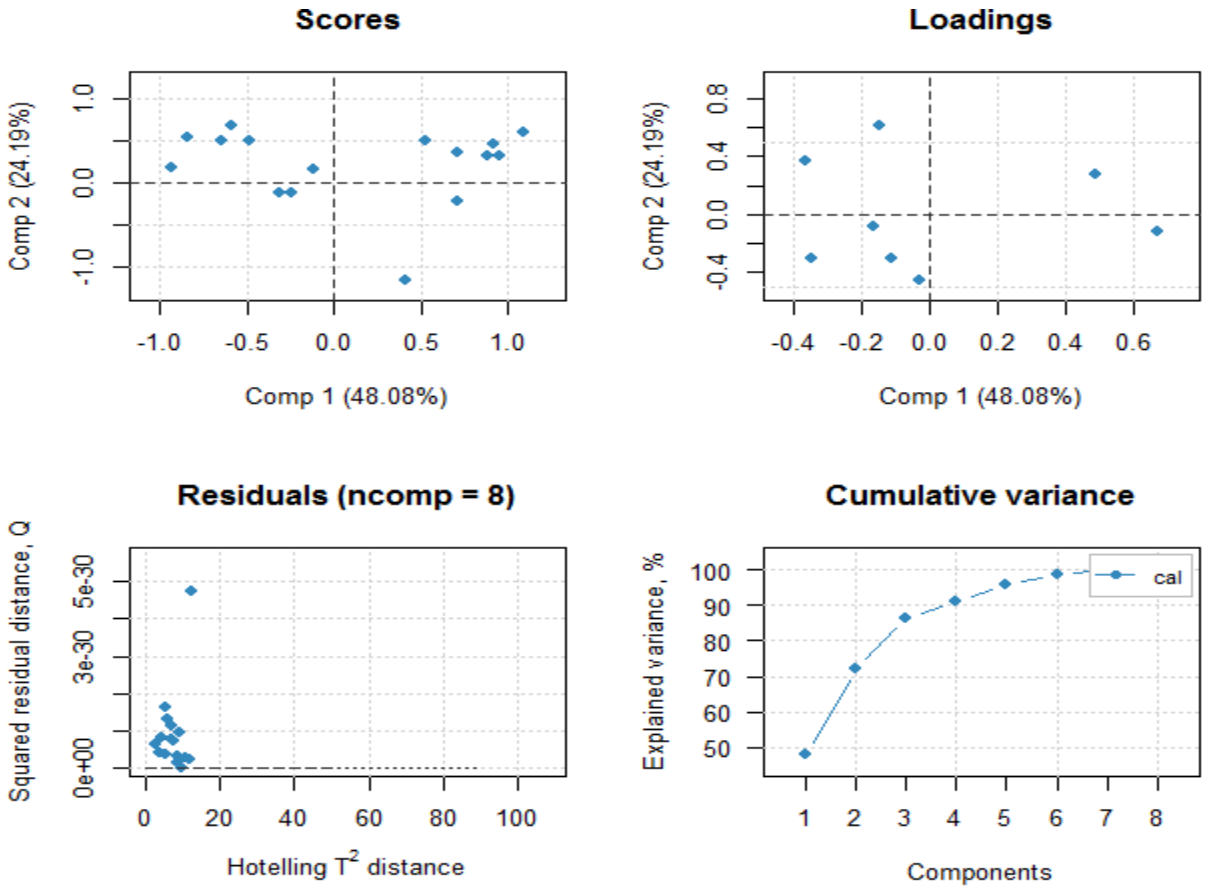
Solution _A		**Solution_B**		**Solution_C**	
Element	**g/L**	**Element**	**g/L**	**Element**	**mg/L**
$Mg(NO_3)_2\ 6H_2O$	4.94	KH_2PO_4	2.67	H_3BO_3	128.8
NH_4NO_3	8.48	K_2HPO_4	1.64	$CuCl_2\_2H_2O$	4.84
KNO_3	2.28	K_2SO_4	6.62	$MnCl_2\_4H_2O$	81.1
		Na_2SO_4	0.6	$(NH4)6\ Mo_7O_{24}\_4H_2O$	0.83
		NaCl,	0.33	$ZnCl_2$	23.45
				Ferric citrate pentahydrate	809.84

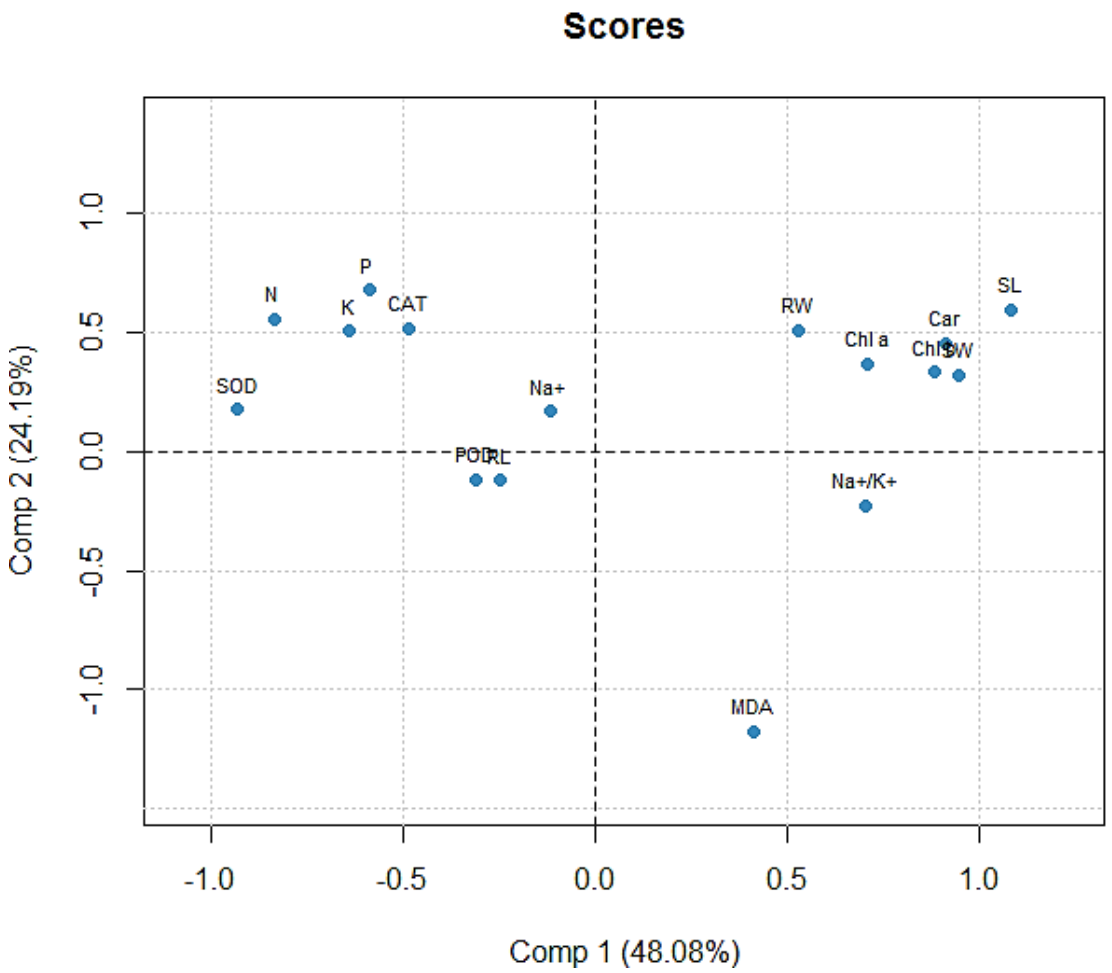
S3: *Leaf concentration of NPK and Ca+ and Na+ Ions in tomato cultures grown in four NaCl concentrations (0mM, 80mM, 120mM and 150mM) and the % increase after treatment with MEF 5%. The values represent the mean standard error of three replicates (n = 3).*

Traitement	**Nitrogen ($mg.g^{-1}$)**	% Increase	**Phosphorus ($mg.g^{-1}$)**	% Increase	**Potassium ($mg.g^{-1}$)**	% Increase	**Sodium ($mg.g^{-1}$)**	% Increase	**Calcium ($mg.g^{-1}$)**	% Increase
Ctl	0.451 ±0.04		0.147 ±0.04		0.074 ±0.01		3.599 ±1.48		3.165 ±0.30	
Ctl. F°5%	0.527 ±0.11	16.91	0.201 ±0.06	36.49	0.314 ±0.11	327.04	3.956 ±2.04	9.916	2.713 ±0.51	-14.29
80mM	0.462 ±0.02		0.178 ±0.06		0.393 ±0.22		6.971 ±2.19		2.26 ±0.68	
80mM.F°5%	1.308 ±0.08	182.95	0.317 ±0.05	78.36	1.041 ±0.20	165.13	8.557 ±2.62	22.744	2.648 ±0.73	17.14

120mM	1.06 ±0.03		0.203 ±0.02		0.568 ±0.13		6.591 ±0.30		2.26 ±0.22	
120mM.F°5%	1.292 ±0.18	21.88	0.304 ±0.06	50	0.902 ±0.51	58.64	3.827 ±1.43	-41.936	2.325 ±0.19	2.86
150mM	1.077 ±0.10		0.244 ±0.03		0.577 ±0.02		2.981 ±0.53		2.583 ±0.11	
150mM.F°5%	0.595 ±0.04	-44.74	0.168 ±0.03	-30.98	0.422 ±0.06	-26.76	2.561 ±0.22	-14.081	2.454 ±0.3	-5

S4*: Generic function for plotting explained variance for data decomposition and Scores of plant growth parameters. Data presents the normalized means of the total replicates for each variable.*




Scores
Comp 2 (24.19%)
Comp 1 (48.08%)
1.0
0.5
0.0
-0.5
-1.0
P
N
K
CAT
SOD
Na+
POD
RL
RW
Chl a
Car
SW
SL
Na+/K+
MDA

S5*: Shows the Profile of common alkanes in 40-day old MEF5%-treated and non-treated tomato plants subjected to different NaCl concentrations under the laboratory conditions. F° indicate plants treated with MEF5% formulation. The values represent the sterol content of homogenized biomass of three biological replicates. Profile of some alkanes in 40-day old tomato plants under normal and soil-salinity conditions. Heat-map represents the normalized mean values of three independent biological replicates of MEF 5% treated plants under 0 mM, 80 mM, 120 mM and 150 mM NaCl as compared with stressed non-treated under the same conditions. Refer to Table S3 for all alkanes detected under different treatments.*

	Concentration µg. g^{-1} FM							
	Ctrl -	**Ctrl.F°**	**80mM**	**80mM.F°**	**120mM**	**120mM.F°**	**150mM**	**150mM.F°**
Hexachloroethane	468.014	220.872	247.828	38.072	4383.188	735.738	61.801	36.165
Hentriacontane	12313.205	3329.658	734.243	453.653	19943.082	3721.634	766.157	344.457
Cyclohexasiloxane, dodecamethyl-	434.133	548.915	602.571	203.816	10226.550	1953.919	125.616	70.337
Cycloheptasiloxane, tetradecamethy-	1161.668	794.695	768.672	432.517	13826.656	2326.803	213.253	654.272
Cyclooctasiloxane, hexadecamethyl-	0.000	1327.599	2147.974	128.789	0.000	2349.560	261.216	885.570
Cyclononasiloxane, octadecamethyl-	735.375	558.165	3874.995	0.000	16832.551	1775.461	135.426	584.622
Total	15112.395	6779.904	8376.282	1256.848	65212.028	12863.115	1563.468	2575.424

S6*: Data of all detected fatty acids in both treated and non-treated tomato plants. Metabolites are expressed in µg/g.*

	Ctrl -	**Ctrl.F°5%**	**80mM**	**80mM.F°5%**	**120mM**	**120mM.F°5%**	**150mM**	**150mM.F°5%**
C6:0	0.000	69.442	0.000	27.463	0.000	0.000	0.000	0.000

C9:0	1253.101	298.487	138.351	49.884	2805.387	556.307	57.290	107.889
C10:0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C12:0	557.414	0.000	0.000	0.000	4088.724	0.000	0.000	0.000
C14:0	3050.472	665.961	440.419	254.272	12626.299	1545.959	261.254	286.115
C16:0	88756.399	21011.373	8451.520	4734.680	364616.616	45808.759	4659.114	5628.807
C16:1	0.000	0.000	0.000	54.534	19340.917	0.000	0.000	0.000
C16:3	8552.267	1680.394	3488.677	2108.289	0.000	0.000	2401.106	2414.350
C17:0	6002.363	1731.938	0.000	195.814	0.000	5544.761	0.000	456.284
C17:3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C18:0	17874.863	4685.496	2089.059	1444.497	71947.898	11340.332	857.361	891.720
C18:1	0.000	0.000	0.000	0.000	2311.055	0.000	0.000	188.036
C18:2	0.000	0.000	0.000	0.000	0.000	3971.558	0.000	0.000
C18:3	48879.291	14515.578	16400.765	10078.791	0.000	10684.092	11398.563	11597.446
C19:0	0.000	0.000	0.000	0.000	0.000	0.000	150.992	0.000
C20:0	4169.906	1363.356	1138.506	143.870	48487.909	5308.078	275.652	296.938
C22:1	0.000	0.000	0.000	0.000	0.000	0.000	184.549	97.171
C22:0	5004.036	883.023	612.817	0.000	9394.557	1305.277	186.525	106.592
C23:0	1630.908	0.000	0.000	0.000	0.000	0.000	80.580	0.000
C24:0	2770.699	727.703	356.809	0.000	10430.802	1254.794	160.164	164.783
C25:0	1483.958	382.394	0.000	0.000	3807.702	623.105	0.000	0.000

C26:0	2012.692	560.915	0.000	0.000	0.000	0.000	0.000	55.931
Hexadecanoic acid, 2-hydroxy-, met	6925.285	2674.997	0.000	0.000	21478.488	3505.174	0.000	0.000
Hexanoic acid, 4-oxo-, methyl ester	256.250	73.621	0.000	13.804	0.000	0.000	0.000	0.000
Methyl 2-hydroxy-tetracosanoate	3215.473	1207.458	321.748	326.233	11642.209	1478.664	204.029	154.850
Nonanoic acid, 9-oxo-, methyl ester	0.000	0.000	0.000	36.073	0.000	0.000	37.706	0.000
2-Hydroxyethyl palmitate,	0.000	0.000	0.000	6376.853	0.000	0.000	0.000	0.000
Butanedioic acid, dimethyl ester	390.486	0.000	0.000	0.000	0.000	0.000	0.000	0.000
17-Octadecynoic acid, methyl ester	0.000	147.523	0.000	0.000	0.000	0.000	0.000	0.000
Total	202395.4	52532.14	33438.67	25845.06	582978.56	92926.86	20914.88	22446.91

S7*: Data of all detected alkanes in both treated and non-treated tomato plants. Metabolites are expressed in µg/g.*

	Ctrl -	**Ctrl.F°5%**	**80mM**	**80mM.F°5%**	**120mM**	**120mM.F°5%**	**150mM**	**150mM.F°5%**
Ethane, hexachloro-	468.014	220.872	247.828	38.072	4383.188	735.738	61.801	36.165
Dodecane	18737.500	18737.500	18737.500	18737.500	18737.500	18737.500	18737.500	18737.500
Hexadecane	0.000	0.000	0.000	0.000	0.000	3260.533	0.000	0.000
Octadecane	0.000	0.000	241.004	0.000	0.000	0.000	0.000	229.534
Eicosane	6127.816	1516.779	699.687	0.000	28776.695	0.000	0.000	429.252
Tricosane	0.000	0.000	449.889	0.000	0.000	0.000	0.000	0.000
Tetracosane	0.000	154.122	0.000	0.000	0.000	2296.696	0.000	0.000
Heptacosane	0.000	0.000	0.000	0.000	0.000	0.000	0.000	26.718

Octacosane	1246.352	158.289	0.000	351.928	0.000	0.000	147.730	173.425
Nonacosane	0.000	0.000	0.000	610.426	0.000	0.000	0.000	0.000
Triacontane	3964.454	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Hentriacontane	8316.903	2232.095	0.000	453.653	0.000	1385.795	413.367	0.000
Hexadecane, 1-iodo-	3845.585	0.000	768.231	605.845	14622.156	0.000	194.343	0.000
Octadecane, 1-chloro-	0.000	0.000	0.000	0.000	2404.043	309.892	0.000	0.000
Nonadecane, 1-chloro-	989.212	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Heptacosane, 1-chloro-	0.000	0.000	0.000	0.000	9346.201	632.846	0.000	0.000
Nonacosane, 2-methyl-	0.000	0.000	0.000	0.000	0.000	848.266	0.000	0.000
Triacontane, 1-bromo-	0.000	717.897	0.000	0.000	0.000	0.000	0.000	0.000
Hentriacontane, 3-methyl-	3996.301	1097.563	734.243	0.000	19943.082	2335.838	352.790	344.457
Dotriacontane, 2-methyl-	0.000	0.000	0.000	0.000	13767.251	0.000	0.000	0.000
Cyclotriacontane	0.000	0.000	128.203	0.000	0.000	0.000	0.000	0.000
Cyclotetrasiloxane, octamethyl-	0.000	38.270	0.000	0.000	302.760	57.254	0.000	0.000
Cyclopentasiloxane, decamethyl	0.000	48.068	0.000	0.000	1368.599	225.425	0.000	0.000
Cyclopentanetridecanoic acid, meth	0.000	0.000	0.000	0.000	14564.684	0.000	0.000	0.000
Cyclohexasiloxane, dodecamethyl-	434.133	548.915	602.571	203.816	10226.550	1953.919	125.616	70.337
Cycloheptasiloxane, tetradecamethy	1161.668	794.695	768.672	432.517	13826.656	2326.803	213.253	654.272
Cyclooctasiloxane, hexadecamethyl-	0.000	1327.599	2147.974	128.789	0.000	2349.560	261.216	885.570
Cyclononasiloxane, octadecamethyl-	735.375	558.165	3874.995	0.000	16832.551	1775.461	135.426	584.622

Cyclodecasiloxane, eicosamethyl-	0.000	0.000	2028.422	0.000	0.000	1814.137	0.000	0.000
Tetracosamethyl-cyclododecasiloxan	0	1441.694	446.0716	0	0	1142.282	73.9855	0
D-Homoandrostane, (5.alpha.,13.alp 134876054482-31-4 95	0	0	101.7957	0	0	0	0	0
6.beta.Bicyclo[4.3.0]nonane, 5.bet	0	259.6868	0	0	0	0	0	0
1,2-15,16-Diepoxyhexadecane	0	3928.261	0	0	0	0	0	0
Heptasiloxane, hexadecamethyl-	0	525.8105	288.5481	0	0	0	0	286.0463
Cyclononasiloxane, octadecamethyl-	0	0	0	0	0	0	135.4259	0
Tetracosamethyl-cyclododecasiloxan	0	451.7046	446.0716	0	0	1142.282	266.1706	0
Silane, trichlorooctadecyl-	0	0	0	0	0	0	0	16.27096
Total	50023.31	34757.99	32711.71	21562.55	169101.92	43330.23	21118.62	22474.17

RESUME

Les microalgues et les cyanobactéries sont une source prometteuse de nouveaux biostimulants due à leur capacité de produire une variété des molécules biologiquement actives. Cette étude met en évidence l'effet des extraits des microalgues et des cyanobactéries sur les voies moléculaires, métaboliques et biochimiques liées à la croissance et la tolérance de la tomate (*Solanum lycopersicum* L.). Le traitement de la tomate avec les extraits des microalgues et des cyanobactéries a significativement amélioré la teneur en chlorophylle dans les feuilles, le poids sec (aérienne et racinaire), la croissance racinaire et l'absorption racinaire des nutriments (azote, phosphore et potassium), par rapport aux plantes non traitées. Les plantes traitées ont démontré une accumulation plus élevée des acides gras C16 et C18 (indiquant une stimulation de la synthèse lipidique de novo), et d'autres métabolites lipophiles essentiels dans la biosynthèse des vitamines (la pyridine-3-carboxamide), et des hormones (l'acide linolénique, le précurseur clé dans la voie de la biosynthèse des jasmonates). Les extraits des microalgues et des cyanobactéries ont stimulé la croissance et la tolérance de la tomate dans les conditions du stress salin modéré par l'amélioration de l'activité des enzymes antioxydantes et l'absorption des nutriments, notamment le K+, dont l'accumulation foliaire a induit l'augmentation du rapport K+/Na+ cytosolique. Les extraits ont également augmenté l'efficacité de l'absorption du P par la stimulation de la croissance racinaire. Une faible expression des gènes codant les protéines transporteurs du phosphate a été détectée dans les racines des plantes traitées soumises à l'engrais TSP (0,3 mM et 0,6 mM de P).

Mots clés : Microalgues, cyanobactéries, biostimulants, *Solanum lycopersicum* L, croissance, stress abiotique.

ABSTRACT

Microalgae and cyanobacteria are a promising source of new biostimulants, due to their ability to produce a variety of biologically active molecules. This study investigates the biostimulant properties of microalgae and cyanobacteria extracts as enhancers of molecular, metabolic and biochemical mechanisms related to the growth and stress tolerance of tomato plants (*Solanum lycopersicum* L.). Treatment of tomato plants with microalgae and cyanobacteria extracts significantly improved chlorophyll concentrations in leaves, as well as the root length, nutrient uptake (nitrogen, phosphorus and potassium) and shoot and root dry weights of treated plants compared with the control. Treated plants accumulated higher levels of C16 and C18 fatty acids (indicating improved de novo lipid synthesis), and of other lipophilic metabolites essential in the biosynthesis of vitamins (pyridine-3-carboxamide), and hormones (linolenic acid, a key precursor in the biosynthetic pathway of jasmonates). Microalgae and cyanobacteria extracts also enhanced antioxidant enzyme activities and NPK absorption in plants subjected to low and moderate salinity. Enhanced K+ uptake and leaf accumulation in treated plants improved their cytosolic K+/Na+ ratio and tolerance to low and moderate salinity. Microalgae and cyanobacteria extracts also enhanced P-absorption efficiency by stimulating root growth, resulting in increased root surface area for P absorption. Low level expression of genes encoding phosphate transporter proteins in roots was also detected in treated plants subjected to TSP fertilizer (0.3 mM and 0.6 mM P).

Key words: Microalgae, cyanobacteria, biostimulants, *Solanum lycopersicum* L, plant growth, abiotic stress