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Biostimulating effects of silicon on the tolerance of fenugreek (*Trigonella foenum-graecum* L.) to salt stress under biological nitrogen fixation: Agrophysiological and biochemical responses

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Abstract

Although, aromatic and medicinal plants have a crucial role in the world's health, but their growth and production are drastically affected by various abiotic stresses such as salinity and water deficit. In this context, the present work aimed at improving the tolerance of fenugreek (*Trigonella foenum graecum* L.) and its rhizobial symbiosis to salt stress by using exogenous silicon (Si). The experiments were carried out at different plant development stages. Results showed that salt stress reduced seed germination and embryo viability and altered seed reserve mobilization. The reduction in seed germination was significantly correlated with the induction of oxidative stress, reflected by high levels of malonyldialdehyde (MDA) and hydrogen peroxide (H_2O_2). However, Si supply induced a significant accumulation of compatible osmolytes and improved seed germination, embryo viability and seed reserve mobilization process. Si also triggered high activity of both enzymatic and non-enzymatic antioxidant systems, reducing as a result MDA and H_2O_2 contents in seedling. During vegetative stage, salinity also reduced plant growth and nodulation. This reduction was accompanied with a significant decrease in leaf chlorophyll content, relative water content, nutrient uptake and photosynthesis machinery in terms of the activity of photosystems. However, the exogenous supply of Si resulted in a considerable accumulation of some compatible solutes together with enhanced antioxidant systems, reflected by a significant accumulation of polyphenols and flavonoids and a high activity of antioxidant enzymes, such as superoxide dismutase, catalase, polyphenol oxidase and guaiacol peroxidase. These latter improved leaf relative water content and reduced oxidative stress and therefore restored growth and photosynthetic activity in salt-stressed fenugreek plants. Similarly, under field conditions, the obtained results showed that seed priming with Si significantly improved seed germination, plant biomass and yield related parameters, such as number of pods per plant, hundred seed weight, and harvest index, in salt stressed fenugreek. More interestingly, Si priming not only enhances plant biomass and yield parameters but also was able to improve the nutritional quality and antioxidant activity of harvested seeds under salt stress. Taken together, treatment with exogenous Si could be a promising and ecofriendly technic to alleviate the drastic effects of salinity on germination, growth and yield of fenugreek.

Key words: Salinity, Fenugreek, Symbiosis, Silicon, Germination, Antioxidant systems, Growth, Photosystems, Field conditions, Priming, Harvest index.

Résumé

Bien que les plantes aromatiques et médicinales jouent un rôle important dans la santé mondiale, leur croissance et leur production sont fortement affectées par divers stress abiotiques tels que la salinité et le déficit hydrique. Dans ce contexte, ce travail de thèse vise à améliorer la tolérance du fenugrec (*Trigonella foenum graecum* L.) et sa symbiose rhizobienne au stress salin *via* l'apport exogène du silicium (Si). Les expériences ont été menées à différents stades de développement, notamment la germination, la croissance et le rendement grainier. Les résultats ont montré que le stress salin a réduit la germination et la viabilité des embryons et a perturbé la mobilisation des réserves embryonnaires. La réduction de la germination a été corrélée à une induction significative du stress oxydatif, reflétée par des niveaux élevés en malonyldialdéhyde (MDA) et en peroxyde d'hydrogène (H_2O_2). Cependant, l'application du Si a induit une accumulation importante des solutés compatibles et a amélioré la germination des graines, la viabilité des embryons et la mobilisation des réserves embryonnaires. Le Si a également induit une activité élevée des systèmes antioxydants enzymatiques et non enzymatiques, réduisant ainsi les teneurs en MDA et en H_2O_2. Au stade plante, la salinité a également réduit la croissance des plantes et la nodulation. Cette réduction a été accompagnée d'une diminution significative de la teneur des feuilles en chlorophylle, de la teneur relative en eau ainsi que l'efficacité photosynthétique des photosystèmes. Cependant, l'apport exogène de Si a significativement induit une accumulation des solutés compatibles et une accumulation des polyphénols totaux et de flavonoïdes et une augmenation des activités des enzymes antioxydantes. Ces réponses ont augmenté la teneur relative en eau des feuilles, réduit le stress oxydatif et rétabli ainsi la croissance et l'activité photosynthétique des plantes stressées de fenugrec. De même, sous conditions naturelles, les résultats obtenus ont montré que le prétraitement des graines avec de Si a amélioré considérablement la germination des graines, la biomasse végétale et le rendement, notamment, le poids de 100 graines, sous conditions du stress. Le pretratiement germiniatif des graines avec du Si a amelioré aussi la qualité nutritionnelle et le pouvoir antioxydant des graines récoltées sous conditions du sel. Ainsi, le traitement avec le Si pourrait être une technique écologique prometteuse pour atténuer les effets drastiques de la salinité sur la germination, la croissance et le rendement du fenugrec.

Mot clés : Salinité, Fenugrec, Symbiose, Silicium, Germination, Systèmes antioxydants, Croissance, Photosynthèse, Conditions du champ, Prétraitement des graines.

ملخص

على الرغم من أن النباتات العطرية والطبية لها دور مهم على الصحة، إلا أن نموها وإنتاجها يتأثران بشكل كبير بالعديد من الضغوطات البيئية مثل الجفاف ونقص الاسمدة والملوحة. في هذا السياق، يهدف محتوى هده الاطروحة إلى تعزيز تحمل وإنتاج الحلبة (*Trigonella foenum graecum* L.) وتكافلها الريزوبي للملوحة عن طريق إضافة السليسيوم إلى الوسط. التجارب تمت في ظروف جد محكمة وخلال مختلف مراحل النمو، بما في ذلك إنبات البذور ونموها وإنتاجيتها. أظهرت النتائج المحصل عليها ان الملوحة قد أثرت سلبا على انبات البذور، قابلية الجنين للحياة وقدرته على استهلاك المخزون الطاقي. التأثير السلبي للملوحة على إنبات البذور كان مرفقا بأكسدة الأغشية ناتجة عن إنتاج كميات كبيرة من مالونديالديهيد والماء الأوكسيجيني. على خلاف ذلك، إضافة السليسيوم في الوسط أدى إلى إنتاج كميات كبيرة من الجزيئات المتوافقة مثلا البرولين مما أدى الى تحسين إنبات البذور، قابلية الجنين للحياة وقدرته على استهلاك المخزون الطاقي. إضافة السليسيوم أدت كذلك الى تحسين نشاط بعض الأنظمة الأنزيمية وغير الأنزمية مما ادى إلى تحسين جودة الأغشية والنقص من إنتاج الماء الأوكسيجيني. أثناء نموالنبات، أدت الملوحة أيضا إلى إنخفاظ مهم في نموالنبات وتكون العقد للحلبة. ترافق هذا الإنخفاض مع إنخفاض كبيرفي محتوى الأوراق من اليخضور، المحتوى النسبي للماء، إمتصاص العناصر الغذائية والتركيب الضوئي من حيث تقليل النشاط النظامي الضوئي. في حين أن إضافة السليسيوم إلى الوسط أدت إلى تراكم كبير لبعض المواد المذابة المتوافقة مع مسارات مضادات الأكسدة، والتي انعكست من خلال التراكم الكبير للبوليفينول والفلافونويد ونشاط بعض الإنزيمات المرتفع، مثل السيبر أوكسيد ديسموتاز، الكطلاز، بوليفينول أوكسيداز وغاياكول بيروكسيداز، مما أدى هذا الأخير إلى تحسين المحتوى المائي النسبي للأوراق، وتقليل التأثير التأكسدي، وبالتالي استعادة النمو والتركيب الضوئي لنباتات الحلبة المتأثرة بالملوحة. بالمثل، في ظل الظروف الحقلية، أظهرت النتائج المتحصل عليها أن تحضير بذور الحلبة بمادة السليسيوم قد أدى إلى تحسين إنبات البذور والكتلة الحيوية للنبات والمعايير المتعلقة بالإنتاجية، مثل وزن مائة بذرة ودليل الحصاد للحلبة تحت تأثير الملوحة. علاوة على ذالك، معالجة البذور بسليسيوم لم تحسن من نمو ومردودية الحلبة فحسب، بل كانت أيضًا قادرة على تعزيز الجودة الغذائية والنشاط المضاد للأكسدة للبذور المحصودة في البيئات المتأثرة بالملوحة. نظرا لنتائج المحصل عليها، يمكن أن تكون المعالجة بالسليسيوم تقنية مشجعة وصديقة للبيئة للتخفيف من تأثير الملوحة على إنبات البذور، ونمو وإنتاجية الحلبة.

كلمات المفتاح: الملوحة، الحلبة، التكافل، السليسيوم، الإنبات، أنظمة مضادات الأكسدة، النمو، التركيب الضوئي، الظروف الميدانية، معالجة البذور.

Foreword

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Thesis title: Biostimulating effects of silicon on the tolerance of fenugreek (*Trigonella foenum-graecum* L.) to salt stress under biological nitrogen fixation: Agrophysiological and biochemical responses

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A) Publications

1- **Nadia Lamsaadi,** Ahmed El Moukhtari, Aziz Oubenali, Mohamed Farissi (2022). Exogenous silicon improves salt tolerance of Fenugreek (*Trigonella foenum-graecum* L.) during seed germination and early seedling stages. *Biologia*. https://doi.org/10.1007/s11756-022-01035-5

2- **Nadia Lamsaadi**, Ahmed El Moukhtari, Ziati Irouane, Mohammed Mouradi, Majida El Hassni, Cherki Ghoulam, Mohamed Farissi (2022). Beneficial role of exogenous silicon on yield, antioxidant systems, osmoregulation and oxidative stress in fenugreek (*Trigonella foenum-graecum* L.) under salinity stress. *Silicon*. https://doi.org/10.1007/s12633-022-020346

3- **Lamsaadi Nadia**, Hidri Rabaa, Zorrig Walid, El Moukhtari Ahmed, Debez Ahmed, Savouré Arnould, Abdelly Chedly, Farissi Mohamed. Exogenous silicon alleviates salinity stress in fenugreek (*Trigonella foenum graecum* L.) by enhancing photosystem activities, biological nitrogen fixation and antioxidant defence system. Under review (*Soil Science and Plant Nutrition*).

4- **Nadia Lamsaadi** et al. Silicon seed priming mitigated salinity stress effects and improved fenugreek (*Trigonella foenum-graecum* L.) biomass and yield under field conditions. *In progess*.

5- **Nadia Lamsaadi** et al. New approaches to improve the tolerance of aromatic and medicinal plants under salt stressed conditions. *In progress*.

B) Oral communications

1- **LAMSAADI Nadia,** El MOUKHTARI Ahmed, ZIATI Irouane, FARISSI Mohamed. Bio-stimulant activity of silicon in alleviating salt stress effects on Fenugreek germination, growth and production. Second International Symposium on Medicinal and Aromatic Plants Valorization. Abdelmalek Essaâdi University, Tetouan, Morocco. 11- 13th May 2022.

2- **LAMSAADI Nadia,** ELAASSALI Saad, EL MOUKHTARI Ahmed, CHERKI Ghoulam, FARISSI Mohamed. Exogenous silicon application improves fenugreek (*Trigonella foenum-graecum* L.) tolerance to low phosphorus availability. 2nd International Conference: Strategies toward Green Deal Implementation, Water, Raw Materials & Energy. Online Conference. Poland. 8-10th December 2021.

3- **LAMSAADI Nadia**, EL MOUKHTARI Ahmed, OUBENALI Aziz, FARISSI Mohamed. Exogenous silicon application, as inorganic biostimulant, to improve salt tolerance in fenugreek (*Trigonella foenum-graecum* L.). First international congress onvSustainable Agriculture: Tools and Innovations ‘‘AgriNov2021’’. Faculty of Sciences and Technics, Sultan Moulay Slimane University. Beni Mellal, Morocco. 27-30th October 2021.

4- **LAMSAADI Nadia,** EL MOUKHTARI Ahmed, OUBNALI Aziz, MOURADI Mohamed. GHOULAM Cherki. FARISSI Mohamed. Exogenous silicon improves fenugreek (*Trigonella foenum-graecum* L.) seed germination under salt stress. Second International E-conference on Climate Nexus Perspectives (I2CNP): toward innovative, resilient and sustainable solutions for natural resources and biodiversity management. Higher School of Technology Khenifra, Sultan Moulay Slimane University Morocco (in partnership with the University of Ottawa Canada), June 05th, 2021.

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General introduction

Worldwide, people have a long history of the use of traditional aromatic and medicinal plants (AMPs) for medical and therapeutical purposes and nowadays, this is highly actively promoted (Chaachouay et al. 2019). Indeed, AMPs are widely used in several fields, particularly in pharmacology and traditional medicine to prevent and improve human well-being. The World Health Organization (WHO 2002) estimated that 70-80 % of people worldwide use traditional herbal medicines to meet their primary health care needs. In this context, FAO (2002) noted that more than 50 000 AMP species are cultivated for their active compounds, which are used for different purposes such as prevention of chronic diseases like cancer, diabetes and coronary heart disease (Saxena et al. 2013). In addition, based on their bioactive and antioxidant compounds, Maggi and Benelli (2018) indicated that the essential oils from many AMPs are used as antivirals, antibacterials, insecticides and antifungals for plant protection and they are also used against herbivores by reducing their appetite for some plants. Therefore, AMPs have crucial advantages in both healthy and agricultural fields.

Fenugreek (*Trigonella foenum graecum* L.), is an oldest AMP, where its ancient use is showed in traditional medicine of India, Southern China, Ethiopia and the Middle East (Snehlata and Payal 2012). In fact, following its chemical components, fenugreek is used for prevention and treatment of several disease, like diabetes (Mahjoub et al. 2018), cancer (Alrumaihi et al. 2021), rheumatism and sore muscles (Subhashini et al. 2011). Similarly, it was showed that fenugreek has also demonstrated anti-inflammatory and hepatoprotective properties (Srinivasa and Naidu 2021). Among the bioactive molecules of seeds, trigonelline is one of the valuable alkaloids in fenugreek seeds, which is known for its therapeutic potential for diabetes and central nervous system diseases (Naghdi Badi et al. 2018). Additionally, the presence of phenolic acids, fibers, saponins and proteins in different plant parts (leaves, seeds and roots), fenugreek extracts showed a powerful antioxidant and it acts as a mucus solvent and throat cleanser, which also eases the urge to cough (Snehlata and Payal 2012). On the other hand, this medicinal plant species is a legume, belonging to the Fabaceae family. Thus, it cultivation consitutes an important source of bioactive molecules and improves the soil nitrogen content through its symbiosis with rhizobia as leguminous species. In this way, Singh et al. (2008) estimated that fenugreek rhizobial symbiosis could fix 48% of its total nitrogen during growing season. Hence, the importance of fenugreek needs other studies to improve and preserve the richness of its genetic heritage recently threatened by several factors of genetic erosion such as prolonged periods of drought, salinity and soil nutrient deficiency.

In fact, salt stress has been reported as one of the serious environmental problems, limiting agricultural production all over the world. Currently, it was reported that, about 1125 Million hectares of lands are salt-affected, with 1.5 Million hectares that become unsuitable for agricultural production every year (Sanower 2019). Thus, salinity becomes a serious obstacle for plants production, including AMPs. Indeed, salinity has devastating impacts on AMPs at different growth stages, from germination to fruiting. For example, it was reported that salinity has a depressive effet on seed germination and seedling growth of fenugreek (Hojjat and Kamyab 2017). The same results were reported in many AMPs like *Thymus daenensis*, *Thymus kotschyanus* and *Origanum compactum* (Khoshsokhan et al. 2012; Laghmouchi et al. 2017). Indeed, during seed germination, salinity inhibits hydrolytic enzyme activities and reduces seed reserve mobilization affecting as a result seed germination (Dash and Panda 2000; Sebei et al. 2007). Likewise, salinity has also reported to reduce plant height, number of branches, fresh and dry flower, root fresh and dry weight and root moisture content in many AMPs such as *Lavandula angustifolia* Mill. (Chrysargyris et al. 2018a), *Matricaria chamomila* (Razmjoo et al. 2008) and *Ocimum basilicum* L. (Heidari 2012). Reducing photosynthesis, nutrient uptake and flowering process have also been reported under salt stress in several AMPs species like *Salvia officinalis* L. (Siler et al. 2007), *Matricaria recutita* L. (Dadkhah 2010) and *Ocimum basilicum* L. (Said-Al Ahl and Mahmoud 2010). These negative effects of salt stress could be explained by the fact that salt stress affects cell integrity and ions homeostasis, which are disrupted by the accumulation of sodium (Na^+) and chloride (Cl^-) ions in plant cells (Hanana et al. 2011; Mbarki et al. 2018). Thus, salinity is a serious obstacle for AMPs production, affecting as a result several worldwide research field, such as health and agriculture, where the use of AMPs has a great interest. Therefore, finding eco-friendly and effective strategies to alleviate the drastic effects of this environmental problem is a priority. In this context, to minimize the negative effects of salinity on plant crop production worldwide, a set of strategies and approaches are investigated recently. One of these strategies focused on the conventional breeding techniques for developing salt tolerant verities, but due to its multigenic traits, this approach has not been successful (İbrahimova et al. 2021). The direct application of exogenous substances or the pretreatment of seeds with different biostimulant compounds, which is known as seed priming, constitutes promising technics to mitigate the salinity effects on plants (Molassiotis et al. 2010; Rizwan et al. 2015; Mahmoudi et al. 2019; El-Serafy et al. 2021; El Moukhtari et al. 2021b).

The beneficial effect of seed priming in the tolerance of plant to salt stress was the subject and the aim of many studies (Cayuela et al. 1996; Iqbal et al. 2006; Caparrotta et al. 2018). In this way, Johnson and Puthur (2021) reported that seed priming induces different defence mechanisms in seeds/seedlings to enhance their tolerance against salinity stress.

Silicon (Si), as the second most abundant element in the earth's crust after oxygen, has often been described for its protective effects against many abiotic stresses (Feng Ma 2004; Debona et al. 2017; Coskun et al. 2019). In fact, under salt stress Si showed most beneficial effects on seed germination, photosynthesis, osmotic adjustment, membrane stability, oxidative stress detoxification and biological nitrogen fixation (Etesami and Adl 2020; El Moukhtari et al. 2021b; Putra et al. 2021). However, in case of AMPs, the effect of exogenous Si application and Si seed priming have not yet received more attention, especially in salt stress tolerance of fenugreek. For example, the mechanisms by which Si mitigate the drastic effects of salinity have not yet detailled, such as its effect on photosynthetic machinery, in terms of photosystem performance, electron transporter rate and energy conversion in two photosystems and the regulation of energy lost during two photosystem functioning in salt-stressed fenugreek, has not been investigated and detailed so far. Furthermore, the effect of seed priming with Si on fenugreek seed germination and yield is unsufficiently studied, especially, under salt-stressed conditions.

The objective of the present thesis is to evaluate the impact of the exogenous supply of Si in improving the tolerance of fenugreek to salt stress. The emphasis was put on the effect of Si on different physiological processes, such as seed reserve mobilization and embryo viability, mineral nutrition, osmoregulation, oxidative stress attenuation, water statuse, biological nitrogen fixation and photosynthesis machinery, in terms of energy conversion and regulation, maximal quantum yield, and electron transporter rate in two photosystems (I and II). Besides, the effect of seed priming with different Si concentrations on germination parameters, embryo viability, plant biomass, yield related parameters and quality of harvested seeds in salt stressed fenugreek was also evaluated under field conditions.

Chapter 1: Review of literature

I. Aromatic and medicinal plants

I.1 Generality

Aromatic and medicinal plants (AMPs) are among the major and crucial group of crops worldwide, supplying a raw materials for the cosmetic and pharmaceutical industries. They have been used for traditional prevention, and diseases treatment and using herbal medicines have a long history (Aghaei and Komatsu 2013). In this context, it was reported that the oldest written evidence of medicinal plants' usage for the preparation of drugs has been found on a Sumerian clay slab from Nagpur, approximately 5000 years old (Petrovska 2012). In fact, based on the World Health Organization (WHO 2002), 70-80 % of people worldwide use traditional herbal medicines to meet their primary health care needs. It was estimated that more than 50 000 AMP species are used for medicinal purposes (FAO 2002). Indeed, most of these plants are used and cultivated for their active compounds, which are used for different purposes such as prevention of chronic diseases like cancer, diabetes and coronary heart disease (Saxena et al. 2013). In addition, their essential oils are often used as an antivirals, antibacterials, insecticides and antifungals for plant protection and they are also used against herbivores by reducing their appetite for some plants (Maggi and Benelli 2018).

According to the WHO, a medicinal plant is a plant containing one or more substances which can be used for therapeutic purposes, or which are precursors of chemo-pharmaceutical technic (Öztekin and Martinov 2007). While, aromatic plant is a plant containing enough of aromatic molecules in one or more producing organs, such as leaves, flowers, stems, fruits, bark, or roots (Neffati and Sghaier 2014).

Several AMPs possessed a wide range of medicinal and therapeutic effects, including antibacterial, antifungal, antiviral, antiparasitic and anticancer, explaining their importance in worldwide healthy (Al-Snafi 2018). Indeed, the use of essential oils and their components is known since long in traditional medicine and aromatherapy for the treatment of various diseases, and is further increased in the recent times. More interestingly, the essential oils from many AMPs, such as *Nigella sativa*, *Acorus gramineus*, *Lavandula angustifolia*, *Eucalyptus globulus*, *Mentha piperita*, *Rosmarinus officinalis* and *Jasminum sambac*, have been reported to have many neuroprotective effects (Ayaz et al. 2017). Dermatological effects also documented in many AMP species like *Agrimonia eupatoria* (Al-Snafi 2015b), *Aloe vera* (Al-Snafi 2015a) and *Calendula officinalis* (Preethi and Kuttan 2009). According to Al-Snafi (2015b), the phytochemical analysis of *Agrimonia eupatoria* revealed that this medicinal plant contain many bioactive chemical groups like glycosides, tannins, terpenoids, phenolic compounds, agrimony, agrimony lactone and oils, which gives it many therapeutically properties such as antibacterial, antiviral, antitumor, analgesic, antioxidant, immunomodulatory, antidiabetic, gastrointestinal, hepatoprotective and many other pharmacological effects. Likewise, Nakisa and Rahbardar (2022) suggested that most of the healing properties of rosemary might be attributed to their antinociceptive, antioxidant and anti-inflammatory effects. On the other hand, there are some medicinal plants, like *Orthosiphon aristatus* and *Momordica charantia* believed to treat diabetes, as they have been proven in research studies to possess antidiabetic properties, such as improved insulin sensitivity and hypoglycemic activities, due to their high level of phenolic compounds, flavonoids, terpenoids, alkaloids and glycosides (Salleh et al. 2021; Arifah et al. 2022). More recently, Yang et al. (2020) reported that some AMPs have been used to treat COVID19. Additionally, following their richness in volatile and aromatic compounds, essential oils play a vital role in plants protection and propagation by acting as an antimicrobials and attractive agents for insects, aiding in pollination process (Khanahmadi et al. 2021).

I.2 Aromatic and medicinal plants in Morocco

Based on its particular geographical situation, Morocco benefits from a diverse bio-climate, allowing rich and diversified vegetation, when more than 4200 vascular species and subspecies are listed, including a hundred endemics (Zrira 2017). Among these 4200 vascular plant species, 382 (9 %) of the total Moroccan flora are used or exported for medicinal and aromatic purposes (Aafi et al. 2009). Almost 100 species are used in the form of dried herbs for food herb trade, while more than 20 species are used for the production of essential oils or other aromatic extracts intended primarily for the perfumery and cosmetic industry and for the preparation of hygienic products and the formulation of flavours (Zrira 2017).

In fact, it was documented that Morocco is ranked among 12th world AMPs's exporter (Lange 2006; Table 1). The main destinations for Moroccan exports of aromatic and medicinal plants are the European Union market (Figure 1). However, opening up to other destinations such as Japan and Switzerland has most intention in recent years (Elachouri 2018). In this context, more than 50% of these exports concern the food sector (carob, spices, aromas), whereas 35% are intended for perfumery and cosmetics, and 5% are used for their medicinal properties (Maazouz 2021).

Table 1 : Major 12 AMPs exporting countries in the world (Lange 2006)

Period 1991-2003		**Period 2004-2008**	
Exporters	**Quantity (tons)**	**Exporters**	**Quantity (tons)**
China	150600	China	204082
Hong Kong	55000	India	47551
India	40400	Mexico	38262
Mexico	37600	Egypt	24600
Germany	15100	Germany	18076
USA	13050	Poland	15775
Egypt	11800	Hong Kong	15011
Bulgaria	10300	USA	13919
Chile	9850	Chile	11627
Morocco	8500	Morocco	10796
Albania	8050	Bulgaria	9338
Singapore	7950	Albania	7770

Besides, in this Mediterranean country, the economic analysis shows that the exported quantities are average of 6805 tons of essential oils and various extracts, for a value of 198 Million Dirhams, while Moroccan importation represent only 1523 tons for a value of 31 Million Dirhams (Aafi et al. 2009). In addition to essential oils, the AMPs sector experiences an export of around one hundred tons of dry grass, for a total value of around 90 Million Dirhams and provides the local population with around 500,000 working days (Aafi et al. 2009). Therefore, in addition to their medicinal and aromatic importance, AMPs have both environmental and economic advantages. In this way, it was reported that around twenty operators located in all regions of Morocco, carry out the operation and packaging of AMPs. Some are in contact with external markets for the sale of their product, while others play the role of intermediaries between the users who carry out the collection, and the processing units (Aafi et al. 2009). Recently, Morocco enhances the valorization process of many AMPs and realizes, each year, important productions, allowing it to occupy a significant place on the international market.

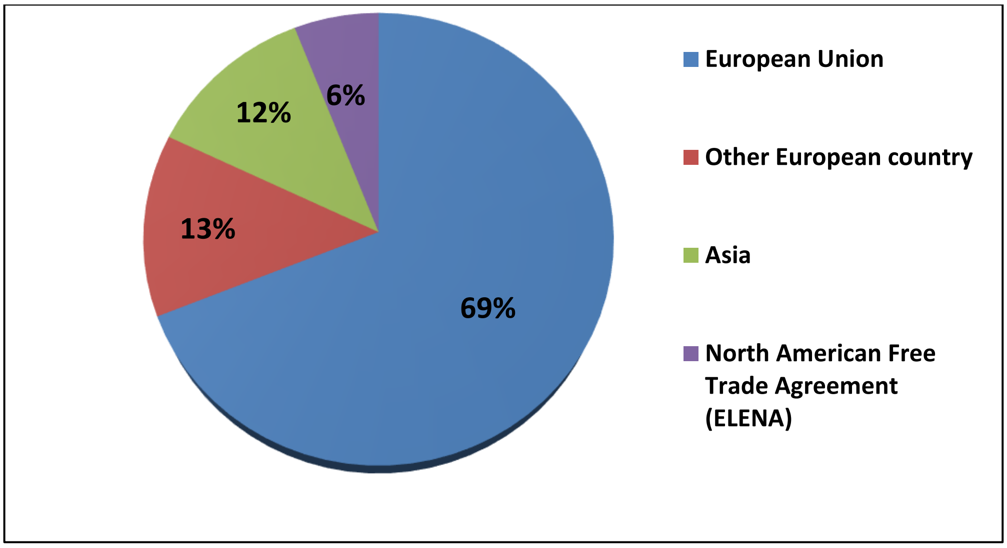

Figure 1 : Important Moroccan AMPs export markets (DEF 2016)

Eddouks et al. (2007) reported that between 50 to 80% of the Moroccan population uses AMPs to treat various diseases and infections, such as digestive disorders, urinary system disorders, cardiovascular diseases and endocrinological disorders. For example, Orch et al. (2015) showed that, in the region of Izarène (northern Morocco), 40 AMPs are traditionally used against diabetes and 30 against hypertension. Of the 40 species used in the treatment of diabetes, 13 are also used to treat high blood pressure. These are: *Ajuga iva*, *Centaurium erythraea*, *Origanum compactum*, *Rosmarinus officinalis*, *Nigella sativa*, *Pistacia lentiscus*, *Tetraclinis articulata*, *Arbutus unedo*, *Trigonella foenum-graecum*, *Lavandula dentata*, *Salvia officinalis*, *Eugenia caryophyllata* and *Olea europaea*. When, the leaves are the most used organs, the decoction is the dominant mode of preparation and administration is done mostly orally (Orch et al. 2015). The same results are found by Tahri et al. (2012) in region of Settat, where 90 AMP species are identifies that traditionally used to treat digestive and respiratory system and the foliage is the most used plant organ and decoction is the dominant technic of preparation. In the Azilal region, among the 89 medicinal plants identified, 25 spontaneous medicinal plants are used for treatment of digestive system, 8 plants to treat urogenital disorders, 5 species are known for their effects on nervous system, 14 plants are used against metabolic disorders, 11 plants to treat diseases of the respiratory system and 26 plants are used for skin and hair care (Alami et al. 2016). In addition to its medicinal use, many AMPs are used in the food industry, where their dry leaves or other organs are used as food flavorings. For example, it was documented that adding *Rosmarinus officinalis* L. to foods as a spice is permitted without limitations (Zrira 2017).

I.3 Presentation of fenugreek (*Trigonella foenum-graecum* L.)

Fenugreek is a AMP with multiple therapeutic properties related to its richness in carotenoids, polyphenols, fatty acids and protein (Abdelhak et al. 2018). This plant is an annual herb, belonging to the Fabaceae family (Nagulapalli et al. 2017). According to Petropoulos (2002), the exact origin of this AMP is difficult to determine, but some authors have suggested that it is native to the Mediterranean region. However, others authors have proposed it as an AMP from Asia (Zandi et al. 2017). Its presence and use are ancient in India, Southern China, Ethiopia and the Middle East (Snehlata and Payal 2012). In addition, it is grown in North Africa, Ukraine, India and China (Zandi et al. 2017).

Fenugreek, scientifically known as *Trigonella foenum graecum* L., belongs to the botanical genus *Trigonella* (Table 2), which comes from the Latin name « trigonum », meaning trigon/triangle, probably referring to the triangular shape of the flowers (Snehlata and Payal 2012). The Latin name for the *foenum-graecum* species means “Greek hay”, referring to the intense fragrance of the dried fenugreek plant (Khorshidian et al. 2016). The leaves of fenugreek are alternate, long petiolated with 2 stipules, composed of 3 oval and denticulate leaflets (Oueslati and Ghédira 2015, Figure 2). The fenugreek flowers, called papillaries, are whitish to yellow, sessile, rather large, composed of a calyx with five sepals not divided, a corolla with five free triangular petals (hence the name of a trigonal flower) and ten stamens and a pluriovulated ovary (Snehlata and Payal 2012, Figure 2). The fruit, containing 10 to 20 seeds, is a linear pod with fine longitudinal veins, ending in a beak (Petropoulos 2002; Oueslati and Ghédira 2015). The stems of fenugreek are erect, hollow, with dark anthocyanin or full green. In fact, it is reported that stems, depending on variety and soil fertility, are either single-branched with no secondary shoots, or multiple branches with multiple shoots from the base (Rahmani et al. 2015).

Table 2 : Phylogenetic classification of fenugreek (Snehlata and Payal 2012)

kingdom	**Plantae**
Division	**Magnoliophyta**
Class	Magnoliopsida
Order	Fabales
Family	*Fabaceae*
Genre	*Trigonella*
Specie	*Trigonella foenum graecum*
Botanical name	*Trigonella foenum graecum* L.

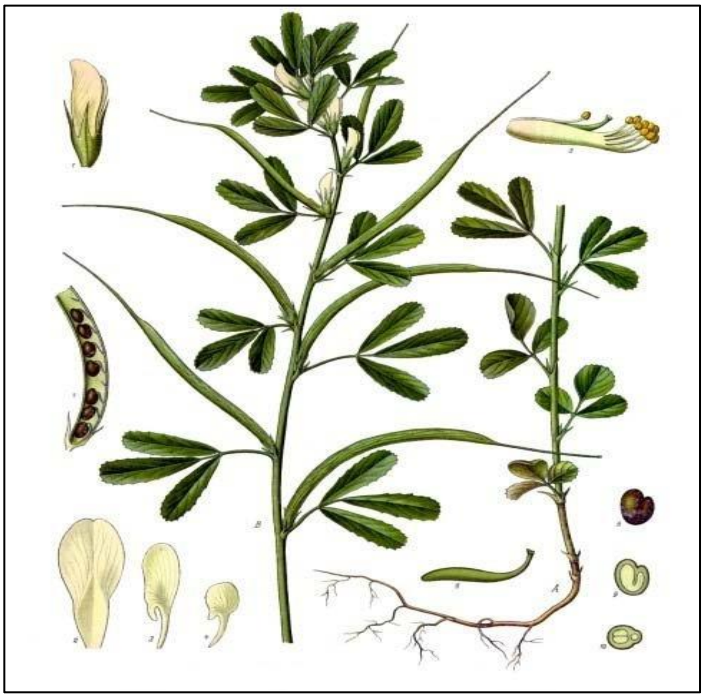

Figure 2: Fenugreek (*Trigonella foenum-graecum* L.) from source of Koehler's Medicinal Plants images.

The used part of fenugreek is essentially seeds that have therapeutic interests. To recover the seeds, the pods are harvested in the fall and then dried for beating so that the seeds come out (Rahmani et al. 2015). In general, use of fenugreek has been documented in Egypt, where it was used in incense and to embalm mummies. In modern Egypt, fenugreek seeds are still used in addition to wheat and corn flour for bread making. Additionally, fenugreek leaves are also used to prepare extracts or powders for medicinal use (Basch et al. 2003).

I.4 Medicinal and agronomic importance of fenugreek

According to their richness on bioactive compounds, fenugreek is documented that used for treatment of several diseases (Table 3). For example, fenugreek is one of the most studied medicinal plants in the treatment of diabetes because of its chemical compounds. In this context, Mahjoub et al. (2018) showed that taking 5 g of fenugreek seeds daily for 3 months improved glycemic balance with an average decrease in glycated hemoglobin (HbA1c) of 0.88%. In addition, Fenugreek seeds showed a hypocholesterolemic and hypotriglycemic effects due to its high fiber content (Sowmya and Rajyalakshmi 1999). The presence of phenolic acids, fibers, saponins and proteins in different parts of the plant, fenugreek is a powerful antioxidant and it acts as a mucus solvent and throat cleanser, which also eases the urge to cough (Snehlata and Payal 2012). Besides, fenugreek has also demonstrated anti-inflammatory and hepatoprotective properties (Srinivasa and Naidu 2021). Seeds act also as a protective agent against liver-induced abnormalities and used to relieve inflammation, rheumatism and sore muscles (Subhashini et al. 2011). In addition to its antidiabetic, cholesterol-lowering, anti-inflammatory and antioxidant effects, fenugreek has the ability to modify cyclophosphamide-induced apoptosis and lipid peroxidation (Ojo Olaiya and Soetan 2014). This latter makes fenugreek as a promising herbal remedy for complementary therapy in cancer patients (Meghwal and Goswami 2013). In this way, in a recent study, which aimed to evaluate the anticancer potential of methanolic fenugreek seed extract, Alrumaihi et al. (2021) showed that fenugreek seed extracts have many bioactive substances with significant cytotoxicity effect for cancer cells.

Table 3: Major chemical components of fenugreek seed (Khorshidian et al. 2016).

Chemical component	Content/percentage
highest saponin	4.63 g / 100g
protein	43.8 g / 100g
total dietary fiber	77.1 g / 100 g
soluble dietary fiber	45.2 g / 100g
total polyphenols	103.8 mg of gallic acid equivalent / g
fixed fatty acids and essential oils	6-8 %

Furthermore, being a forage legume and a natural nitrogen fixer, fenugreek could be easily incorporated into the local crop cycles (short-term rotation) for natural replenishment of soil, for the fixation of nitrogen (N) and for feeding the livestock as hay or silage (Zandi et al. 2017). In this context, Singh et al. (2008) estimated that fenugreek can fix 48% of its total N during growing season. Therefore, fenugreek can be used as a medicinal and aromatic plant, a good soil renovator, and a best green manure friendly to environment.

I.5 Soil and climatic requirements

Although fenugreek is native to the Mediterranean region or Central Asia, their culture in different environments is very satisfactory. Fenugreek is grown well as an annual crop from seeds, by the line sowing method, and is cultivated as a semi-arid crop in the prepared soil (Zandi et al. 2017). It is a cold season crop and is a tolerant plant to frost and very low temperature (Snehlata and Payal 2012). Fenugreek requires well-drained, good soil of medium texture and tolerated a pH range between 5.3 to 8.2. Needs full sunlight, and requires watering during dry periods (Zandi et al. 2017). Generally, the number of irrigation required depends upon the type of the soil and evaporate transpiration potential prevailing during the season (Snehlata and Payal 2012).

I.6 Fenugreek and symbiosis

Recently, because of growing population worldwide, farmers used chemical fertilizers response to food demand. However, these practices are known for their harmful effects on the environment and the human and animal healthy. In this context, it is documented that cultivation of legume plants can improve soil fertility and their composition on mineral elements, where these plant species can fix annually 175 million tons of atmospheric N (Verma et al. 2020). Fenugreek, as forage legume, was reported to fix 48% of its total atmospheric nitrogen during the growing season (Singh et al. 2008). On the other hand, inoculation though fenugreek rhizobium has been reported to increase the biomass of plant and seed production (Singh et al. 2008).

The Rhizobiaceae family consists of a heterogeneous set of gram-negative, aerobic, non-sporulate bacteria that can infect roots and sometimes stems of legumes to form nodules (Verma et al. 2020). Nodule is the site of a symbiotic interaction between the bacterium, which fixes atmospheric N_2 and provides it to the host plant in an assimilable form (NH_3) and, in return the the host plant provides to bacterium an favorable microhabitat and carbohydrate substrates from photosynthesis (Limpens and Bisseling 2003).

Symbiosis is the result of interactions controlled by molecular signals between the plant and its bacterial partner. Under N deficiency, plants produce flavonoids in the rhizospheric area. Once this signal received by the rhizobium, the expression of *nod* genes, encoding for Nod factor synthesis enzymes, induced (Limpens and Bisseling 2003). Nod factor is important in inducing root nodules by causing morphological, physiological and molecular changes in the root cells (Graham and Vance 2003; Kinkema et al. 2006). The last stage of the nodule formation is invasion of the bacteria, from the infection cords, inside the cortical cells followed by the division and differentiation of the rhizobia into nitrogen-fixing cells, recognized as bacteroids (Gage 2004). A peribacteroid membrane envelops these bacteroids, it protects the plant from the ammonia produced and the potential pathogenicity of the bacteria while maintaining a gradient of N, oxygen and nutrients necessary for nitrogen fixation (Limpens and Bisseling 2003; Putra et al. 2020).

II. Salt stress

II.1 Generality

Soil salinization is one of the most environmental problems, because approximately 50% of the irrigated land and 20% of cultivated land are affected by high accumulation of salt ions, causing a considerable decrease in agricultural systems, in terms of production and yield (Hu and Schmidhalter 2004). In fact, salinity not only affected plant production, but also altered chemical and physical properties of soil (Ding et al. 2020), as well as it indirectly affects food security in the world.

Salinity is defined as an excess of salts soluble minerals in irrigation water or in soil solution (Farissi et al. 2014). These salts are largely represented by the combination of three cations (Ca^{2+}, Mg^{2+} and Na^{+}) and three anions (Cl^{-}, SO_4^{2-} and HCO_3^{-}) (Safdar et al. 2019). Historically, soils have been classified as saline, sodic or saline-sodic based on total salt concentration and the ratio of Na^{+}/Ca^{2+} and Na^{+}/Mg^{2+} in the soil (Yadav et al. 2011). In general, sodium chlorides (NaCl) are more frequent and account for more than 90% of salt ion in the soil (Farissi et al. 2014). Two types of salinity were described; primary salinity and secondary salinity. Primary salinity is the result of long-term salt accumulation through natural causes such as high level of evaporation, low precipitation, existence of saline parent rock and some hydrological conditions (Hu and Schmidhalter 2004). While, the secondary salinization results from human activities that alter the hydrological balance of soil between applied water (irrigation or rain) and water used by crops (Yadav et al. 2011). Clearing and replacement of perennial vegetation with annual crops, excessive irrigation, irrigation with saline water, inadequate drainage and excessive fertilizer use are the main causes of secondary salinity (Hu and Schmidhalter 2004).

II.2 Effect of salt stress on AMPs

II.2.1 Seed germination of AMPs under salt stress

Seed germination is the first and the most critical stage that determines plants growth and productivity (Parihar et al. 2015). However, this key stage is one of the most sensitive plant growth stages to abiotic stresses, particularly salt stress (Khoshsokhan et al. 2012; Aghaei and Komatsu 2013). Indeed, salt stress caused osmotic stress and ionic toxicity due to Na^{+} and Cl^{-} ions accumulation, inhibiting or delaying seed germination process (Jamil et al. 2006; Sebei et al. 2007).

The negative effects of salt stress on seed germination has been reported in several AMPs such as *Thymus maroccanus Ball* (Belaqziz et al. 2009), *Thymus daenensis, Thymus kotschyanus* (Khoshsokhan et al. 2012), *Origanum compactum* (Laghmouchi et al. 2017) and *Artemisia Annua* L. (Hosein and Keshavarzi 2012). In this line, Saeidnejad et al. (2013) reported that salinity caused a significant decrease in germination characteristics including germination percentage and germination rate of *Ocimum basilicum* L. Similarly, salt stress interestingly reduced germination percentage, germination rate, vigor index, radicle and plumule lengths were significantly reduced in *Linum usitatissimum* L., *Lavandula stricta* Del. and *Limonium bicolor* L. (Kadkhodaie & Bagheri 2011; Sanginabadi & Khorasaninejad 2016; Li et al. 2019; Liu et al. 2019). Furthermore, other authors showed that even low salinity could induce a state of dormancy before germination, while high level of salinity could completely inhibits germination process (Torabi et al. 2011; Zhanwu et al. 2011).

El Moukhtari et al. (2022b) suggested that salt stress could reduce seed germination by altering the embryo metabolism, disturbing hormonal balance and seed reserve mobilization (Figure 3). Indeed, Liu et al. (2019) reported that 200 mM NaCl significantly downregulated the expression of key genes involved in biosynthesis of gibberellic acid (*GA20ox* and *GA3ox*), while the expression of *NCED1* and *NCED3*, key genes involved in biosynthesis of abscisic acid (ABA), was considerably upregulated in salt-stressed *Limonium bicolor* L. seeds. Consequently, salt stress increased the ABA and reduced the GA contents thus, in turn, affected GA/ABA ratio of germinated seeds. These findings is in agreement with the those of Li et al. (2019), indicating that 200 mM NaCl upregulated *lbnced1, lbnced, lbcyp707a1* and *lbcyp707a2,* genes involved in ABA biosynthesis, whereas the expression of *GA20ox* and *GA3ox* genes were markedly downregulated. On the other hand, several studies found that salt stress could alter seed germination and embryo viability by Na^+ and Cl^- ions accumulation (Sebei et al. 2007; Farissi et al. 2011) . Salt stress has also reported to inhibit the activities of some hydrolytic enzymes such as amylases, ATPase and phosphatase, reduced as a result seed reserve mobilization. This reduction is more obvious in the sensitive cultivars than in the tolerant ones (Dash and Panda 2000; Sharma et al. 2004). In the same line, Liu et al. (2019) reported that the activity of amylase was significantly inhibited under salt stress (200 mM NaCl) in *Limonium bicolor.*

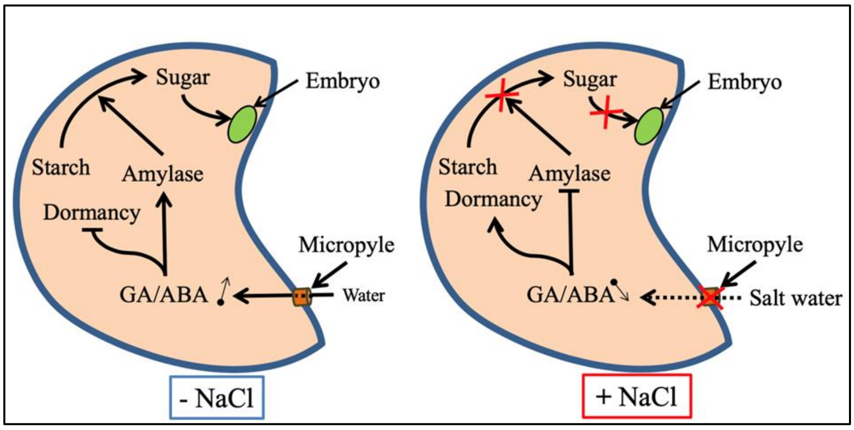

Figure 3: Suggested effects of salinity on seed germination (Adopted from El Moukhtari et al. (2020)).

According to the above studies, salinity affected germination stage by two ways: the first one is caused by an osmotic stress, which retards or prevent the uptake of water necessary for reserve mobilization. The second one is due to salt ions like Na^+ and Cl^-, at high concentrations, causing ionic toxicity and disturbing ion homeostasis in embryo cells (Jamil et al. 2006; Sebei et al. 2007; Said-Al-Ahl and Omer 2011). However, seed germination of some AMPs as *Lepidium sativum* L., *Plantago ovata Forssk* and *Trigonella foenum-graecum* L. showed a significant tolerance to moderate salinity (Muhammad and Hussain 2010). Therefore, the salt tolerance of AMPs, at seed germination, is dependent on the AMPs species and the level of salinity stress.

II.2.2 Growth and flowering stages under salt stress

Salt stress usually restricts plant growth and productivity of many AMPs species by affecting diverse morpho-physiological and biochemical characteristics (Muhammad and Hussain 2010). Chrysargyris et al. (2018) reported the harmful effects of different salt concentrations on plant biomass, shoot height, leaf length and shoot number of *Lavandula angustifolia* Mill., and this negative impacts were more noted under high concentrations of NaCl (100 mM) as compared to the unstressed control. The same results were found by Khanam and Mohammad (2018) in *Mentha piperita* L. under three levels of salinity (50, 100 and 150 mM NaCl). In addition, salinity stress significantly decreased plant height, number of branches, number of capsules, number of roots per plant, roots volume, roots fresh and dry weights, capsules yield and seed yield in *Nigella Sativa* L. (Rashed et al. 2017). Similarly, plant height, flower fresh and dry weight, number of flower and branch per plant, peduncle length and head diameter of *Matricaria chamomile* were severally reduced under salt stressed conditions (Razmjoo et al. 2008). El-Esawi et al. (2017) reported that *Rosmarinus officinalis* L. plant height was reduced from 31 to 19 cm respectively, when NaCl concentration was increased from 640 to 4000 ppm. The same was reported by Hassanpouraghdam et al. (2020) in *Rosmarinus officinalis* L. where salinity stress significantly decreased root growth and above-ground plant growth (fresh and dry weight). In the same line, Elhindi et al. (2017) found that addition of 60 and 120 mM NaCl significantly inhibited the growth and photosynthetic activity of *Ocimum basilicum* L. Also, the morphological traits such as leaf area, total fresh and dry herbs as well as total fresh and dry roots of *Plectranthus amboinicus* (Lour.) were drastically reduced under salt stress (Ahmed and al. 2020). Similarly, Khan et al. (2010) showed that 150 mM NaCl decreased plant height, leaf area, shoot dry biomass and roots dry biomass by 12 %, 23 %, 17 % and 29% respectively, in *Linum usitatissimum* L. The leaf area index was also markedly decreased with increasing salt stress in *Carthamus tinctorius* L. (Ghassemi-Golezani and Hosseinzadeh-Mahootchi 2015). The negative impacts of salt stress on plant growth has also been reported in *Lepidium sativum* L., *Linum usitatissimum* L., *Nigella sativa* L., *Plantago ovata Forssk* and *Trigonella foenum-graecum* L. (Muhammad and Hussain 2010).

The flowering stage is one of the most important growth stages of AMPs, where the harvesting of the flowers or the other plant parts is begins (Ghanmi et al. 2010), for essential oil extraction or other industrial and pharmaceutical purposes. However, this important stage was negatively affected by salt stress in some AMPs like *Borago officinalis* L. (Jaffel et al. 2011) and *Ocimum basilicum* L. (Said-Al Ahl and Mahmoud, 2010). Also, Chrysargyris et al. (2018) showed a significant reduction in flowers number of *Lavandula angustifolia* (Mill.) under salt stress. Similarly, the fresh flower yield was also decreased with increasing salinity levels in *Matricaria recutita* L. (Baghalian et al. 2008) and *Calendula officinalis* L. (Khalid and Teixeira da Silva 2010; Bayat et al. 2012). The same results was found in *Matricaria chamomile,* where the flowers number and the fresh flower yield were significantly decreased under salt stress (Razmjoo et al. 2008; Dadkhah 2010).

Growth reduction due to salt stress is attributed to several factors, such as decreasing of water uptake and accumulation of Na^+ and Cl^- ions, at toxic concentrations, in the different plant parts (Zhu 2003; Aghaei and Komatsu 2013; Hasanuzzaman et al. 2017). Indeed, Kotagiri and Kolluru (2017) reported that excessive salt ions accumulation in leaves led to a reduction of carbohydrate supply and growth hormones synthesis in other plant parts, and consequently affected plant growth process. More than that, Pessarakli and Touchane (2006) indicated that salt stress might result in limited cell division, and hence it decreased the rate of plant development. Another study showed that depressed growth by salinity could be explained by an imbalance between the root and the shoot hormones and a reduction of photosynthesis products available to growth during exposition to salt stressed conditions (Said-Al Ahl and Mahmoud 2010).

II.2.3 Photosynthesis in AMPs under salt stress

Under salt stressed conditions, it was reported that the accumulation of Na^+ and Cl^- in the chloroplast decreased the electron transport activity of photosystem II (PSII), reduced the leaf photosynthetic pigment contents and inhibited the uptake of potassium (K^+), which is necessary for osmoregulation and stimulation of photosynthesis (Chérel 2004; Ashraf and Harris 2013; Chrysargyris et al. 2016; Bouassaba and Chougui 2018; Chrysargyris et al. 2018). In addition, the osmotic stress caused the stomata closure to prevent water loss by transpiration, restricting as a result the rate of CO_2 assimilation (Yang et al. 2006; Figure 4). This decrease in internal CO_2 concentration in the leaf, reduced in turn the CO_2 assimilation by Rubisco, the key enzyme in the Calvin cycle, (Reddy et al. 2004; Dias and Brüggemann 2010), and as a result the reduction of the photosynthesis performance. Also, one of the major consequences of the deceleration in the Calvin cycle is a decrease in the consumption of reduced cofactors (NADPH) by this cycle, and therefore a decrease in the same oxidized cofactors ($NADP^+$). Consequently, the NADPH produced by photosystems is not recycled to $NADP^+$ induced in turn, a blockage in the electron transport chain. This has the consequence of preventing the chlorophyll from returning to an excited state and can finally induce degradation of the photosystems (Dias and Brüggemann 2010), therefore inhibition the plant photosynthetic activity.

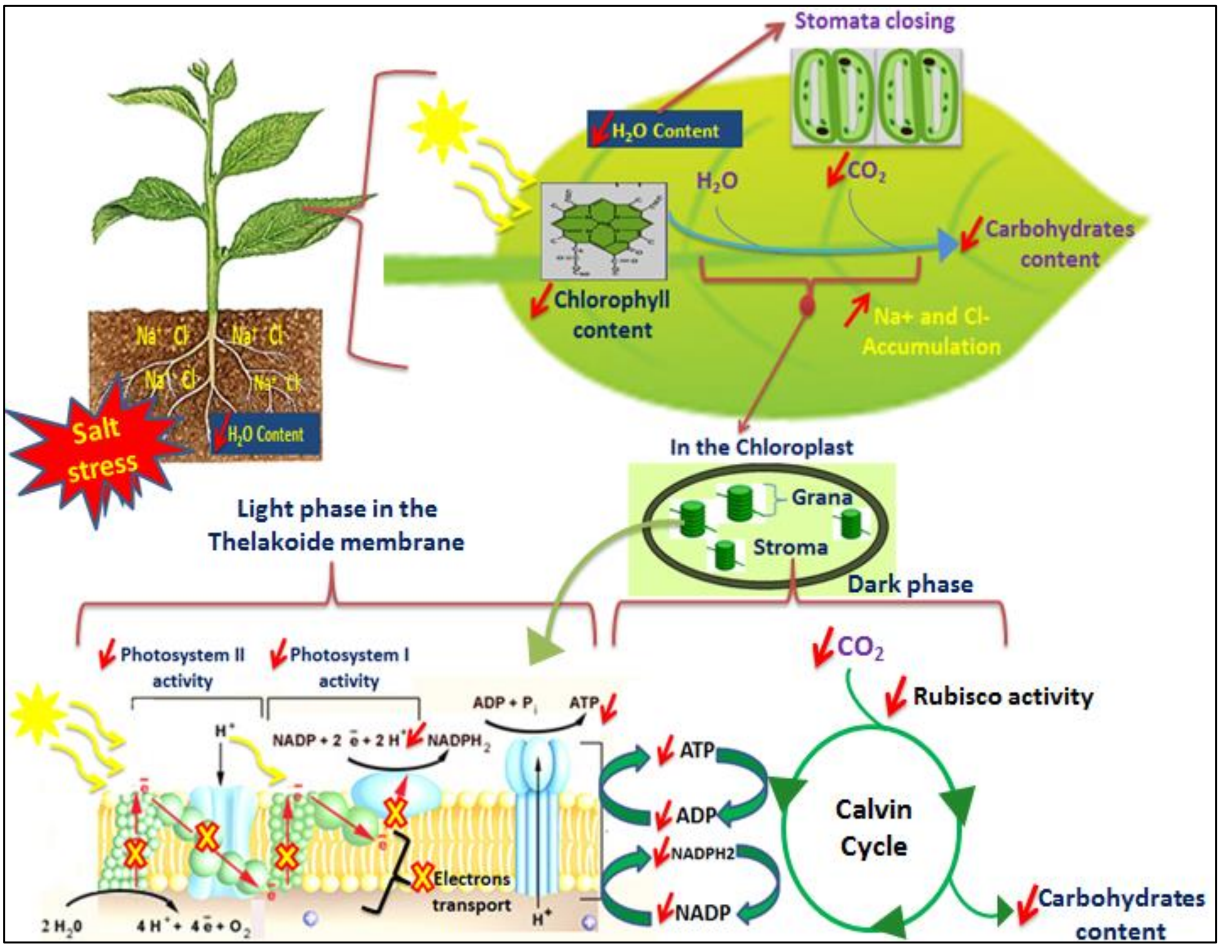

Figure 4: Proposed effects of salt stress on the two photosynthesis phases. × = indicate a blockage, ↓= indicate a decrease, ↑= indicate an increase.

The effects of salt stress on photosynthesis in AMPs, such as *Centaurium erythraea* Rafn., *Plantago coronopus* L., *Lonicera japonica* L., *Ammi majus* L. and *Ocimum basilicum* L., were reflected by low net photosynthesis, stomatal conductance, chlorophyll content and photosynthetic efficiency (Ashraf et al. 2004; Siler et al. 2007; Tarchoune et al. 2012 ; Koyro et al. 2015). The same was found by Tounekti et al. (2012) on *Salvia officinalis* L., where the addition of 100 mM NaCl to the nutrient solution increased Na^+ concentration in leaves and reduced net photosynthesis, stomatal conductance, net CO_2 assimilation and chlorophyll content. Similarly, Elhindi et al. (2017) showed that 60 and 120 mM NaCl-treatment significantly reduced net CO_2 assimilation rate, transpiration rate, stomatal conductance and substomatal CO_2 concentration of *Ocimum basilicum* L. In addition to these results, the contents of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were also decreased in *Egletes viscosa* L. plant under 40 and 80 mM NaCl stress (Batista et al. 2019). Exposure of *Melissa officinalis* L. plants to 100 mM NaCl stress reduced the levels of chlorophyll a and b by 41 % and 54 % respectively (Safari et al. 2020). Also, increasing salinity level from 0 to 4000 ppm NaCl in the soil solution of *Nigella sativa* L. caused a gradual decrease in chlorophyll a and b (Rashed et al. 2017). The same result was reported in *Rosmarinus officinalis* L. (Hassanpouraghdam et al. 2020), *Carthamus tinctorius* L. (Shaki et al. 2018) and *Moringa oleifera* (Farouk et al. 2018). Likewise, in *Centaurium erythraea* Rafn*.*, the net photosynthesis and PSII efficiency, reflected by *Fv/Fm* ratio, were decreased with increasing NaCl levels (Siler et al. 2007). In *Rosmarinus officinalis* L*.*, the results showed that the total chlorophyll content and the quantum yield of the PSII were significantly decreased under salt stressed conditions (Chetouani et al. 2019). In the same way, Messedi et al. (2016) found that salt stress above 100 mM NaCl significantly reduced photosynthetic activity and quantum yield of PSII, while it increased the non-photochemical quenching, along with a significant inhibition of the linear electron transport rate at 400 mM NaCl in *Cakile maritime*.

Carbonic anhydrase has an important role in the functioning of photosynthetic pathway in plant, it catalyzes the reversible hydration of atmospheric CO_2 (Jebanathirajah and Coleman 1998), which is an important reaction for CO_2 integration into the Calvin cycle. However, the activity of this key enzyme was suppressed by 150 mM NaCl stress in *Linum usitatissimum* L., this was accompanied with a significant reduction of net photosynthesis, stomatal conductance and chlorophyll content, which suggest the important role of this enzyme under salt stress (Khan et al. 2010). Similarly, in salt stressed *Mentha piperita* L., there was a reverse relationship between salinity levels (50, 100 and 150 mM NaCl) and the activity of carbonic anhydrase as well as the net photosynthesis, stomatal conductance, transpiration rate and internal CO_2 concentration (Khanam and Mohammad 2018). Altogether, above studies suggest that salt stress significantly alters chloroplast structure and inhibits the key enzymes involved in photosynthesis process, which explains the growth reduction of AMPs under salt stressed conditions.

II.2.4 Salt stress effect on nutrient uptake in AMPs

High salinity levels in the soil lead to nutrient unbalance by increasing the plant content in Na^+ and Cl^- and reducing the essential ions such as K^+, calcium (Ca^{2+}), and magnesium (Mg^{2+}) (Karimi et al. 2005). Indeed, Oueslati et al. (2010) found that increasing level of Na^+ in plant was accompanied by restricting K^+ uptake, which in turn induced a decrease in *Mentha pulegium* growth. As a result of Na^+ increase and K^+ decrease, salinity decreased K^+/Na^+ ratio in various AMPs such as *Mentha suaveolens* Subsp. (Kasrati et al. 2014) and *Carthamus tinctorius* L. (Gengmao et al. 2015a). Similarly, the contents of *Foeniculum vulgare* Mill Subsp. and *Matricaria recutita* L. in Na^+ and Cl^- were found increased in both shoots and roots, whereas the content of K^+, Mg^{2+} and Ca^{2+} was significantly reduced under salt stress (Abd El-Wahab 2006). Exposure of *Linum usitatissimum* L. plant to 150 mM NaCl stress significantly increased Na^+ content but decreased K^+ content, leading to a decrease in K^+/Na^+ ratio (Khan et al. 2010; Amraee et al. 2020). The same results were obtained in *Egletes viscosa* plant, where the content of Na^+ was increased, while the K^+ content was reduced in leaves and roots of salt-stressed plants (Batista et al. 2019). In addition, salt stress (50, 100 and 200 mM NaCl) enhanced Na^+ concentration but decreased K^+, Ca^{2+} and Mg^{2+} concentrations as well as the ratio of K^+/Na^{2+}, Ca^{2+}/Na^+ and Mg^{2+}/Na^+ in *Lonicera japonica* (Huang et al. 2019). The same was found in *Aloe* L. (Xu et al. 2015), *Thymus vulgaris* (Zrig et al. 2019) and *Carthamus tinctorius* L. (Shaki et al. 2019) about K^+/Na^+ and Ca^{2+}/Na^+ ratios and Na^+ content under salt stressed conditions. Also, Hassanpouraghdam et al. (2020) found that 75, 150, and 225 mM NaCl stress significantly decreased K^+ and zinc (Zn^{2+}) contents and raised the content of Na^+ in plant, which decreased as a result K^+/Na^+ ratio in *Rosmarinus officinalis* L.

Elhindi et al. (2017) reported that the uptake of nitrogen (N), phosphorus (P), iron (Fe^{2+}), manganese (Mn^{2+}), copper (Cu^{2+}), K^+, Mg^{2+} and Zn^{2+} was strongly inhibited, whereas Na^+ and Cl^- uptake was considerably enhanced under 60 and 120 mM NaCl stress in *Ocimum basilicum* L. In concordance, Chrysargyris et al. (2018) reported that salinity significantly affected the content of leaf and the root on macro- and micronutrients such as N, K^+, Mg^{2+} and Ca^{2+} in *Lavandula angustifolia* Mill. Mineral contents in terms of N, P, K^+, Mg^{2+}, Zn^{2+}, Fe^{2+} and Mn^{2+} have been also decreased in *Melissa officinalis* L., when the salinity level was increased (Khalid and Cai 2011). In the same line, N, P and K accumulation was significantly affected by

NaCl stress in *Mentha piperita* L. (Khanam and Mohammad 2018) and *Plectranthus amboinicus lour* (Ahmed et al. 2020).

Nutrient deficiency or imbalance caused by salt stress could be explained by the competition of Na^+ and Cl^- with other nutrients for membrane transporters (Said-Al Ahl and Omer 2011). Indeed, due to the similarity in physicochemical properties between K^+ and Na^+, at high concentration, Na^+ inhibited the uptake of K^+ (Nawaz et al. 2010) by blocking K^+ transporters (Almeida et al. 2017). However, some AMPs as *Trachyspermum Ammi* L. maintained markedly higher K^+/Na^+ and Ca^{2+}/Na^+ ratios under salt stressed conditions (Ashraf and Orooj 2006). These findings confirmed by Abdoli et al. (2020), which also reported the increase of K^+/Na^+ ratio at 40, 80 and 120 mM NaCl in *Trachyspermum Ammi* L. Consequently, these species are moderately salt tolerant owing to their ability to maintain high K^+/Na^+ and Ca^{2+}/Na^+ ratios in both shoots and roots under salt stress.

II.2.5 Salt tress and primary metabolites biosynthesis

Plant primary metabolites have been reported to be affected by salt stress. Proteins, carbohydrates, lipids and amino acids are one, among others, of the most affected primary metabolites in AMPs under salt stressed conditions (Said-Al Ahl and Omer 2011). Indeed, total carbohydrates were adversely affected by salinity in *Foeniculum vulgare Mill Subsp.* (Abd El-Wahab 2006). The same result was reported in salt stressed *Rosmarinus officinalis* L., where the percentage of total carbohydrates were decreased as the salinity level were increased from 2000 to 3000 or 4000 ppm NaCl (Abdelkader et al. 2019). Similarly, 100 and 200 mM NaCl-treatment negatively affected soluble proteins contents of *Carthamus tinctorius* L. plant (Shaki et al. 2018). In addition, proteins content was significantly decreased in salt stressed *Chamomilla recutita*, *Origanum majorana* (Ali et al. 2007) and *Catharanthus roseus* (Osman et al. 2007). Regarding lipid metabolites, the total fatty acid, α-linolenic and linoleic acids content were significantly decreased in *Coriandrum sativum* weather raising NaCl levels in the soil solution (Neffati and Marzouk 2008).

In contrast, the content of AMPs of certain amino acids was significantly increased during response to salt stress (Said-Al Ahl and Omer 2011). For example, proline content was accumulated in leaves as a result of salinity stress in many AMPs such as sweet fennel cultivars (Zaki et al. 2009), *Salvia officinalis* L. (Hendawy and Khalid 2005), *Catharanthus roseus* L. (Osman et al. 2007), *Carthamus tinctorius* L. (Shaki et al. 2018), *Chamomilla recutita* and *Origanum majorana* (Ali et al. 2007). Furthermore, in *Mentha pulegium*, the proline content was increased by 19 % as a response to 75 mM NaCl stress, and this was explained by the increase in the activity of pyrroline-5-carboxylate reductase and the reduction of proline oxidase activity (Farhadi and Ghassemi-Golezani 2020). Total soluble sugars content was also enhanced in *Melissa officinalis* L. (Khalid and Cai 2011) and *Lavendula officinalis* L. (Nasab et al. 2018) during response to salt stress. In addition to proline, glycine betaine content was also improved by 9 % in salt stressed *Mentha pulegium* plants (Farhadi and Ghassemi-Golezani 2020). In the same way, Abdoli et al. (2020) reported that the contents of proline, glycine betaine, soluble sugars and soluble proteins was significantly raised in exposed *Trachyspermum ammi* L. to salinity stress. In fact, AMPs improved the content of primary metabolites in terms of proline, glycine betaine and carbohydrates, as the important compatible solutes, to reduce osmotic stress induced by salt stress, and in turn to enhance their antioxidant activity and tolerance to salt stressed conditions.

II.2.6 Salt stress and secondary metabolite production

Secondary metabolites are mainly produced from the primary metabolites i.e. carbohydrates, amino acids, lipids and nucleotides. These bioactive compounds are well known for their aromatic and medicinal properties and their role in plant defence to environmental constraints (Mahajan et al. 2020). However, it has been reported that the biosynthesis and accumulation of these metabolites were affected by several environmental stressors, including salinity (Aghaei and Komatsu 2013). Indeed, the accumulation of secondary metabolites was enhanced in some AMPs, but it was negatively affected in others under salt stress, depending on the plant species and stress level. For example, total phenolic contents were improved under salt stress in *Achillea fragratissima* (Abd EL-Azim and Ahmed 2009), *Carthamus tinctorius* L. (Salem et al. 2014), *Mentha pulegium* (Oueslati et al. 2010), *Nigella sativa* (Bourgou et al. 2010), *Stevia rebaudiana* (Sharuti et al. 2014), *Ocimum basilicum* L. (Bekhradi et al. 2015), *Thymus vulgaris* L. and *Thymus daenensis* (Bistgani et al. 2019), but the content of these secondary metabolites were decreased after NaCl-treatment in *Rosmarinus officinalis* L. (Hassanpouraghdam et al. 2020) and *Lavandula angustifolia* Mill. (Chrysargyris et al. 2018).

The exposure of *Ocimum basilicum* L. to 100 mM NaCl stress markedly decreased the content of caffeic acid by 65 % (Bahcesular et al. 2020). Conversely, this compound was significantly increased under 60 mM NaCl stress in *Thymus vulgaris* L. and *Thymus daenensis Celak*, but it then decreased with higher salinity level (Bistgani et al. 2019). Tounekti et al. (2011) found that addition of 100 mM NaCl to the nutrient solution reduced the carnosic acid and 12-O-methyl carnosic acid contents by 20 % in the leaves of *Salvia officinalis*. However, an increase of some secondary metabolites, such as reserpine, vincristine and solasodine, has been observed respectively, in *Rauvolfia tetraphylla* (Anitha and Ranjitha Kumari 2006), *Catharanthus roseus* (Misra and Gupta 2006) and *Solanum nigrum* (Bhat et al. 2008) during response to salinity. In addition, in leaves of *Artemisia annua*, the maximum concentration of artemisinin has been observed under salt stress due to the upregulation of related-gene expression (Vashisth et al. 2018). Also, Aghighi et al. (2019) showed that the treatment with 30 mM NaCl increased the percentage of stevioside and rebaudioside-A in *Stevia rebaudiana*. Likewise, salt stress significantly increased rosmarinic acid content in *Thymus vulgaris* L. (Bistgani et al. 2019). Altogether, there was a different in secondary metabolite biosynthesis and accumulation among AMPs during response to salt stress, all dependent on the response mechanisms of AMPs and the severity of salt stress.

II.2.7 Effect of salt stress on essential oils yield in AMPs

Salt stress significantly influences the production of essential oils in AMPs as shown in table 4. For example, in *Matricaria chamomile,* the essential oils yield was remarkably decreased under salt stress conditions (336 mM NaCl) (Razmjoo et al. 2008). In agreement with that finding, Aziz et al. (2008) found that the essential oils yield of *Mentha x piperita* L., *Mentha pulegium* L. and *Mentha Suaveolens Ehrh* was adversely affected by 50 mM NaCl application. Çoban and Göktürk Baydar (2016) confirmed this decreased essential oils yield in *Mentha piperita* L., which was severely hampered under 150 mM stress. In the same species, Khalvandi et al. (2019) also confirmed that salt stress considerably inhibited essential oils biosynthesis. Furthermore, the essential oils content and menthol concentration was strongly reduced in salt-treated *Mentha canadensis* plant (Yu et al. 2015). The same result was demonstrated by Hassanvand et al. (2019) in *Pelargonium graveolens*, where the essential oils content and yield were decreased from 1.35 mL to 0.55 mL per plant respectively, in plant control (1.8 dS m^{-1} NaCl) and in salt stressed plants (6 dS m^{-1} NaCl). On the other hand, salinity was able to affect the essential oils constituents. Indeed, salt stress caused by 640, 2000 and 4000 ppm NaCl affected the specific major essential oils compounds of *Rosmarinus officinallis* L. via causing a reductions in α-pinene, β-pinene and cineole contents, along with an increases in linalool, camphor, borneol and verbenone contents (El-Esawi et al. 2017). Similarly, the exposure of *Ocimum basilicum* L. to 100 mM NaCl stress influenced the concentration of the major essential oils compounds by decreasing eugenol and methly eugenol concentrations, whereas linalool concentration was increased. While, the amount of terpenoids, α-terpinyl acetate, 1,8-cineole and linalyl acetate was increased in *Salvia mirzayanii* under salt stress (Valifard et al. 2018). In addition, the total content of monoterpenoids and sesquiterpenoids of essential oils was increased by NaCl salinity treatment in *Artemisia annua* (Yadav et al. 2017b).

In contrast, in others plant species such as *Salvia officinalis*, Taarit et al. (2010) showed that under low NaCl concentration (100 mM NaCl), the essential oils yield was significantly increased as compared to the untreated control. Similar results were found in *Ocimum Basilicum* L., when salt stress caused a significant increase in the essential oils yield (Said-Al Ahl and Mahmoud 2010). This result is recently confirmed by Gohari et al. (2019), who reported that 50 mM NaCl stress significantly improved *Ocimum basilicum* L. essential oils content compared with control conditions. Likewise, Neffati & Marzouk (2008) found that low NaCl concentrations (25 and 50 mM) caused an increase in the essential oils yield, which would be decreased under high salinity level (75 Mm NaCl) in *Coriandrum sativum* L. In addition, Khalid and Cai (2011) reported that essential oils percentage was increased from 0.14 % to 0.23 % at all saline irrigation water used (1.56, 3.13 and 4.69 dS m^{-1}) in *Melissa officinalis* L. At flowering stage of *Calendula officinalis* L., 9.38 dS m^{-1} NaCl-treatment increased the essential oils percentage from 0.11 % to 0.26 % (Khalid and Teixeira da Silva 2010). Similarly, Ahmed et al. (2020) showed that salt stress, caused by 2 and 4 g L^{-1} NaCl, increased essential oils yield of *Plectranthus amboinicus lour*.

The essential oils yield reduction during response to salt stress was partially explained by Ashraf et al. (2004), on the fact that increasing salt concentration caused a significant reduction in the fresh and dry masses of both shoots and roots, as well as seed yield. Similarly, Khanam and Mohammad (2018) reported that the decrease in essential oils yield could be because of the observed reduction in growth characters, photosynthesis and nutrient contents in *Mentha piperita* L. under 50, 100 and 150 mM NaCl stress. However, others studies have shown that salinity limited plant growth and caused a decline in the primary metabolites formation, but it encouraged secondary metabolites synthesis (Kulak et al. 2020). Thus, it enhanced secondary metabolites accumulation in plant. In the same line, Taarit et al. (2010) reported that in *salvia officinalis*, although salt constraint (100 mM NaCl) led to a reduction of plant growth and total fatty acids content, it caused an increase in essential oils production. These effects may be the results of a new pattern of resource partitioning, providing more carbon skeletons for terpene biosynthesis.

Generally, the contradictory findings between AMPs in biosynthesis and accumulation of primary and secondary metabolites, as essential oils (Table 4), could be explained by the differences in the mechanisms involved in the response to salt stress. On the other hand, it was reported that the effects of salinity on these metabolites vary depending on the tolerance of AMPs species or genotype as well as the intensity and duration of salt stress (Bahcesular et al. 2020). For example, in Genove and Rubi, two cultivar of *Ocimum basilicum* L. species, the maximum oils yield was attained under 60 mM NaCl in Rubi cultivar, while in Genove cultivar it was achieved under 30 mM NaCl (Talebi et al. 2018). Therefore, the variation in the essential oils content between these two cultivar used of basil (Genove and Rubi) could attributed with the genetic difference between them.

Table 4: Essential oils yield of aromatic and medicinal plants under salt stress

AMPs	**NaCl-treatment**	**Essential oils yield**	**References**
Calendula officinalis L.	93.8 mM NaCl	Increased from 0.11 % to 0.26 %	Khalid and Teixeira da Silva (2010)
Coriandrum sativum L.	50 mM NaCl	Increased from 0.12 % to 0.18 %	Neffati and Marzouk (2008)
Cymbopogon flexuosus Steud. Wats. (Neema cultivar)	150 mM NaCl	Decreased from 0.59 % to 0.50 %	Idrees et al. (2012)
Matricaria chamomila	336 mM NaCl	Decreased from 1.00 % to 0.29 %	Razmjoo et al. (2008)
Cymbopogon flexuosus Steud. Wats. (Krishna cultivar)	150 mM NaCl	Decreased from 0.61 % to 0.53 %	Idrees et al. (2012)
Melissa officinalis L.	46.9 mM NaCl	Increased from 0.14 % to 0.23 %	Khalid and Cai (2011)
Melissa officinalis L.	60 mM NaCl	Decreased from 0.15 % to 0.12 %	Ozturk et al. 2004
Mentha x piperita L.	51.3 mM NaCl	Decreased from 0.11 % to 0.04 %	Aziz et al. (2008)
Mentha piperita L.	150 mM NaCl	Decreased from 2.59 % to 0.39 %	Çoban and Göktürk Baydar (2016)
Mentha pulegium L.	51.3 mM NaCl	Decreased from 0.11 % to 0.07 %	Aziz et al. (2008)
Mentha Suaveolens Ehrh	51.3 mM NaCl	Decreased from 0.16 % to 0.05 %	Aziz et al. (2008)
Mentha suaveolens subsp	150 mM NaCl	Decreased from 0.77 % to 0.38 %	Kasrati et al. (2014)
Ocimum Basilicum L.	49.5 mM NaCl	Increased from 0.59 % to 0.76 %	Said-Al Ahl and Mahmoud (2010)
Salvia officinalis L.	100 mM NaCl	Increased from 0.66 % to 1.68 %	Taarit et al. (2010)

II.3 How aromatic and medicinal plants respond to salt stress

II.3.1 Regulation of ion homeostasis

Under saline conditions, the high accumulation of some salt ions (Na^+ and Cl^-) disturb those of K^+, because of the molecular similarity between Na^+ and K^+ and maintaining cellular balance between K^+ and Na^+ is one of the most important mechanism required for plant survival under salinity (Timperio et al. 2008; Aghaei and Komatsu 2013; Benito et al. 2014; Van Zelm et al. 2020). Indeed, tolerant plants are able to maintain Na^+ cell homeostasis by rejecting it from the cytosol or its compartmentalization in the vacuole (Peng et al. 2016). For example, in some aromatic and medicinal plants like *Ocimum Basilicum* L. Ning et al. (2015) found that plants could grow up under 0,5%, 10%, 20% and 40% seawater and this explained by its ability to Na^+ compartmentalization into vacuole and maintain high K^+/Na^+ and Ca^{2+}/K^+ ratios in the leaves. Similar results have been found by Ashraf & Orooj (2006) on *Trachyspermum Ammi* L. and by Ashraf et al. (2004) on *Ammi majus* L. In addition, Abdoli et al. (2020) reported that *Trachyspermum ammi* L. maintained high K^+/Na^+ ratio under 40, 80 and 120 mM NaCl stress. The salt-tolerant plants have been reported to have tonoplast AtNHX transporter, which is an Na^+/H^+ antiporter helping in Na^+ vacuolar compartmentalization and in maintaining intracellular K^+ status (Yokoi et al. 2002). According to Barkla et al. (1994) the activity of the vacuolar Na^+/H^+ antiporter increased with increasing NaCl concentrations in the growth medium suggesting the important role of this transporter in salt stress tolerance. In the line with this, the members of the high affinity K^+ transporter (HKT1 of HKTs) family and the plasma membrane salt overly sensitive 1 (SOS1) also help plant to keep Na^+ at low concentrations in the above-ground tissues under salt stress. HKT1 facilitates Na^+ homeostasis in plant and by this function modulates K^+ nutrient status (Rus et al. 2004). SOS1 is an Na^+/H^+ antiporter involved in the exclusion of Na^+ ions to the soil (Ji et al. 2013). Consequently, *Carthamus tinctorius* L. plant reduced toxic Na^+ in the cytosol by increasing *SOS1* and *NHX1* genes expression in plants under 100 and 200 mM NaCl stress (Shaki et al. 2018, 2019).

Additionally, the Pump-ATPase has been described to have a critical role in plant adaptation to salt conditions. Indeed, higher Pumps-ATPase activity under salt stress repolarizes the NaCl-induced depolarization of the plasma membrane (Mansour 2014) and energizes the active transport that excludes Na^+ from root cells, a process dependent of the SOS1 Na^+/H^+ antiporter (Gaxiola et al. 2007). Furthermore, increase of Pumps-ATPase activities was strongly associated with salt stress tolerance of plant (Mansour 2014). For example, Xu et al. (2015) suggested that the tolerance of *aloe* L. under 100 mM NaCl was highly associated with the activity of H^+–ATPase in the plasma membrane and tonoplast, and the activity of H^+-pyrophosphates in cells of the aloe root tip.

On the other hand, some AMPs acclimate to salt stressed conditions by secreting excessive salt ions through salt-secreting structures as salt glands, excreting salt ions directly by pores existing in the cuticle surface (Oi et al. 2014). Such as, Ning et al. (2015) suggested that the tolerance of *Ocimum Basilicum* L. to salt stress was associated with the compartmentalization of Na^+ in roots and the balance of K^+, Na^+ and Ca^{2+} in leaves by the leaf glands. Also, among the species of the Plumbaginaceae family, *Plumbago auriculata* Lam. characterized by the presence of salt secreting glands and trichomes which explain its high adaptation to salt conditions (Caperta et al. 2020).

II.3.2 Induction of antioxidant system

Generally, high salt content in the soil causes an accumulation of salt ions (Na^+ and Cl^-) into the plant cells, which leads to reactive oxygen species (ROS) formation, including singlet oxygen ($'O_2$), superoxide (O_2^-), hydroxyl radicals (HOº) and hydrogen peroxide (H_2O_2). These ROS induce an oxidative stress and disrupt as a result cell integrity and homeostasis by damaging lipids, proteins and nucleic acids (Quiles and López 2004; Said-Al Ahl and Omer 2011; Aghaei and Komatsu 2013b). In literature, tolerant plants had efficient antioxidant systems for scavenging ROS during oxidative stress (Foyer et al. 1994). Among these antioxidant systems, many antioxidant enzymes such as catalase (CAT), glutathione reductase (GR), superoxide dismutase (SOD) and glutathione-S-transferase (GST) have been described among the efficient ROS scavengers under salinity-mediated oxidative damage (Foyer et al. 1994; Omamt et al. 2006). Indeed, Omamt et al. (2006) suggested that the plants with high levels of antioxidant enzyme activities have greater resistance to salt stress.

Like other plant species, some AMPs such as *Linum usitatissimum* L. (El-Beltagi and El Hariri 2008), *Hyssopus officinalis* (Jahantigh et al. 2016), *Plantago ovata* Forsk. (Kala 2015), *Catharanthus roseus* (Osman et al. 2007) and *Carthamus tinctorius* L. (Gengmao et al. 2015b) its salt tolerance capacity might be closely related with the increased activity of antioxidant enzymes like peroxidase (APX), CAT and SOD to scavenge ROS and limit oxidative damages. Similarly, Khalvandi et al. (2019) reported that *Mentha piperita* L. plants exposed to 9 dS/m increased lipid peroxidation. In order to reduce this damage, it significantly enhanced the DPPH-scavenging activity contents and CAT activity. Also, Khanam & Mohammad (2018) found that this AMP respond to salt stress by enhancing CAT, SOD and peroxidase (POX) and activities under 50, 100 and 150 mM NaCl stress. In addition, during response to 4,8 and 12 dS/m NaCl stress, the activity of SOD, CAT, POX and polyphenol oxidase (PPO) was enhanced to decreased lipid peroxidation by reducing H_2O_2 and O_2^- generation in *Trachyspermum ammi* L. (Abdoli et al. 2020). In the same way, the activities of some antioxidant enzymes such as CAT, SOD, POX, APX and glutathione peroxidase (GPX) were improved in response to 25, 50 and 75 mM NaCl-treatment in *Lavendula officinalis* L. (Nasab et al. 2018). Likewise, the activity of SOD and APX enzymes was enhanced in response to the application of salinity (2 and 4 g L^{-1} NaCl) in *Plectranthus amboinicus lour*. (Ahmed et al. 2020). Also, it has been reported that the DPPH-scavenging activity content was significantly increased in response to salt stress in *Melissa officinalis* L. (Safari et al. 2020) and *Cakile maritima* (Messedi et al. 2016).

II.3.3 Biosynthesis of secondary metabolites

Beside enzymatic antioxidant systems, salt tolerant plants are also equipped by non-enzymatic antioxidant activities (Ahmad et al. 2010). Production of secondary metabolites like flavonoids, phenols and alkaloids is one among the non-enzymatic antioxidant systems induced under salt stress (Aghaei and Komatsu 2013). In fact, it has been reported that these secondary metabolites have a high potential to scavenge ROS and in turn reduced or inhibited the oxidative damages induced by salt stress (Bartwal et al. 2013). In this line, in several AMPs, like *Hyssopus officinalis* (Jahantigh et al. 2016), *Mentha pulegium* (Oueslati et al. 2010) and *Achillea fragratissima Forssk* (Abd EL-Azim and and Ahmed 2009) there was a significant positive correlation between the accumulation of phenol and anthocyanine in plants and their tolerance to salinity. In addition, a significant accumulation of flavonoids, phenolic monoterpenes, phenolic acids and cinnamic acid in *Thymus vulgaris* treated with 60 mM NaCl was noted and this was accompanied with a high tolerance to salt stress (Bistgani et al. 2019). Similar results have been reported in *Melissa officinalis* L. under 100 mM NaCl where the accumulation of phenolic and flavonoid compounds was elevated by 14 and 30%, respectively in NaCl-treated plants (Safari et al. 2020). Likewise, salt stress caused by 100 and 150 mM NaCl application significantly improved the total phenolic contents in *Mentha piperita* L.(Çoban and Göktürk Baydar 2016), and this finding was confirmed by Khalvandi et al. (2019) which reported that salinity (3, 6 and 9 dS/m) remarkably increased total phenols and anthocyanin in this AMP. Also, the total phenol content was improved in response to salt stress in *Mentha pulegium* (Farhadi and Ghassemi-Golezani 2020), *Cakile maritima* (Messedi et al. 2016) and *Rosmarinus officinallis* L. (El-Esawi et al. 2017). Alkaloides, as an antioxidant molecules (Hanana et al.

2011), its concentration was also increased in response to salt stress in some AMPs such as *Chelidonium majus* L. (Yahyazadeh et al. 2018) and *Catharanthus roseus* (Osman et al. 2007).

II.3.4 Biosynthesis and accumulation of osmoprotectants

To maintain normal cell turgor under salinity-mediated osmotic stress, many plant species naturally synthesize and accumulate glycinebetaine and proline as major organic osmolytes (Ashraf and Foolad 2007). Indeed, these compatible solutes are reported to play adaptive roles in mediating osmotic adjustment and protecting subcellular structures in stressed plants through their contribution to cellular osmotic adjustment, detoxification of ROS, protection of membrane integrity and protein stabilization (Bohnert and Jensen 1996; Ashraf and Foolad 2007). More than that, it has been reported that proline content is an accepted parameter for measuring the plant tolerance under salt stress (Nouman et al. 2018). In *Trachyspermum ammi* L., the response to salt stress was correlated with the accumulation of proline in salt stressed plants (Ashraf and Orooj 2006). The same was observed in *Ammi majus* L. (Ashraf et al. 2004), *Hyssopus officinalis* (Jahantigh et al. 2016) and *Rosmarinus officinalis* (Chetouani et al. 2018). In the same way, Farhadi and Ghassemi-Golezani (2020) found that *Mentha pulegium* was responded to 75 mM NaCl stress by increasing the activity of pyrroline-5-carboxylate reductase up to 60% and decreasing significantly proline oxidase activity, explaining the increase of proline content by 19% in salt stressed plants. Likewise, during adaptation to 100 mM NaCl stress, the content of glycine, glutamine, ornithine and proline was significantly increased by 20% , 10%, 15% and 64%, respectively, in *Thymus vulgaris* (Zrig et al. 2019). Moreover, proline values showed significant increase associated with increased salinity levels in *Rosmarinus officinallis* L. (El-Esawi et al. 2017; Hassanpouraghdam et al. 2020), *Cakile maritime* (Messedi et al. 2016) and *Carthamus tinctorius* L. (Shaki et al. 2018). In addition, exposure to 150 mM NaCl enhanced the proline content by 36.49% in *Mentha piperita* L. (Çoban and Göktürk Baydar 2016). Regarding glycine betaine, the production and the accumulation of this neutral osmolyte were increased in *Linum usitatissimum* L. during response to salt stressed conditions (Khan et al. 2010).

In addition to proline and glycine betaine, polyols also act as a compatible solute and have a crucial role during response to salt stress, based on their significant antioxidant capacity (Williamson et al. 2002). Among these osmoprotectant molecules, the soluble sugars content was increased during response to salt stressed conditions in some AMP species like *Lavendula officinalis* L. (Nasab et al. 2018), *Melissa officinalis* L. (Khalid and Cai 2011) and *Thymus vulgaris* (Zrig et al. 2019). Similarly, in *Carthamus tinctorius* L. the soluble sugars and proteins contents in leaves were much higher in salt stressed plant than in the control and this increased significantly with increasing salinity levels (Gengmao et al. 2015). Similar results were observed in *Plectranthus amboinicus lour*. when subjected to 2 and 4 g L^{-1} NaCl stress (Ahmed et al. 2020). In addition, Abdoli et al. (2020) suggested that *Trachyspermum ammi* L. tolerated 4, 8 and 12 dS/m NaCl stress by enhancing the contents of proline, glycine betaine, soluble sugars and soluble proteins.

III. Silicon

In the soil and on the surface of Earth's crust, silicon (Si) is the second most abundant element after oxygen, comprising approximately 28% of the earth's crust (Datnoff and Rodrigues 2005). In the plant, Si is not considered as an essential element for the plant growth, although its content in plant are comparable to the level of many macronutrients like nitrogen, potassium and phosphorus (Epstein 1994, 1999). However, during last few years, it has go much attention, where several reviews are documented that Si could improve the plant tolerance to different biotic and abiotic stressors and its beneficial effects depend on the ability of plants to absorb it from the soil solution (Feng Ma 2004; Debona et al. 2017; Coskun et al. 2019; El Moukhtari et al. 2021b).

III.1 Solubility and forms of silicon in soil

Generally, in the soil, Si amount varies from 400 to 500 g Si Kg^{-1} soil, making it the most abundant element, after oxygen, in the earth's crust (Rizwan et al. 2015). Silicon dioxide (SiO_2) is the usually form of Si in the soil, presenting about 50 to 70% of the soil mass (Epstein 1994; Matichenkov and Bocharnikova 2001). Additionally, in the solid phase of soil, Si compounds are divided into amorphous and crystalline forms (Epstein 1994; Sommer et al. 2006; Figure 4). While, in the soil solution, there are three main groups of soluble Si compounds, monosilicic acids, polysilicic acids and organosilicon compounds (Rizwan et al. 2015; Figure 5). Orthosilicic acid (H_4SiO_4) and its anion ($H_3SiO_4^-$) are the most widely distributed varieties of monosilicic acids and its concentration ranged from 0.1 to 0.6 mM (Epstein 1994, 1999). The maximum solubility of H_4SiO_4 is 1.7 mM at 25°C and pH < 9, it is the form of Si accessible to plants (Epstein 1994; Rizwan et al. 2015).

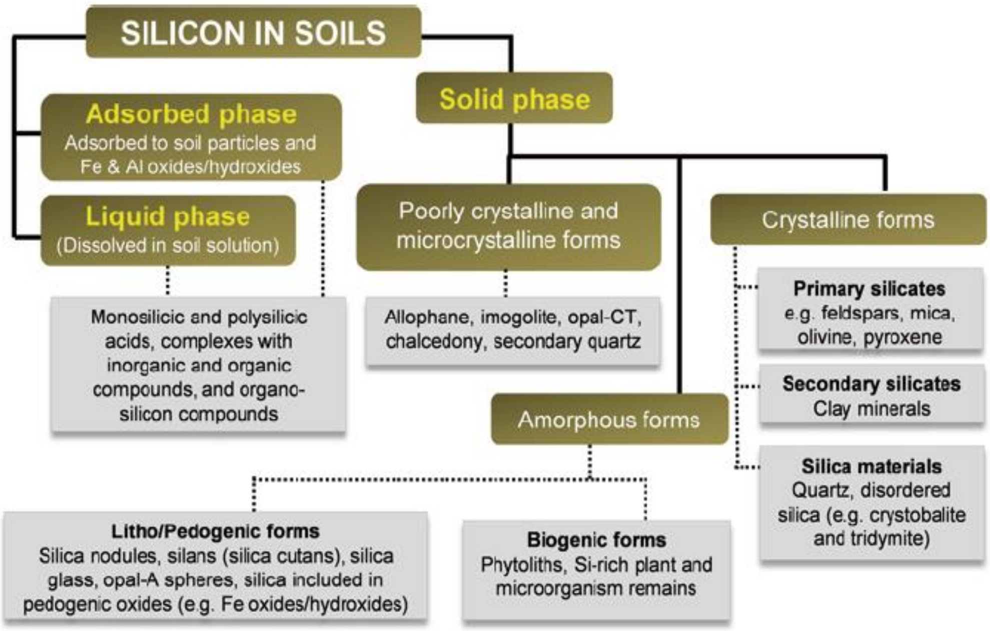

Figure 5: Classification of Si compounds in soil (adopted from Cornelis et al. (2011))

The primary sources of H_4SiO_4 in the soil solution are the various forms of silicon dioxide, silicate minerals and plant residuum (Matichenkov and Bocharnikova 2001). However, the solubility and the content of Si are dependent on the physico-chemical properties of soil like pH, temperature, particle size, water and organic matter contents, redox potential and adsorption-desorption processes (Epstein 1999; Rodrigues and Datnoff 2015). For example, soil pH is the most factor indicating Si compounds solubility (Matichenkov and Bocharnikova 2001). In fact, the solubility of both the crystalline and the amorphous silica is approximately constant between pH values 2 and 8.5, but increases rapidly at pH ~9 (Rodrigues and Datnoff 2015). Likewise, the uncharged H_4SiO_4 is available in soils with pH values <8 ant it dissociates into $H^+ + H_3SiO_4^-$ at pH values above 9 and further dissociates into $2H^+ + H_2SiO_4^{2-}$ at pH values above 11 (Rodrigues and Datnoff 2015). On the other hand, the phytoliths are mainly composed of about 92% silica and 6% water with small amounts of carbon and traces of aluminum and iron (Meunier et al. 2001). For this compound, Fraysse et al. (2009) showed that phytoliths dissolve faster than other silicates at pH > 4 and its solubility is about 17 times higher than that of quartz. Thus, its presence in the soils can be a major source of bioavailable Si for plants in natural ecosystems.

III.2 Silicon uptake and accumulation in plant

In most agricultural soils, plants take up Si from the soil solution in the form of H_4SiO_4, where its concentration ranged from 0.1 to 0.6 mM (Epstein 1994; Rizwan et al. 2015). While, Zhu and Gong (2013) documented that plants could uptake Si from the soil solution in the form of SiO_2. It was documented that Si assimilation is achieved by lateral roots of rice, while the root hairs have not role in Si absorption (Ma et al. 2001). Si assimilate by lateral roots and translocate to shoot parts by involving an influx and efflux transporters, encoded by genes called low-silicon (*Lsi*) genes such as the Lsi1 and Lsi2 in roots and the Lsi6 in shoots (Epstein 1994; El Moukhtari et al. 2021b). In fact, Si transport from soil solution to the root cells is ensured by Lsi1, which is an aquaporin as an influx transporter, encoded by *Lsi1* gene. Lsi2 was shown to encode a local plasma membrane transporter, which ensures Si transport from exodermal cells to the apoplast (Coskun et al. 2019). For translocation to aerial parts, Ma et al. (2001) have reported that Si is translocated to the shoot parts through the xylem using transpiration water flux. In this aerial parts, Ma and Yamaji (2008) reported that Lsi6 is a Si transporter responsible for xylem unloading and subsequently affects the distribution of Si in the leaf. In addition, this latter transporter is identified to be localizes at the xylem parenchyma cells of leaf blades and sheaths (Ma and Yamaji 2008; Rizwan et al. 2015).

After assimilation and translocation, Si is reported to depose and accumulate in the leaf epidermal surfaces, when it is condensed into a polymerized silica gel (SiO_2, H_2O), knowing as a phytolith (Rodrigues and Datnoff 2015). The phytoliths are found in specific cells, named the silica cells, where the deposed Si is immobile and not redistributed (Rizwan et al. 2015). In graminaceous plant species such as maize and sorghum, silica is deposited as silica bodies in the epidermis as well as in the stomata and leaf sheath (Currie and Perry 2007). Likewise, Mecfel et al. (2007) showed that in wheat plants Si was predominately deposited in the epidermis cells of the leaves and their cell walls. In addition to their accumulation in epidermis of shoots, Si is also can occur in cell walls of root endodermis, but the maximum deposition of Si is takes place in the leaf apical region (Ma and Yamaji 2008; Zhu and Gong 2013; Rizwan et al. 2015).

The content of Si in plant can reach up to 10 % of their total dry weight, and this amount varied between plant species according to its Si absorption capacity (Epstein 1994). In this context, Coskun et al. (2019) classed plants as high Si accumulator, intermediate or non-accumulator based on their content on Si. However, Takahashi at al (1990) proposed another classification, grouping plants into three groups, active, passive and rejective Si, based on the mechanism by which plant root assimilate Si from the soil solution. On the other hand, others like Henriet et al. (2006) have reported the coexistence of both active and passive transport within the same plant. The difference between xylem and growth medium about Si concentration is the technic to distingue between active, passive and rejective Si. Indeed, plants that take up Si by active process tend to have higher Si concentrations in the xylem than that of growth medium, those that take up Si by passive process have similar Si concentration both in xylem sap and in growth medium and those that reject have a lower Si in xylem than that in the growth medium (Takahashi et al. 1990; Feng Ma 2004; Rizwan et al. 2015).

In the high and/or active Si accumulator plants like rice and wheat, the Si content is between 1.5–10 % of their total dry weight (Epstein 1994; Ma and Yamaji 2008). The intermediate and/or passive Si accumulator such as cucumber and melon, represent between 0.5 % and 1.5 % of Si of their total dry weight (Coskun et al. 2019). For other plant species, for example, legumes are described as non-accumulator and/or rejective, where their shoots have been reported to contain a Si amount not exceed 0.5% of total dry weight (Meena et al. 2014). In contrast, Ahmad et al. (2019) and Liang et al. (2007) have described soybean and *Vigna radiate*, among legume species, as a Si passive and active accumulator plants, respectively.

III.3 Silicon functions in plants

The number of studies that showed the benefic effect of Si on the growth and development of many plants is vast and continues to increase, although it has not been considered as an essential element for plants. Indeed, the deposition of biogenic silica in shoots increases the structural component of the plant and creates a hard outer layer, diminishing as a result water loss via transpiration and playing as a mechanical wall for plant protection against insects (Epstein 1994; Ma and Yamaji 2008). More than that, it is documented that the beneficial effects of Si become more evident when plants are in stressed environments (Zhu and Gong 2013; Hu et al. 2020; El Moukhtari et al. 2021b).

III.4 Effect of silicon application on plant tolerance to abiotic stresses

Recently the climate change has given rise to several abiotic stresses, like salinity and drought, where the growth and yield of many plants species, including AMPs are greatly destructed. For this reason, several studies are conducted to maintain or improve the production of most agricultural systems by application of different exogenous compounds. Among these, Si had documented to enhance the production of a wide variety of plants under stressed conditions. For example, Si application increased plant growth, biomass, photosynthetic pigments, straw and grain yield, and quality of various plants species under different abiotic stressors like salinity (El Moukhtari et al. 2021a), drought (Alamri et al. 2020), phosphorus deficiency (Hu et al. 2020) and heavy metals accumulation (Liang et al. 2007).

It is known that large amounts of P exist in soils bound in unavailable form (Yang and Post 2011). The effect of Si on P deficiency was initially thought to be associated with the enhancement of P availability in soil (Hu et al. 2020). In this line, numerous works have shown that the increased soil P availability under high Si content can be explained by Si competition with P for binding at the surface of soil minerals, resulting in P mobilization (Hu et al. 2020; Neu et al. 2017). On the other hand, Si supplementation induced a strong root production and exudation du malate and citrate, as organic acids, for acidification of soil solution and in return to solubilize P (Hu et al. 2020). Furthermore, Si has been reported to upregulate the expressions of Pi transporter genes (*TaPHT1;1* and *TaPHT1;2*) in wheat roots at low P availibility stress (Kostic et al. 2017). About plant growth, the high plant biomass was showed in treated plans through exogenous Si under low P availability stress (Zhang et al. 2019; El Moukhtari et al. 2022a). The positive effect of Si under P deficiency was significantly correlated with a significant decrease in oxidative stress indicators and an important increase in antioxidant activity (Zhang et al. 2019; Alamri et al. 2020; Liang et al. 2020). Similarly, several studies have shown the ameliorative effect of Si under drought stress (Zhu and Gong 2013; Namjoyan et al. 2020). Indeed, Ahmad et al. (2007) reported that Si application in soil, 50 and 150 mg kg^{-1} soil, increased shoot and root biomass, and spike weight of drought-stressed wheat. Application of Si increased also grain yield of drought-stressed plants grown both in hydroponics (Pei et al. 2010), in pots (Kaya et al. 2007) and in field condition (Rizwan et al. 2015). Likewise, Shi et al. (2014) showed that Si treatment significantly improved seed germination and alleviated oxidative stress of bud seedlings in tomato under water deficit stress.

In addition to above cited abiotic stresses, Si application also have a significant ameliorative effect under heavy metals accumulation. For example, it has been shown that Si application in the soil affects the soil properties, which in turn control heavy metals availability to plants (Liang et al. 2007; Kim et al. 2017). As a results, Si supply increased the dry weight of both shoots and roots of many plant species such as maize (Liang et al. 2005), lettuce (Pereira et al. 2021), cotton (Farooq et al. 2013) rice (Nwugo and Huerta 2008) and wheat (Rizwan et al. 2012) plants grown in Cd contaminated soils. Also in cucumber plants, Si supply also inhibited the Mn toxicity symptoms and improved the plant growth (Shi et al. 2005). In fact, one of the major effects of Si on the reduction of metal toxicity is reducing the metal uptake by plants. In this context, it has been reported that Si may stimulate production of root exudates, which can chelate metals and reduce their uptake by roots (Fan et al. 2016; Bali et al. 2020). Moreover, the physical barrier formed by the deposition of Si in the vicinity of the endoderm may reduce the cell wall porosity of inner root tissues, thus reducing the metal concentration in the xylem (Shi et al. 2005; Fan et al. 2016). Additionally, Si treatment also increases the plants tolerance to heat stress (Agarie et al. 1998; Shalaby et al. 2021). Regarding salt stress, the mechanisms of Si-mediated alleviation of salinity stress in plants at physiological, morphological, and biochemical levels have been summarized below.

III.5 Beneficial effect of silicon on plant salt tolerance

III.5.1 Effect of silicon on seed germination under salt stress

Seed germination play a crucial role in plant life cycle, where it is the conditioned stage for plant development. However, this crucial stage is very sensitive to abiotic stressors, like salinity, which caused both an osmotic stress accompanied with an oxidative stress, and inhibited, as a result, seed germination. For this reason, numerous studies are realized to improve seed germination under salt stressed conditions by application of exogenous Si. The obtained results showed that Si treatment significantly alleviated the harmful effects of salt stress on many germination traits, such as germination percentage, germination speed and the growth and biomass of stressed seedlings of *glycyrrhiza uralensis* (Zhang et al. 2015), tomato (Haghighi et al. 2012), wheat (Azeem et al. 2015) and barley (Ellouzi et al. 2022). Similarly, Gou et al. (2020) observed that 0.3 mM Si could raise seed germination percentage and germination index of cucumber under NaCl stress. Si mediated the negative effects of salt stress on germination by increasing embryo viability and decreasing salt ions accumulation in embryonic cells. Others correlated the Si benefits with improving seed reserve mobilization in Si treated salt stressed seeds (Gou et al. 2020; Zhang et al. 2020). In addition, GA/ABA ratio is a key ratio regulating seed germination (Shuai et al. 2017). In this context, Si has been reported to mediate *Cucumis sativus* L. seed germination under high level of salt stress (up to 250 mM NaCl) by inhibiting the expressing of *GA20ox* catabolism gene as well as those responsible for ABA anabolism such as *NCED1* and *NCED2* (Gou et al. 2020). Antioxidant activity is also improved though Si treatment to reduce and regulate oxidative stress markers production in salt stressed seedlings. In this context, Zhang et al. (2015) suggested that supplemented Si enhanced seed germination of *Glycyrrhiza uralensis* by reducing MDA content and improving antioxidant enzyme activity under salt stress. Therefore, Si is directly involved in the central mechanisms to enhance seed germination, and their application it might be a potential approach to improve seed germination under salt stressed conditions.

III.5.2 Effect of silicon on plant growth and biomass under salt stress

Plant biomass is severally affected under salt stressed conditions, whereas, Si supply was shown to enhance plant growth at different agronomical, morphological and physiological levels (El Moukhtari et al. 2021b). Indeed, Meng et al. (2020) found that exogenous Si could mitigate the adverse effects of NaCl on *Medicago sativa* growth by greater shoot and root dry weight by 16 % and 11 %, respectively. Similar results have been observed in salt-stressed cowpea and kidney beans, where 1 mM $CaSiO_3$ significantly improved root dry weight, stem dry weight, shoot dry weight and whole plant dry weight in the two evaluated plant species (Murillo-Amador et al. 2007). El Moukhtari et al. (2021a) found that 3 mM Si could alleviate *Medicago sativa* damage caused by 120 mM NaCl and improve plant fresh and dry weight, as well as the number of leaves. In addition to the number of leaves, Si was also found to be able to delay the premature leaf senescence under salt stress (Alamri et al. 2020). Adding Si to fenugreek was found effective in alleviating the negative effects of salinity on shoot dry weight and root dry weight (Nasseri et al. 2012). Moreover, supplementation of exogenous Si to salt-stressed *Phaseolus vulgaris* resulted in an increase of seed number, 100-seed weight and yield (Parande et al. 2013). According to the above findings, the treatment with Si is strongly ameliorates plant growth and production under salt stressed environments, and the mechanisms by which Si induced these benefits is detailed below.

III.5.3 Effect of silicon on photosynthesis under salt stress

Photosynthesis is among the key physiological processes for plant development, and their activity and performance is evaluated by many parameters like chlorophyll content, net photosynthesis, leaf area, stomatal conductance and photosystems efficiency. However, it is documented that exposition to salt stress significantly reduced all of photosynthetic pigments content, stomatal conductance and photosystem II efficiency in many plants species like alfalfa (Meng et al. 2020), tomato (Almutairi 2016), basil (Robatjazi et al. 2020) and liquorice (Shen et al. 2022). Conversely, supplementation of Si to the growth medium reversed this negative effect of salt stress on photosynthetic parameters (Meng et al. 2020; El Moukhtari et al. 2021b). In this context, El Moukhtari et al. (2021a) showed that added Si significantly increased plant biomass, chlorophyll content, leaf area, chlorophyll stability index, photosystem II efficiency and stomatal conductance in salt stressed *Medicago sativa*. The beneficial effects of Si on chlorophylls content under salt stress has been studied by Alamri et al. (2020) in *Brassica juncea*. The results showed that Si could increase the activity of some chlorophyll synthesis enzymes, including δ-aminolevulinic acid dehydratase and porphobilinogen deaminase, while it inhibits those responsible of chlorophyll degradation, such as chlorophyllase, chlorophyll-degrading peroxidase and pheophytinase. In addition, under salt conditions, Si was also reported to increase stomatal number and improves RuBisCO activity, as a result increased internal CO_2, this supports a key role of Si in photosynthetic activity (Murillo-Amador et al. 2007; Esmaeili et al. 2015). However, there are other photosynthesis related parameters like quantum yield of photochemical energy conversion (Y(II)), quantum yield of non-regulated non-photochemical energy loss (Y(NO)), quantum yield of regulated non-photochemical energy loss (Y(NPQ)) and photosystem I oxidation. But, the effect of Si treatment on these latter traits remained poorly understood and needs further investigation to clarify and understand the role of Si in photosynthesis machinery under salt stressed environments.

III.5.4 Effect of silicon on water statute and compatible solutes accumulation under salt stress

The negative effects of salt stress on plant growth could be the results of an osmotic stress, as the absorption and content of water were reported to diminish in salt stressed plants (Rasool et al. 2012). However, it reported that exogenous Si supply enhanced relative water content (RWC) under salt stress in several plant species, including cucumber (Mousavi et al. 2022), maize (Rohanipoor et al. 2013; Ali et al. 2021b;), turfgrass (Esmaeili et al. 2015) and wheat (Hajiboland et al. 2017). In the same line, Siddiqui et al. (2014) and Avestan et al. (2019) showed that Si supply along with NaCl significantly improved RWC, enhancing as a result plant growth performance. Similarly, in salt stressed alfalfa, El Moukhtari et al. (2021a) found that Si treatment remarkably increased leaves RWC and this increase was significantly correlated with an improvement in plant biomass, photosynthetic machinery and antioxidant activity. To explain the enhanced RWC in Si treated, Epstein (1994) and Wang et al. (2022) suggested that, after the uptake, Si accumulates on the epidermis of various plant tissues mainly as a polymer of hydrated amorphous silica, and consequently raised the wax content of the plant epidermis to overcome water loss by transpiration especially under osmotic stress conditions. On the other hand, Si has been reported to upregulate aquaporin gene expression, lead, as a result, to an increase in root conductivity and water uptake under stressed conditions (Rios et al. 2017).

Several reports documented that accumulation of compatible solutes is one among the important mechanisms to maintained water statue in plant tissues (Suprasanna et al. 2016; Meena et al. 2019; El Moukhtari et al. 2020). In this context, it was reported that application of exogenous Si caused a significant accumulation of osmolytes compounds, like proline and glycine betaine, where this accumulation was correlated with an increase of relative water content under salt stressed conditions (Abbas et al. 2015; Alamri et al. 2020; El Moukhtari et al. 2021b). Likewise, Pereira et al. (2013) reported that Si-mediated incensement in proline, glycine betaine and total free amino acids content contribute to the osmotic adjustment in two *Capsicum annuum* cultivars subjected to salt stress. In addition to their role in osmoregulation, proline acts also as an antioxidant molecule and their accumulation is known as a tolerance index, because its concentration has been shown to be generally higher in salt tolerant than in salt sensitive plants. According to Abbas et al. (2015), exogenously applied Si was responsible for high accumulation of proline in the salt sensitive genotype of *Abelmoschus esculentus.* Similarly, exposed *Onobrychis viciaefolia* and *Vigna radiata* L. plants to salinity stress have higher proline contents when treated with exogenous Si (Wu et al. 2017; Ahmad et al. 2019). Zhang et al. (2018a; b) reported that treatment of salt-stressed *Glycyrrhiza uralensis* by Si led to a significant accumulation of endogenous proline. Conversely, in a study carried out by Lee et al. (2010), the authors found that the application of exogenous Si, at 2.5 mM, to the soil solution of 80 mM NaCl-stressed *Glycine max* L. reduced proline content as compared to Si-untreated salt-stressed plants. Furthermore, Zamani and Izanloo (2017) also reported the negative correlation between Si addition and proline content in salt stressed chickpea.

In addition to proline and glycine betaine, soluble sugars act also as compatible solutes and its content was interestingly improved after Si treatment. Indeed, under 100 mM stress, the amount of glucose, sucrose and fructose was increased by 20–30 % in root of *Onabrychis viciaefolia* plants when treated with 1 mM Na_2SiO_3 as compared to salt stressed control (Wu et al. 2017). The same was reported by Zhang et al. (2022) in alfalfa, where the results showed that soluble sugars content was found to be elevated after exogenously applying Si under salt stress.

III.5.5 Effect of silicon on salt ions accumulation and nutrient uptake under salt stress

Salt stress destroys the growth and development of plants through many harmful effects, like induction of osmotic and oxidative stress and ionic toxicity. Absorption and accumulation of salt ions, like Na^+ and Cl^-, and inhibition of nutrient uptake, such as K^+, Ca^{2+} and P, induced an ionic toxicity and desturb as a result plant development (Karimi et al. 2005). In fact, during response to salt stress, inhibition or reduction of Na^+ uptake and/or its compartmentation into vacuole represent an important adaptive strategy used by tolerant plants to reduce salt ionic toxicity, as well as increase their tolerance to this abiotic constraints (Peng et al. 2016). Importantly, it was documented that Si was able to alleviate the deleterious effects of salt stress on plant growth by diluting salt ions concentration in the cells and improving those of essential and beneficial elements (El Moukhtari et al. 2021b). In fact, when *Anethum graveolens* and *Lonicera japonica* L. were treated with exogenous Si, a decrease in Na^+ uptake was observed, leading to an increase of K^+/Na^+ ratio (Gengmao et al. 2015a; Shekari et al. 2017). Similarly, Kafi and Rahimi (2011) showed in *Portulaca oleracea* L. that adding 1 mM Si to the nutrient solution was able to reduce leaf Na^+ and Cl^- content with an increase in K^+/Na^+ ratio under salt stressed conditions. Liu et al. (2018) reported that Si priming significantly accumulated more Na^+ in the roots, but led to a decrease in Na^+ accumulation and an increase in K^+ accumulation in the alfalfa leaves under alkaline stress. In contrast, Murillo-Amador et al. (2007) showed a low shoot/root Na^+ ratio in *Phaseolus vulgaris* and *Vigna unguiculata*. Besides, in *Phaseolus vulgaris,* Zuccarini (2008) reported that Si reduced Na^+ in the leaves more than in the roots. Thus, these latter demonstrate that Si not only reduced the uptake of Na^+ by roots, but also its translocation to aerial parts. Two Na^+/H^+ antiporters were reported during Na^+ compartmentation in vacuole or its exclusion to cytosol, SOS1 localized in the plasma membrane and NHX in the tonoplaste (Keisham et al. 2018). In this context, Bosnic et al. (2018) showed that, in salt-stressed *Zea mays,* 1.5 mM of exogenous Si upregulated both SOS and NHX transcript levels under 40 mM NaCl and decreased root Na^+ content. Additionally, K^+/H^+, a symporter plays an important role in maintaining K^+ homeostasis, Si was also documented to increase the activity of this symporter under salt stress, increasing K^+ content and in return helping to maintain osmotic homeostasis (Rizwan et al. 2015; El Moukhtari et al. 2021b; Figure 6). These findings could explain the fact that Si decreased salt ions and increased K^+ in salt stressed plants.

Si treatment was also reported to improve the content of other essential and benefic elements, like N, P, and Ca. In fact, Garg and Bhandari (2016) reported that Si application increased the content of N, P, Mg and K in *Cicer arietinum* L. under salt stress. Similarly, 3 mM of exogenous Si significantly improved the tolerance of *Medicago sativa* to 120 mM NaCl and increased Ca^{2+} K^+ and N contents (El Moukhtari et al. 2021a). Increased the uptake of K^+ and Ca^{2+} following application of Si were also reported in salt-stressed cowpea, kidney bean (Murillo-Amador et al. 2007) and *Vigna radiata* (Ahmad et al. 2019).

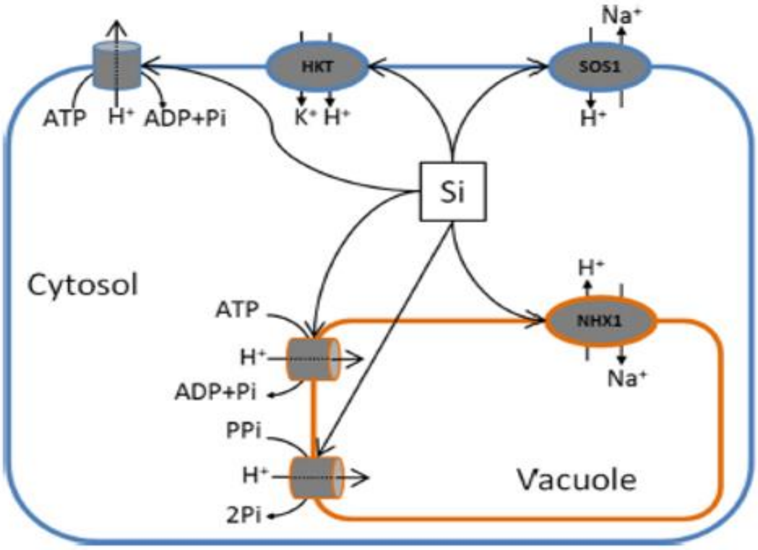

Figure 6: Proposed mechanisms of silicon in the alleviation of salt ions accumulation in plant cell (adopted from El Moukhtari et al. (2021b).

III.5.6 Effect of silicon on oxidative stress and antioxidant activity under salt stress

The content of MDA and ROS, in terms of H_2O_2 and O_2^- are widely used as oxidative stress markers. Application of exogenous Si alleviated the oxidative damages of salt stress by reducing the accumulation of MDA, H_2O_2 and O_2^- in salt stressed alfalfa, where this reduction was accompanied with a significant increase in plant growth parameters and photosynthetic attributes (El Moukhtari et al. 2021b). Similarly, many studies showed that ROS accumulation and membrane cell permeability were significantly declined in response to exogenous Si application under salt stressed conditions (Al-aghabary et al. 2007; Avestan et al. 2019; Robatjazi et al. 2020). In addition, Siddiqui et al. (2014) suggested that the increase in plant germination and growth characteristics through application of nano-SiO_2 might reflect a reduction in oxidative damages in *Cucurbita pepo* L. Similar findings were reported by Mahmood et al. (2016), who showed that electrolyte leakage was reduced by 24% in salt-stressed mung bean as a response to 2 kg K_2SiO_3 ha^{-1} applied as a foliar spray. Likewise, exposure of *Glycyrrhiza uralensis* to NaCl, increased MDA content and membrane permeability in plants after 150 days of treatment and this was found to be mitigated by 0.6 g K_2SiO_3 Kg^{-1} soil supplementation (Zhang et al. 2018b). Additionally, exogenous Si-mediated membrane stability and lipid peroxidation under salt stress was also reported in alfalfa (Meng et al. 2020), *Momordica charantia* (Wang et al. 2010) and *Anethum graveolens* (Shekari et al. 2017).

To overcome salinity-mediated oxidative stress, tolerant plants adopt some tolerant strategies. This includes the induction of the enzymatic and non-enzymatic antioxidant systems (Aghaei and Komatsu 2013). Accumulation of ascorbic acid, total polyphenols and flavonoids, act as non-enzymatic antioxidant compounds, was significantly increased in several plants species in response to Si application under salt stressed conditions (Kim et al. 2017; Kubi et al. 2021; El Moukhtari et al. 2021b). In the same line, Robatjazi et al. (2020) reported that Si supply detoxifies the accumulation of ROS by increasing the level of polyphenols and radical scavenging activities in the aerial parts of basil under salinity constraints. Additionally, induction of enzymatic antioxidant pathway has also improved after Si incorporation to alleviate oxidative stress under salt stress (Rizwan et al. 2015; Kim et al. 2017). In this context, under salt stress, it was reported that the activity of some antioxidant enzymes like, CAT, SOD and POD, was raised in many Si treated plants such as alfalfa (Meng et al. 2020), *Brassica juncea* (Alamri et al. 2020), maize (Abdel Latef and Tran 2016) and *Glycyrrhiza uralensis* (Zhang et al. 2017). Similar findings have been documented by Wang et al. (2011) in 120 mM NaCl-treated *Medicago sativa*, where the supplementation of 1 mM K_2SiO_3 to the growth medium significantly increased the activity of APX, CAT and POD in roots, shoots and leaves. According to Liu et al. (2015), Si inhibited the increase in the root H_2O_2 levels by enhancing the activities of antioxidant enzymes in salt stressed *Sorghum bicolor* L. In addition, in a study conducted by Zhang et al. (2017) on *Glycyrrhiza uralensis*, where AsA and GSH contents was increased and the activities of APX, SOD, POD and CAT were improved by 1 mM of Si under 100 mM NaCl stress. Application of exogenous Si increased plant germination and growth characteristics through a reduction in oxidative damage and expression of antioxidant enzymes, such as CAT, POD, GR and SOD in *Cucurbita pepo* L. (Siddiqui et al. 2014). According to the above-cited studies, Si could reduce oxidative stress in plant under salinity by inducing both enzymatic and non-enzymatic antioxidant systems.

III.5.7 Effect of silicon on symbiosis establishment under salt stress

As other plant species, salinity has devastating impacts on legume plants by affecting germination, reproductive stage, and the ability of biological nitrogen fixation (BNF) in legumes (Nadeem et al. 2019). In fact, BNF in legumes has proved in many studies to be limited under salt stress, because of the high salt sensibility of nodulation process and nitrogenase activity (El Moukhtari et al. 2021b). Importantly, Si treatment has been documented as one of the important approaches to alleviated harmful effects of salt stress on BNF by improving nodulation and N_2 fixation particularly (Etesami and Adl 2020; Putra et al. 2020). In this context, enhancing BNF by Si supply was reported under salt stress in *Sesbania aculeata* (Kurdali et al. 2018) and alfalfa (El Moukhtari et al. 2021a). Putra et al. (2021) found that the activity of nitrogenase in *Medicago truncatula* root nodules was raised by more than 85 % upon Si-treatment, which potentially reflects an increase in N fixation mediated by rhizobia. In the same study, the authors suggested that increased BNF after Si application might be related to the ability of Si to accelerate exchanges of solutes and gasses between the soil and the plant (Putra et al. 2021). In the same way, Putra et al. (2020) explained the increased number of nodules upon Si treatment by induction of some key symbiotic signals such as flavonoid compounds required for symbiosis establishment. On the other hand, it was reported that Si also have a positive structural effect inside nodules by increasing the number of bacteroids and symbiosomes (Nelwamondo et al. 2001). Taken together, Si application could be a promoting technic to enhance legume growth performance by improving BNF, but the mechanisms by which exogenous Si increase nodulation and N fixation are not yet detailed.

III.5.8 Effect of silicon on aromatic and medicinal plants production under salt stressed conditions

Si, as a beneficial element, is exogenously used to improve the salt stress tolerance of some AMPs, such as *Anethum graveolens* (Shekari et al. 2017), *Portulaca oleracea* L. (Kafi and Rahimi 2011b) and *calendula officinalis* L. (Bayat et al. 2013). Gengmao et al. (2015a) showed that Si application helped to maintain the integrity of chloroplast ultrastructure, enhanced the activities of SOD and CAT and increased K^+/Na^+ ratio of *Lonicera japonica* L. under 100 and 200 mM NaCl stress. in the same way, Xu et al. (2015) reported that exogenous application of Si significantly improved the activity of plasma membrane H^+-ATPase, tonoplast H^+-ATPase and H^+-pyrophosphates of *aloe* L. root and leaf under 100 mM NaCl stress. Thus, it keeps cells homeostasis under salt stress conditions. In addition, Si supplementation could induce also the tolerance of *Ocimum basilicum* L. (Robatjazi et al. 2020), *Trigonella foenum-graecum* L. (Nasseri et al. 2012) and *Pelargonium graveolens* (Hassanvand et al. 2019) against salt stress. However, the mechanism by which Si treatment mitigated the negative effects of salinity on the growth and development of AMPs is not yet detailed so far. For this reason, future studies are needed to assess the beneficial effect of Si application on the tolerance of AMPs under different abiotic constraints.

Chapter 2: Exogenous silicon improves salt tolerance of fenugreek (*Trigonella foenum-graecum* L.) during seed germination and early seedling stages

Abstract

In the present study, we investigated the effects of exogenous silicon (Si) treatment on seed germination, embryo viability, seedling growth, antioxidant molecules and osmotic regulation in fenugreek under salt stress. The seeds were germinated in Petri dishes at 25 ± 1 °C for 8 days under 0 mM (control) versus 200 mM NaCl (salt stress) with or without 3 mM Si treatment. The obtained results showed that salt stress significantly reduced final germination percentage, germination speed, velocity index, germination energy, peak value, germination value and vitality index, but increased the mean germination time of fenugreek seeds. Salinity also significantly impaired seedling length and fresh weight. However, Si supplementation contracted the negative impacts of salinity and significantly increased all of the studied germination traits except of mean germination time. Additionally, Si alleviated the negative effects of salt on embryo viability and improved its ability to mobilize sugar and protein reserves. Furthermore, 200 mM NaCl increased Na^+ content and induced the oxidative stress reflected by high malonyldialdehyde content, reactive oxygen species accumulation and electrolyte leakage percentage. However, salt-mediated oxidative stress was alleviated by Si treatment through a significant decrease of Na^+ content and an increase of endogenous Si content, which correlated with a significant induction of enzymatic and non-enzymatic molecules with antioxidant function. Altogether, these findings suggested that, the exogenous Si treatment could be a potential method to enhance salt tolerance of fenugreek during seed germination.

Keywords: Antioxidant activity; Embryo viability; Fenugreek; Salinity; Seed reserve mobilization; Silicon.

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I. Introduction

In Morocco, one among the major fenugreek-producing countries, fenugreek (*Trigonella foenumgraecum* L.) has been used as a spice crop for a long time. It is also widely used in traditional medicine as a remedy against fever and anemia, and for appetite stimulation (Haddad et al. 2003; Benayad et al. 2014a). Indeed, it was documented that fenugreek has various medicinal and therapeutic properties, explained by it richness in bioactive molecules such as alkaloids, flavonoids, amino acids, tannins, saponins and some steroidal glycosides (James and Devi 2021). The seeds of this species are used against several diseases like hypercholesterolemia, cancer, constipation and hypertriglyceridemia (Basch et al. 2003; Khorshidian et al. 2016; Hozzein et al. 2020). Also, the extracts of fenugreek seeds contain many molecules with cytotoxicity effect for cancer cells (Alrumaihi et al. 2021). On the other hand, as leguminous species, fenugreek may fix 48% of its total nitrogen (N) during growing season by its symbiotic N-fixation involving soil bacteria called rhizobia (Singh et al. 2008). This process contributes towards soil fertility improvement and reducing the inputs of chemical N fertilizers.

Despite the aforementioned advantages, the productivity and growth stages, including germination and early seedling stages, of this important aromatic and medicinal plant are negatively affected by several abiotic stresses such as salinity stress. In fact, due to climate change, salinization has become a global environmental problem. It was predicted that more than 50% of the total arable land will have salinity problems by 2050 due to the annual increase from 1 to 2% rate of salt-affected soils in the world (Bianco and Defez 2009; El Moukhtari et al. 2020), thus as a result, the plants productivity, including aromatic and medicinal plants, will considerably decrease.

Germination is a key stage in the life cycle of plants. Once germinated, seedling establishment is of critical importance to crop productivity, especially under stressed conditions. Salinity grievously affected the seed germination of many aromatic and medicinal plants such as *Trigonella foenumgraecum*, *Thymus daenensis* Celak., *Thymus kotschyanus* Boiss. and *Origanum compactum* Benth. (Khoshsokhan et al. 2012; Laghmouchi et al. 2017; Mahmoudi et al. 2019). The harmful effects of salt stress on seed germination could be explained by inhibition of seed reserve mobilization and activity of some hydrolytic enzymes like α-amylase, β-amylase and α-glycosidase (Sidari et al. 2008). Salinity has also been reported to cause an ionic toxicity through the accumulation of Na^+ and Cl^- ions, disturbing the ion homeostasis

(Farissi et al. 2011). Furthermore, Liu et al. (2019) reported that application of 200 mM NaCl stress significantly decreased gibberellic acid (GA)/abscisic acid (ABA) ratio in *Limonium bicolor* Bag. by down-regulating the expression of *GA20ox* and *GA3ox* (the key genes involved in the biosynthesis of GA), while the expression of *NCED1* and *NCED3* (the key genes involved in the biosynthesis of ABA) was significantly up-regulated in salt stressed seeds. Hence, salt stress might also affect the embryo metabolism by disturbing hormonal balance. On the other hand, Luo et al. (2021) suggested that salinity actives the transcription of NADPH oxidase genes such as *RbohD*, which leads to uncontrolled production and accumulation of reactive oxygen species (ROS), resulting in cell membrane damage and a decrease in seed vigor.

Currently, many strategies are used to alleviate salinity problems on crop production worldwide. One of these strategies focused on the conventional breeding techniques for developing salt tolerant verities, but due to its multigenic traits, this approach has not been successful (İbrahimova et al. 2021). Another approach looks at the use of exogenous substances, as organic or inorganic stimulants, helping plant to tolerate drastic conditions of environment (Hassanvand et al. 2019; Zrig et al. 2019; Robatjazi et al. 2020). However, the mechanism by which these exogenous compounds improved plant tolerance to abiotic stresses is still poorly understood. Likewise, regarding silicon (Si) application, as the second most abundant mineral after oxygen in the Earth’s crust (Yan et al. 2018), Ivani et al. (2018) reported that nanosized SiO_2 application significantly increased the germination percentage of the fenugreek seeds and enhanced their vigor under salt stress. But, the mechanism by which Si improved salt tolerance of fenugreek during seed germination, are still not fully understood and have not been detailed (Shi et al. 2014; Sun et al. 2021). Therefore, in this study, we investigated the effects of applying exogenous Si on the seed germination of fenugreek under salt stress. Our work focuses on different physiological and biochemical properties associated with fenugreek salt tolerance. The role of Si in salt stress mitigation with link to seed reserve mobilization, embryo viability, enzymatic and non-enzymatic antioxidant system and organic osmolytes were detailed.

II. Materials and methods

II.1 Plant material and treatments

Fenugreek (*Trigonella foenum-graecum* L.) seeds, supplied by the National Institute of Agronomic Research (INRA Morocco), were surface-disinfected for 5 min with sodium hypochlorite solution (6%) and rinsed thoroughly with sterile distilled water (El Moukhtari et al. 2021a). After disinfection, every forty seeds were transferred into 9 cm diameter sterile Petri dishes containing two layers of Whatman paper No. 1 and were then moistened with 7 mL of 200 mM NaCl with or without 3 mM Si. Others were soaked with 7 mL of sterile distilled water or 3 mM Si and served as controls. Seeds were germinated in the dark at 25 ± 1 °C and 60% - 80% relative humidity for 8 days, with five repetitions per treatment. 200 mM NaCl and 3 mM Si were used according to the results of our preliminary experiments, showing that the germination of fenugreek seeds was not affected by the NaCl concentrations lower than 200 mM, and the 3 mM Si was the effective concentration of Si to alleviate the negative effects of 200 mM NaCl on fenugreek seed germination.

II.2 Assessment of germination traits

The number of germinated fenugreek seeds, with extended radicle for at least 2 mm, was recorded every 24 h. After 8 days of germination, seedling fresh weight (FW) and total seedling length were determined and some germination parameters were calculated by using the formulas indicated in Table 5.

Table 5: All studied germination parameters

Germination parameters	Formula	References
Final germination percentage *(FGP)*	$FGP = (n/N)*100$ Where n is the number of germinated seeds and N is the total number of tested seeds	(Farissi et al. 2011)
Germination speed *(GS)*	$GS = \sum (ni/ti)$ Where ni is the number of germinated seeds on day ti and ti represents the corresponding day of germination	(Hojjat and Kamyab 2017)
Mean germination time *(MGT)*	$MGT = \sum Dn/\sum n$ Where n is the number of seeds newly germinated at time D and D is the days from the beginning of the germination test	(Moradi Dezfuli et al. 2008)
Velocity index *(VI)*	$VI = \sum (G/t)$ Where G is the germination percentage from 2 days and t is the total time of germination	(Khan and Ungar 1984; Farissi et al. 2011)
Germination energy *(GE)*	$GE = \frac{N1}{D1} + \frac{N2 - N1}{D2} + \cdots + \frac{Nj - Ni}{Dj}$ Where N is the number of germinated seeds on the counting date and D is the number of days	(Calone et al. 2020)
Peak value *(PV)*	$PV = M_{ag}/ D$ Where M_{ag} is the maximum of seeds accumulative germination and D is the germination time	(Czabator 1962)
Germination value *(GV)*	$GV = PV*MDG$ Where MDG = number of germinated seeds/ number of days	(Czabator 1962)
Vitality index	$\text{Vitality index} = S*GI$ Where S is the seedling length, and GI is the germination index	(Wang et al. 2010)

II.3 Seed reserve mobilization

During seed germination, representative samples of germinated and non-germinated seeds were taken at different germination times and stored at -20 °C. The seed reserve mobilization was evaluated by measuring soluble proteins and sugar contents at 0, 2, 4, 6 and 8 days of germination.

Extraction of soluble proteins was assessed by homogenization of 100 mg fresh samples in 4 mL Tris-HCL buffer (0.1 M, pH 7.5). After centrifugation at 14,000 rpm for 15 min at 4 °C, 2 mL of Bradford reagent was added to 2 mL of supernatant. The content of soluble proteins was determined after optic density (OD) measurement at 595 nm by referring to standard curve prepared with bovine serum albumin solutions (Bradford 1976).

Assessment of soluble sugars content was realized by grinding 100 mg fresh samples in 4 mL of 80% ethanol. Then, the mixture was centrifuged at 5,000 rpm for 15 min at 4 °C and 1 mL of 5% phenol and 5 mL of concentrated sulfuric acid were added to 1 mL of supernatant. After cooling, the OD was measured at 485 nm and the content of soluble sugars was calculated from a standard curve prepared with glucose solutions (Dubois et al. 2002).

II.4 Evaluation of embryo viability

Embryo viability of the non-germinated seeds was assessed by a cytochemical method using 2,3,5 triphenyltetrazolium chloride (TTC). TTC assay is based on the fact that viable embryos are red stained due to the reduction of TTC by cell respiratory activity (Verma and Majee 2013). 10 non-germinated seeds from each treatment were taken at different germination times (0 h, 24 h and 48 h), immersed in 10 mg mL-1 TTC and incubated at 30 °C in the dark for 24 h. After draining of the TTC solution, seeds were washed three times with distilled water and the embryos were isolated under a binocular magnifying glass with 10x magnifications and photographed.

II.5 Membrane cell stability and oxidative stress markers

Electrolyte leakage percentage (EL) of 8-day-old fenugreek seedlings was determined accordingly to the method used by Ghoulam et al. (2002). Fresh seedling segments were washed and placed in 10 mL of distilled water and then incubated under agitation for 24 h at 25 °C. After incubation, electrical conductivity was measured before (EC1) and after autoclaving and cooling at 25 °C (EC2) to calculate the EL by the following formula:

$$\mathbf{EL\ (\%) = (EC1/EC2) * 100}$$

Malonyldialdehyde (MDA) content in fenugreek seedlings were estimated using the thiobarbituric acid (TBA) method as described previously by Heath and Packer (1968). Briefly, 100 mg fresh weight were ground in 1 mL of extraction solution, containing 0.5% TBA and 20% trichloroacetic acid (TCA) and incubated at 95 °C for 30 min. After centrifugation at 14,000 rpm for 10 min, the OD was determined at 532 nm and 600 nm, and then the content of MDA was calculated using its extinction coefficient of 155 mM^{-1} cm^{-1}.

Hydrogen peroxide (H_2O_2) of 8-day-old fenugreek seedlings was determined following the procedure developed by Brennan and Frenkel (1977) with some modifications. 100 mg fresh samples were homogenized in 2 mL of cold acetone and centrifuged at 5,000 rpm for 15 min at 4 °C. Then, 150 µL of titanium (20% titanic tetrachloride in concentrated hydrochloric acid (HCl), v/v) were added to 1350 µL of supernatant, afterward 300 µL of concentrated ammonia was added to the mixture to precipitate the peroxide-titanium complex. After centrifugation at 10,000 rpm for 10 min, the supernatant was discarded and the precipitate was washed five times with cold acetone. The H_2O_2 precipitated was solubilized in 3 mL of 2N sulfuric acid and its content was determined after OD measurement at 410 nm.

Cytochemical detection of superoxide anion (O_2^-) in the 8-day-old fenugreek seedlings was assessed using the nitro blue tetrazolium chloride (NBT) method (Kumar et al. 2014). For this purpose, 12 seedlings from each treatment were immersed in 0.2% NBT solution, prepared in 50 mM sodium phosphate buffer (pH 7.5), and then they were incubated overnight in the dark. The reaction between O2- of seedlings and NBT has given a dark blue stain in the reaction site. After staining, samples were transferred on a paper towel saturated with 60% glycerol until they were photographed using a digital camera.

II.6 Determination of antioxidant enzymes activities

Superoxide dismutase (SOD) extraction was assessed by grinding 0.1 g of fresh materials in 1 mL of 50 mM phosphate buffer (pH 7.8), containing 1% of insoluble polyvinylpyrrolidone (PVP) and 0.1 mM ethylenediaminetetraacetic acid. After centrifugation at 12,000 × g for 20 min at 4 °C, SOD activity was measured using the NBT method (Beyer and Fridovich 1987). One enzymatic unit of SOD was defined as the content of enzyme required to inhibit the reduction of 50% NBT and SOD activity was expressed as an enzymatic unit (U) mg^{-1} proteins.

Polyphenol oxidase (PPO) enzyme was extracted by homogenizing 100 mg of fresh samples in 1 mL of 50 mM phosphate buffer (pH 6), containing 5% of PVP. Afterwards, the PPO activity was assayed according to Hori et al. (1997) by following the oxidation of the 10 mM catechol for 3 min at 410 nm. One unit of PPO activity was defined as the amount of enzyme causing 0.01 absorbance increases.

For both antioxidant enzymes, the content of enzymatic proteins of extracts was determined according to Bradford (1976) method.

II.7 Determination of total polyphenols and flavonoids

Total polyphenols and flavonoids were extracted by grinding 100 mg of fresh samples in 1 mL of methanol (80%). After centrifugation at 12,000 × g for 20 min at 4 °C, the supernatant was stored at -20 °C until total polyphenols and flavonoids determination. The content of total polyphenols was determined through the Folin-Ciocalteu method and their concentration was described as mg gallic acid equivalents g^{-1} FW (Singleton and Rossi 1965). However, the content of flavonoids was assessed by using the method described by Chang et al. (2002), where the flavonoids content was calculated referring to a standard range of different concentrations of quercetin and expressed as mg quercetin g^{-1} FW.

II.8 Determination of organic osmolytes

Method of Bates et al. (1973) was used for proline content determination. 100 mg of fresh matter were homogenized in 1 mL of aqueous sulfosalicylic acid (3%) and centrifuged at 14,000 rpm for 10 min at 4 °C. To 400 µL of resulted supernatant, 400 µL of ninhydrine reagent and 400 µL of concentrated acetic acid were added and the mixture was incubated at 95 °C for 1 h. After cooling down, 800 µL of toluene was added and the OD of the pink phase was read at 520 nm. Afterward, the proline content was calculated from a standard curve prepared with proline.

Regarding glycine betaine measurement, the method descripted by Grieve and Grattan (1983) was used with some modifications. Indeed, 500 mg of dried materials were mechanically shaken with 15 mL of distilled water for 48 h at 25 °C. After filtration, the filtrate was diluted 1:1 with 2N sulfuric acid, and then the mixture was incubated under agitation and cooled in ice water for 1 h. Then, 0.4 mL of cooled potassium iodide-iodine ($KI-I_2$) reagent was added to 1 mL of mixture and incubated at 4 °C for 16 h. After centrifugation at 10,000 rpm for 15 min at 0 °C, the supernatant was carefully aspirated and the precipitate was dissolved in 6 mL of 1.2 dichloroethane. The OD of the dichloroethanic phase was measured at 365 nm and the content of glycine betaine was determined from a standard curve.

II.9 Determination of Si, potassium (K^+), sodium (Na^+) and calcium (Ca^{2+}) contents

The mineral analysis of 8-day-old fenugreek seedlings was conducted according to Liu et al. (2013) with some modifications. Briefly, 100 mg of dry weights were incinerated at 600 °C for 6 h in a Protherm Furnaces (PLF 120/12). Afterward, the obtained ashes were acid digested by using a mixture of 3 mL of concentrated nitric acid and 6 mL of concentrated HCl, then the mixture was heated for 1 h at 200 °C. The content of Si, K^+, Na^+ and Ca^{2+} was determined using inductively coupled plasma optical emission spectroscopy (Optima 8000 ICP-OES).

II.10 Statistical analysis

Data were analyzed by two-way analysis of variance (ANOVA II) using SPSS version 22. Tukey’s test was used to compare the means of control and treatments and the difference at $P < 0.05$ was considered as significant. XLSTAT version 2014.5.03 was used to perform the Pearson's correlation matrix at $p < 0.05$.

III. Results

III.1 Effect of Si and salt stress on germination traits

The effects of 200 mM NaCl stress and 3 mM Si treatment on germination traits, including FGP, GS, VI, GE, PV, GV, MGT and vitality index (For key to abbreviations see table 5), are shown in table 6. The obtained results indicated that exposure of fenugreek seeds to salt stress significantly ($p < 0.001$) reduced FGP, GS, VI, GE, PV, GV and vitality index by 32%, 56%, 44%, 58%, 46%, 63% and 77%, respectively. However, the MGT was significantly ($p < 0.001$) increased by 87.5% compared to control. However, Si supply alleviated the negative impacts of salt stress on seed germination. In fact, FGP, GS, VI, GE, PV, GV and vitality index were 1.29, 1.2, 1.28, 1.24, 1.23, 1.60 and 2.11-fold higher in Si-treated and salt-stressed seeds as compared to Si-untreated ones. Results also showed that Si supplementation to salt- stressed seeds reduced MGT by 20% as compared to salt-stressed seeds. In addition, under non-stress conditions, no significant difference was observed between Si-treated and untreated seeds ($p > 0.05$) for all studied germination parameters.

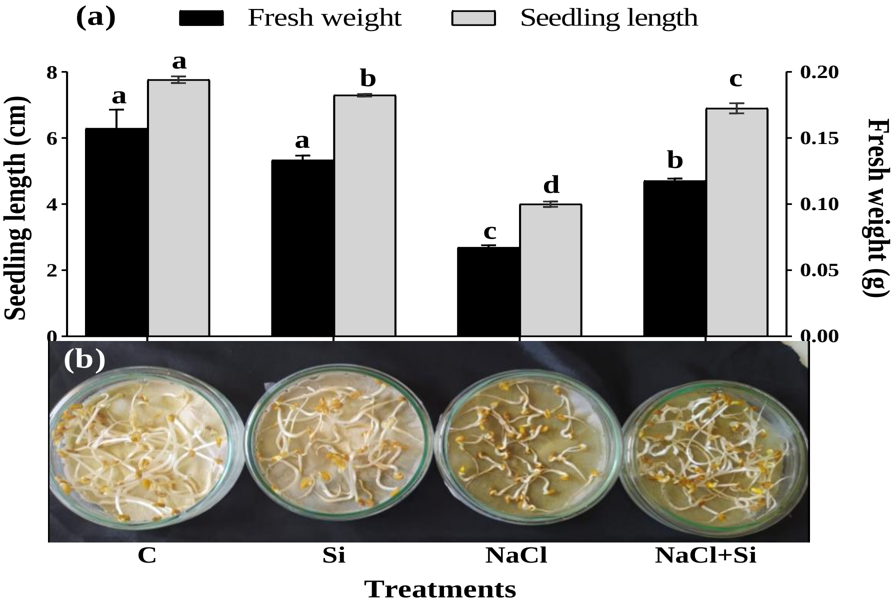


Figure 7: Effect of silicon (Si) and salt stress on fresh weight (a), seedlings length (a) and phenotype (b) of 8-day-old fenugreek seedlings. Bars represent standard errors of three replicates and the values followed by different small letters are significantly different at $p < 0.05$. C: Control; Si: 3 mM Si; NaCl: 200 mM NaCl.

The seedling fresh weight and length was significantly ($p < 0.001$) reduced from 0.15 g and 7.7 cm to 0.06 g and 4 cm, respectively under salt stress in comparison with control (Figure 7).

However, Si supplementation to rooting medium alleviated significantly ($p < 0.05$) the salt stress-induced reduction in fenugreek seedling fresh weight and length. Under unstressed conditions, Si supplementation has no significant effect ($p > 0.05$) on seedling growth as compared to the untreated control.

Table 6: Effect of silicon (Si) and salt stress on germination parameters of fenugreek seeds. The represented data are the means of three replicates ± standard error (SE). Between treatments, the means followed by the same letters at same parameters are not significantly different at $p < 0.05$

Germination parameters	Treatments		
		- Si	+ Si
FGP	- NaCl	100.00±0.00^{a}	100.00±0.00^{a}
	+ NaCl	67.50±0.96^{c}	87.50±0.96^{b}
GS	- NaCl	55.90±0.90^{a}	55.06±0.71^{a}
	+ NaCl	24.51±0.27^{c}	29.93±0.85^{b}
VI	- NaCl	38.02±0.64^{a}	36.90±0.36^{a}
	+ NaCl	21.04±0.28^{c}	27.00±0.56^{b}
MGT	- NaCl	0.16±0.00^{c}	0.16±0.00^{c}
	+ NaCl	0.30±0.00^{a}	0.24±0.00^{b}
GE	- NaCl	18.67±0.17^{a}	18.83±0.34^{a}
	+ NaCl	7.69±0.06^{c}	9.57±0.20^{b}
PV	- NaCl	70.62±1.20^{a}	68.43±0.36^{a}
	+ NaCl	37.81±0.60^{c}	46.77±1.00^{b}
GV	- NaCl	353.12±6.01^{a}	342.18±1.80^{a}
	+ NaCl	127.73±3.81^{c}	204.66±5.16^{b}
Vitality index	- NaCl	434.18±10.85^{a}	401.88±3.29^{a}
	+ NaCl	97.98±1.50^{c}	206.86±9.44^{b}

-NaCl: 0 mM NaCl; +NaCl: 200 mM NaCl; -Si: 0 mM Si; +Si: 3 mM Si; FGP: Final germination percentage; GS: Germination speed; VI: Velocity index; MGT: Mean germination time; GE: Germination energy; PV: Peak value; GV: Germination value.

III.2 Effect of Si and salt stress on seed reserve mobilization

The content of soluble proteins and soluble sugars was followed in fenugreek seeds during 8 days of germination to evaluate the effect of salt and Si treatment on seed reserve mobilization. From the beginning to the end of germination period, the protein content was decreased by 26% in salt-stressed seedlings, whereas this reserve was more decreased by 65% and 30%, respectively in Si-treated and untreated seeds germinated under non-stressed conditions (Figure 8a). Therefore, salt stress hinders the mobilization of protein reserves by the embryo during the germination process. However, the Si treatment enhanced seed protein mobilization in salt-stressed seeds. In fact, the content of soluble proteins was decreased from 2.48 µg mg^{-1} FW, during the germination initiation, to 1.75 µg mg^{-1} FW at the end of the germination period in Si-treated and salt-stressed seeds.

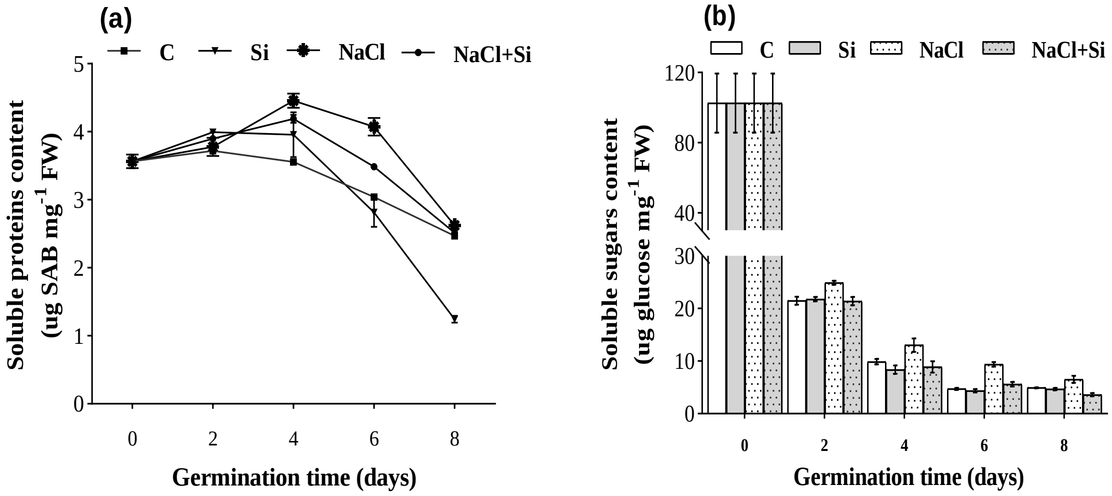


Figure 8: Effect of silicon (Si) and salt stress on use of soluble proteins (a) and soluble sugars (b) by embryo of fenugreek seeds during seed germination. Bars represent standard errors of three replicates. C: Control; Si: 3 mM Si; NaCl: 200 mM NaCl

Likewise, salt stress affected the mobilization process of soluble sugars as compared to control. Indeed, the soluble sugars were more mobilized under non stressed conditions, from 205 µg mg^{-1} FW, during the germination initiation, to 9 µg mg^{-1} FW at the end of the experiment, whereas, it was from 205 µg mg^{-1} FW to 13 µg mg^{-1} FW in stressed seeds (Figure 8b). Conversely, Si treatment enhanced the use of sugar reserves by the embryo under salt stress, which was explained by a reducing in the content of soluble sugars by 96% from the beginning to the end of the germination test.

III.3 Effect of Si and salt stress on embryo viability

The effect of salt and exogenous Si application on embryo viability are presented in figure 9. Results showed that, dependent on the treatment and time of exposition to the applied treatments, the embryo showed different staining intensity. At the beginning of seed germination, embryos from different treatments were bright red. However, the embryo from the seeds treated with 200 mM NaCl has become yellow after 24 h of germination. Therefore, the embryo could become unviable after 48 h of salt stress. However, this effect was alleviated by exogenous Si supplementation. Indeed, when Si was applied to 200 mM NaCl stressed-seeds, the embryo retained a red color during the whole experiment. The same was observed under control or Si-treatment alone, where the embryos were bright red during the whole experiment.

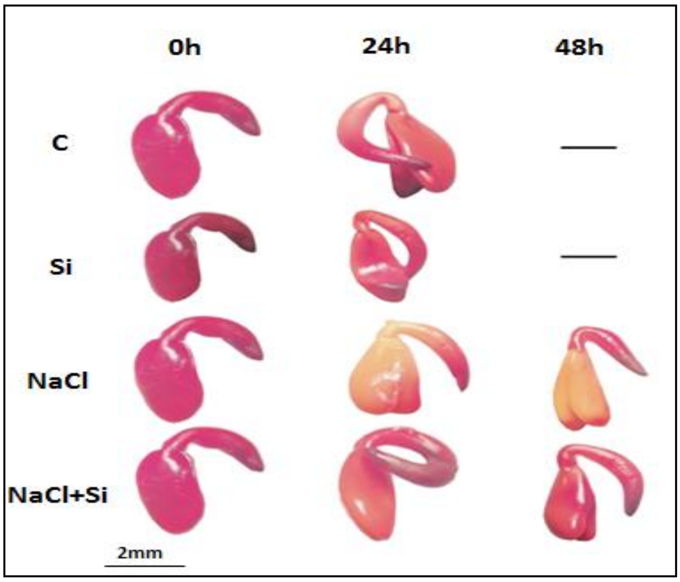


Figure 9: Effect of silicon (Si) and salt stress on embryo viability of fenugreek seeds at different germination times (0 h, 24 h and 48 h after the beginning of seed germination). The embryo viability was evaluated by staining pattern and red color intensity of embryo after their immersion in 2,3,5 triphenyltetrazolium chloride (TTC) solution. Living embryo tissues are red stained, while dead embryo tissues are unstained with red. At 48 h of germination time, hyphens mean that all tested seeds have been germinated. C: Control; Si: 3 mM Si; NaCl: 200 mM NaCl

III.4 Effect of Si and salt stress on cell membrane integrity and oxidative stress markers

Salt stress induced significant effects on cell membrane integrity and oxidative stress markers. As compared to control, EL, MDA and H_2O_2 contents were significantly ($p < 0.05$) increased by 270%, 53% and 400% under salt stress. However, the presence of Si in rooting medium remarkably reduced all of the EL (22%), MDA (23%) and H_2O_2 (48%) relative to salt-stressed seedlings. Under non-stressed conditions, there was no significant difference ($p > 0.05$) between control and Si-treatment alone for EL and MDA content (Figure 10a and b). However, for H_2O_2 content (Figure 10c), Si supplementation obviously reduced H_2O_2 content by 60% relative to Si-untreated seedlings.

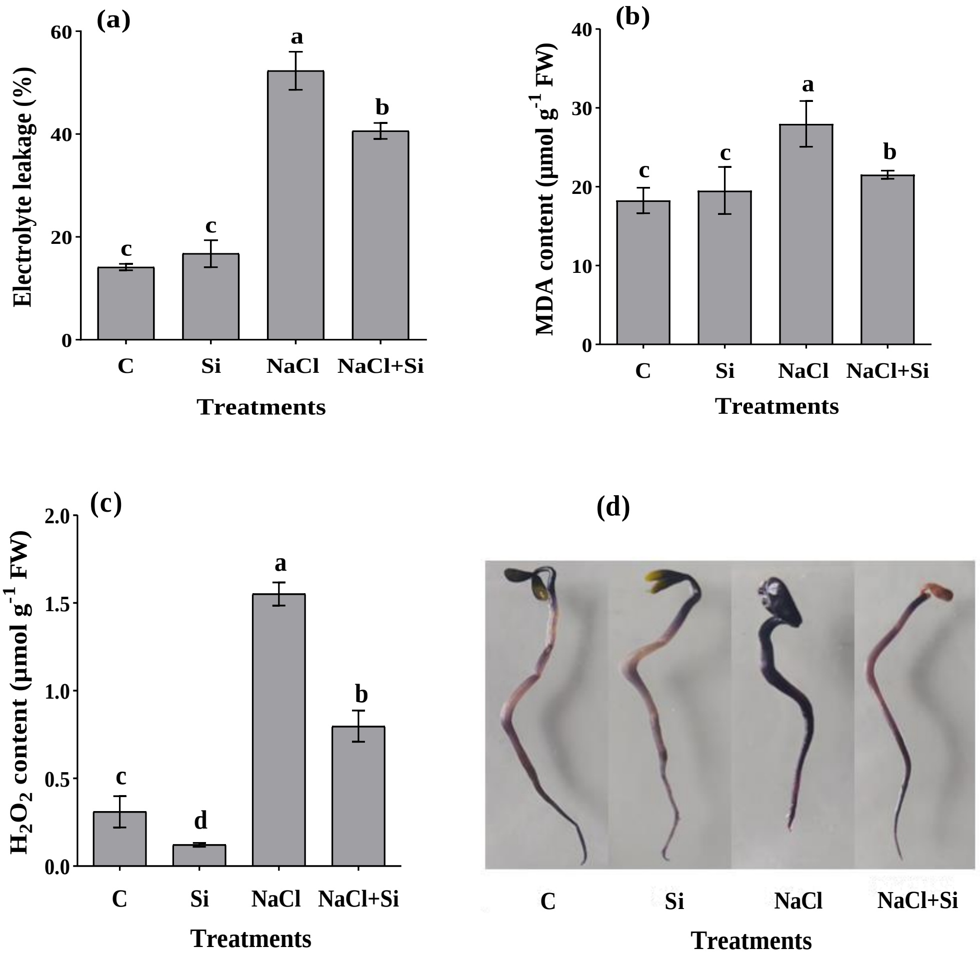


Figure 10: Effect of silicon (Si) and salt stress on electrolyte leakage percentage (a), malonyldialdehyde (MDA) (b) and hydrogen peroxide (H_2O_2) (c) contents and superoxide anion (O_2^-) accumulation (d) in 8-day-old fenugreek seedlings. Histochemical detection of O_2^- was assessed by staining with nitro blue tetrazolium chloride (NBT). Bars represent standard errors of three replicates and the values followed by different small letters are significantly different at $p < 0.05$. C: Control; Si: 3 mM Si; NaCl: 200 mM NaCl.

Results of histochemical staining indicated a significant accumulation of O_2^- in the fenugreek seedlings, which was represented by high intensity of the dark blue stain when seedlings are exposed to salt stress (Figure 10d). However, the exogenous supplementation of Si remarkably reduced the intensity of the dark blue stain of formazan in salt-stressed seedlings. Furthermore, under unstressed conditions, no difference was noted between Si-treated and untreated fenugreek seedlings.

III.5 Effect of Si and salt stress on enzymatic and non-enzymatic antioxidant activity

Salinity and exogenous Si affect significantly ($p < 0.001$) the antioxidant activity of 8-day-old fenugreek seedlings as depicted in Table 7. The results indicated that salt stress significantly ($p < 0.001$) decreased the activity of both SOD and PPO by 75% and 73%, respectively as compared to control. However, Si treatment markedly ($p < 0.001$) enhanced the activity of SOD and PPO by 85% and 158% under salt stress, respectively in comparison to Si-untreated salt-stressed seedlings. However, when compared to control, the treatment with Si alone has no significant effect ($p > 0.05$) on SOD and PPO activities.

Table 7: Effect of silicon (Si) and salt stress on enzymatic and non-enzymatic antioxidant activity of 8-day-old fenugreek seedlings. The represented data are the means of three replicates ± standard error (SE). Between treatments, the means followed by the same letters at same parameters are not significantly different at $p < 0.05$

Treatments	Non-enzymatic antioxidant activity		Enzymatic antioxidant activity	
	Total polyphenols content (mg gallic acid.g^{-1} FW)	Flavonoids content (mg quercetin.g^{-1} FW)	SOD activity (U.mg^{-1}protein)	PPO activity (U.min^{-1}.mg^{-1} protein)
C	$3.06±0.10^{c}$	$1.05±0.06^{b}$	$96.22±0.37^{a}$	$1.19±0.01^{a}$
Si	$5.40±0.25^{b}$	$1.33±0.40^{b}$	$93.81±0.80^{a}$	$0.90±0.00^{b}$
NaCl	$2.44±0.01^{d}$	$1.33±0.00^{b}$	$23.83±0.00^{c}$	$0.31±0.00^{d}$
NaCl+Si	$9.02±0.14^{a}$	$3.61±0.26^{a}$	$44.11±2.32^{b}$	$0.80±0.01^{c}$

-NaCl: 0 mM NaCl; +NaCl: 200 mM NaCl; -Si: 0 mM Si; +Si: 3 mM Si; SOD: Superoxide dismutase; PPO: Polyphenol oxidase.

Regarding non-enzymatic antioxidant molecules, Si treatment significantly ($p < 0.001$) increased total polyphenol content. The increase was more furthered when Si was applied simultaneously with NaCl. Indeed, upon Si treatment, total polyphenol content was increased by 76% in Si-treated seedlings alone and 194% in Si treated salt-stressed seedlings as compared to control. Under salt stress, total polyphenol content was decreased by more than 20% ($p < 0.001$). However, for flavonoid content, although Si and salt treatment alone had no significant effect, the simultaneous application of Si and NaCl significantly ($p \leq 0.001$) enhanced the content of flavonoid by more than 243% and 171%, respectively as compared to control and NaCl-treated seedlings (Table 7).

III.6 Effect of Si and salt on compatible solutes

Glycine betaine and proline contents in 8-day-old fenugreek seedlings are presented in figure 11. The obtained data showed that glycine betaine content was significantly ($p < 0.001$) reduced by 79% under salt stress. However, 3 mM Si application significantly ($p < 0.001$) alleviated this effect and increased the glycine betaine content in salt-stressed seedlings by up to 122% as compared to salt-stressed and Si-untreated seedlings. In contrast, the content of this compatible solute was decreased after Si supplementation under non-stressed conditions.

For proline content, relative to control, a significant ($p < 0.001$) increase of 95% was observed upon salt stress and interestingly this increase was further important when Si was combined with NaCl (147%). However, no significant ($p > 0.05$) difference was found between Si-treated and untreated seedlings under non-stressed conditions (Figure 11).

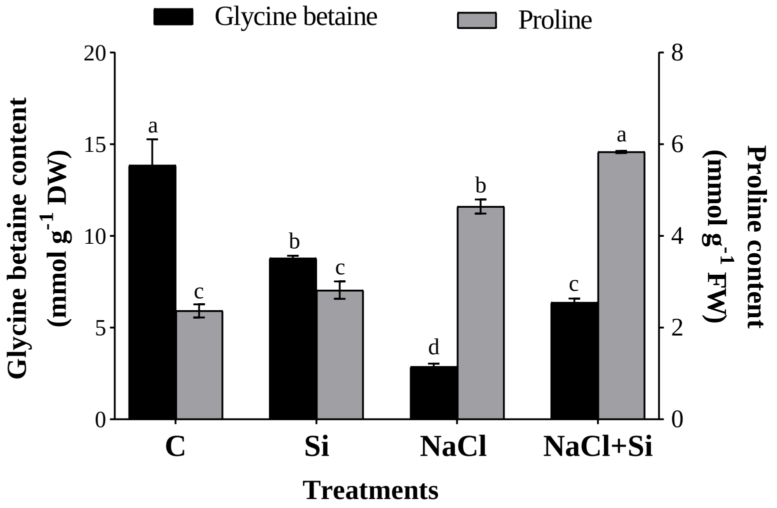


Figure 11: Effect of silicon (Si) and salt stress on glycine betaine and proline contents in 8 day-old fenugreek seedlings. Bars represent standard errors of three replicates and the values followed by different small letters are significantly different at $p < 0.05$. C: Control; Si: 3 mM Si; NaCl: 200 mM NaCl.

III.7 Effect of Si and salt stress on Si, Ca^{2+}, Na^{+} and K^{+} contents

The effects of NaCl stress and Si treatment on nutrient contents in 8-day-old fenugreek seedlings are presented in Table 8. As compared to control, Si content was significantly increased in Si-treated and stressed seedlings ($p \leq 0.001$) or non-stressed ones ($P < 0.001$). In addition, our results showed that Si application has no significant ($p > 0.05$) effect on Ca^{2+}, K^+ and Na^+ contents as compared to Si-untreated seedlings under unstressed conditions. However, in comparison to the control, the content of Ca^{2+}, K^+ and Na^+ was respectively 2.48, 2.65 and 10.72-fold higher in the presence of NaCl in rooting medium. The application of Si to stressed seedlings decreased all of the Ca^{2+}, K^+ and Na^+ contents by 20%, 68% and 70%, respectively relative to salt stress.

Table 8: Effect of silicon (Si) and salt stress on Si, calcium (Ca^{2+}), sodium (Na^+) and potassium (K^+) contents in 8-day-old fenugreek seedlings. The represented data followed by the same letters at same parameters are not significantly different at $p < 0.05$

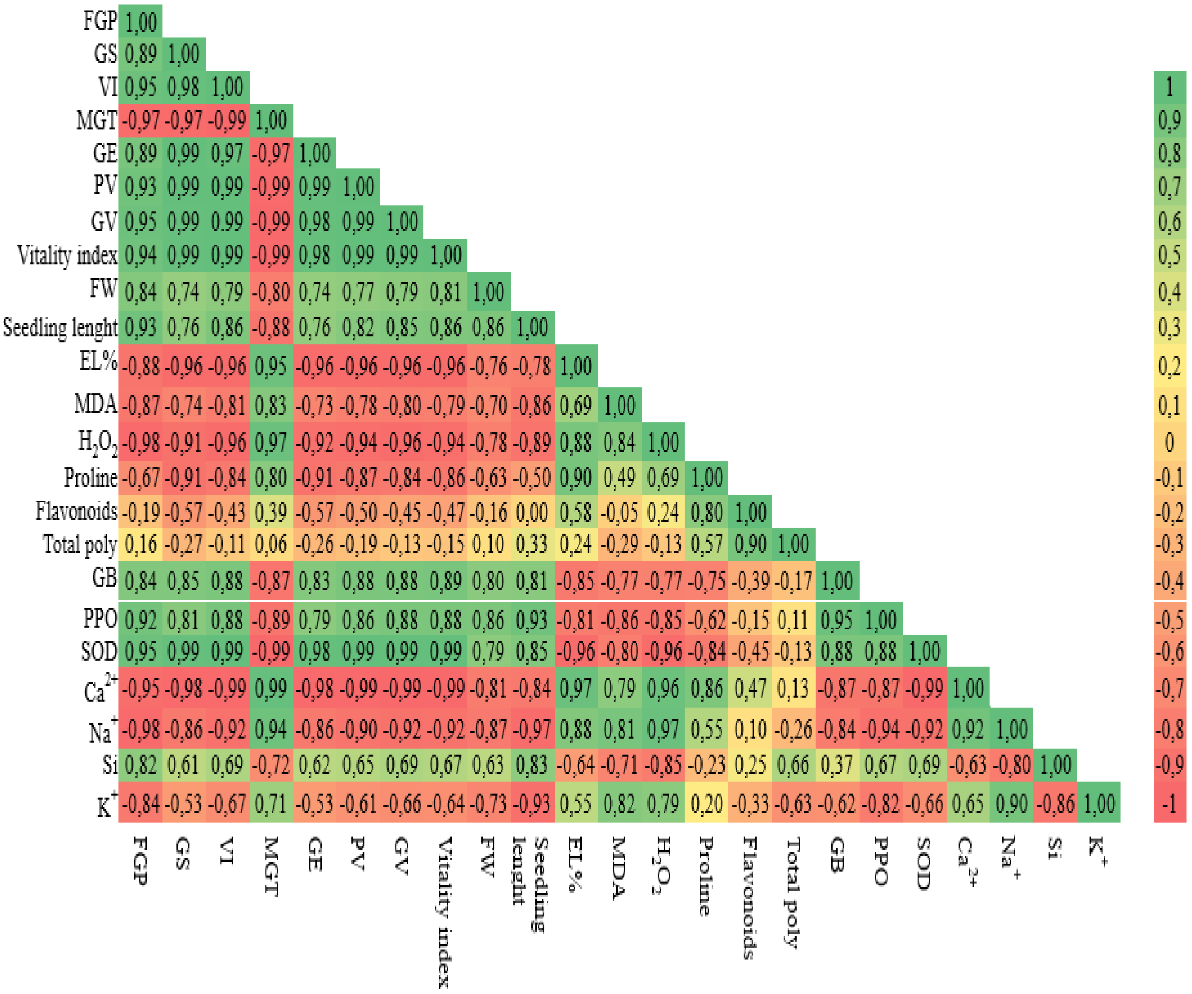
Treatments	Ions content							
	Si (mg g^{-1} DW)		Ca^{2+} (mg g^{-1} DW)		Na^+ (mg g^{-1} DW)		K^+ (mg g^{-1} DW)	
	- Si	+ Si	- Si	+ Si	- Si	+ Si	- Si	+ Si
- NaCl	70.62c	96.10a	14.41c	14.83c	46.17c	46.20c	102.89b	104.34b
+ NaCl	37.81d	83.43b	35.83a	28.65b	495.28a	147.35b	272.90a	86.15c

-NaCl: 0 mM NaCl; +NaCl: 200 mM NaCl; -Si: 0 mM Si; +Si: 3 mM Si

III.8 Pearson's correlation matrix analysis

Analysis of Pearson's correlation matrix (Figure 12) presented a significant negative correlation between Na^+ content and germination parameters including FGP ($r = -0.98$), GS ($r = -0.86$), VI ($r = -0.92$), GE ($r = -0.86$), PV ($r = -0.90$), GV ($r = -0.92$) and vitality index ($r = -0.92$). Additionally, glycine betaine content ($r = -0.84$) and antioxidant activity, in terms of SOD ($r = -0.92$) and PPO ($r = -0.94$), were also negatively correlated with Na^+ content. However, between Na^+ content and MGT ($r = 0.94$), proline content ($r = 0.55$) and oxidative stress markers, such as MDA ($r = 0.81$), H_2O_2 ($r = 0.97$) and EL ($r = 0.88$), there were significant positive correlations, explaining the harmful effects of 200 mM NaCl stress on seedling length ($r = -0.97$) and fresh weight ($r = -0.87$). In addition, a significant negative correlation ($r = -0.86$) was obtained between Na^+ and Si content. In contrast, our results showed a significant positive correlation between Si content and FGP ($r = 0.82$), GS ($r = 0.61$), VI ($r = 0.69$), GE ($r = 0.62$), PV ($r = 0.65$), GV ($r = 0.69$), vitality index ($r = 0.67$), SOD ($r = 0.69$), PPO ($r = 0.67$) and total polyphenol ($r = 0.66$). Whereas, there were significant negative correlations between MGT (r

= -0.72), oxidative stress indicators (MDA (*r* = -0.71), H_2O_2 (*r* = -0.85) and EL (*r* = -0.64)) and Si content, indicating that Si treatment interestingly improved seedling length (*r* = 0.83) and fresh weight (*r* = 0.63) as well as seed germination of fenugreek.



	FGP	GS	VI	MGT	GE	PV	GV	Vitality index	FW	Seedling lenght	EL%	MDA	H_2O_2	Proline	Flavonoids	Total poly	GB	PPO	SOD	Ca^{2+}	Na^+	Si	K^+
FGP	1,00																						
GS	0,89	1,00																					
VI	0,95	0,98	1,00																				
MGT	-0,97	-0,97	-0,99	1,00																			
GE	0,89	0,99	0,97	-0,97	1,00																		
PV	0,93	0,99	0,99	-0,99	0,99	1,00																	
GV	0,95	0,99	0,99	-0,99	0,98	0,99	1,00																
Vitality index	0,94	0,99	0,99	-0,99	0,98	0,99	0,99	1,00															
FW	0,84	0,74	0,79	-0,80	0,74	0,77	0,79	0,81	1,00														
Seedling lenght	0,93	0,76	0,86	-0,88	0,76	0,82	0,85	0,86	0,86	1,00													
EL%	-0,88	-0,96	-0,96	0,95	-0,96	-0,96	-0,96	-0,96	-0,76	-0,78	1,00												
MDA	-0,87	-0,74	-0,81	0,83	-0,73	-0,78	-0,80	-0,79	-0,70	-0,86	0,69	1,00											
H_2O_2	-0,98	-0,91	-0,96	0,97	-0,92	-0,94	-0,96	-0,94	-0,78	-0,89	0,88	0,84	1,00										
Proline	-0,67	-0,91	-0,84	0,80	-0,91	-0,87	-0,84	-0,86	-0,63	-0,50	0,90	0,49	0,69	1,00									
Flavonoids	-0,19	-0,57	-0,43	0,39	-0,57	-0,50	-0,45	-0,47	-0,16	0,00	0,58	-0,05	0,24	0,80	1,00								
Total poly	0,16	-0,27	-0,11	0,06	-0,26	-0,19	-0,13	-0,15	0,10	0,33	0,24	-0,29	-0,13	0,57	0,90	1,00							
GB	0,84	0,85	0,88	-0,87	0,83	0,88	0,88	0,89	0,80	0,81	-0,85	-0,77	-0,77	-0,75	-0,39	-0,17	1,00						
PPO	0,92	0,81	0,88	-0,89	0,79	0,86	0,88	0,88	0,86	0,93	-0,81	-0,86	-0,85	-0,62	-0,15	0,11	0,95	1,00					
SOD	0,95	0,99	0,99	-0,99	0,98	0,99	0,99	0,99	0,79	0,85	-0,96	-0,80	-0,96	-0,84	-0,45	-0,13	0,88	0,88	1,00				
Ca^{2+}	-0,95	-0,98	-0,99	0,99	-0,98	-0,99	-0,99	-0,99	-0,81	-0,84	0,97	0,79	0,96	0,86	0,47	0,13	-0,87	-0,87	-0,99	1,00			
Na^+	-0,98	-0,86	-0,92	0,94	-0,86	-0,90	-0,92	-0,92	-0,87	-0,97	0,88	0,81	0,97	0,55	0,10	-0,26	-0,84	-0,94	-0,92	0,92	1,00		
Si	0,82	0,61	0,69	-0,72	0,62	0,65	0,69	0,67	0,63	0,83	-0,64	-0,71	-0,85	-0,23	0,25	0,66	0,37	0,67	0,69	-0,63	-0,80	1,00	
K^+	-0,84	-0,53	-0,67	0,71	-0,53	-0,61	-0,66	-0,64	-0,73	-0,93	0,55	0,82	0,79	0,20	-0,33	-0,63	-0,62	-0,82	-0,66	0,65	0,90	-0,86	1,00

Figure 12: Pearson's correlation matrix between germination parameters, oxidative stress markers, compatible osmolyte, ions content and enzymatic and non-enzymatic antioxidant systems of 8 day-old fenugreek seedlings treated with 0 or 200 mM NaCl with or without 3 mM Si. In addition to correlation coefficient (*r*), the positive correlations are displayed in green while red color revealed the negative correlations. FGP: Final germination percentage; GS: Germination speed; VI: Velocity index; MGT: Mean germination time; GE: Germination energy; PV: Peak value; GV: Germination value; GB: Glycine betaine; MDA: Malonyldialdehyde; H_2O_2: Hydrogen peroxide; EL: Electrolyte leakage; SOD: Superoxide dismutase; PPO: Polyphenol oxidase; Si: Silicon; Ca^{2+}: Calcium; Na^+: Sodium; K^+: Potassium; FW: Fresh weight of seedlings.

IV. Discussion

Seed germination, as a critical stage in the plant life cycle, is the most sensitive stage to abiotic stresses including salinity, which causes in the same time osmotic stress and ionic toxicity (Farissi et al. 2011; Peng et al. 2016). For this reason, salinity inhibited seed germination and seedling growth of various aromatic and medicinal plants such as *Thymus daenensis*, *Thymus kotschyanus* and *Origanum compactum* (Khoshsokhan et al. 2012; Laghmouchi et al. 2017). According to Calone et al. (2020), determination of germination parameters is among the most important and suitable criteria to assessing salt stress tolerance in plants. In this context, Gou et al. (2020) found that germination percentage, germination index and seedling vigor was significantly reduced in cucumber under 200 mM NaCl. In addition, Hosein and Keshavarzi (2012) showed that germination traits including plumule length, radicle length, seed vigor and fresh and dry seedling weight of *Artemisia annua* L. were drastically decreased upon salt stress. Similar to the above studies, our findings showed that salinity stress, induced by 200 mM NaCl, significantly ($p < 0.001$) reduced germination traits of fenugreek. Inhibition of seed germination of fenugreek due to salinity was also reported by Mahmoudi et al. (2019). However, in this study, the treatment with exogenous Si markedly improved all tested germination parameters ($p < 0.05$) by reducing significantly MGT ($p < 0.001$). In the same way, Gou et al. (2020) observed that 0.3 mM Si could raise seed germination percentage and germination index of cucumber under NaCl stress. Similarly, the germination rate, germination index and velocity index of salt stressed *Momordica charantia* L. seeds were significantly improved after treatment with exogenous Si (Wang et al. 2010). Also, Zhang et al. (2017) showed that 1 mM Si addition ameliorated germination rate, germination index and seedling vitality index of *Glycyrrhiza uralensis* Fisch. seeds under NaCl stress combined with drought stress. Furthermore, in this work, results recorded for fresh weight and seedling length, suggested that Si treatment markedly enhanced growth and phenotype of fenugreek seedlings under salt stressed conditions. The same results were reported in seedlings of *Glycyrrhiza uralensis* (Zhang et al. 2015), *Lycopersicum esculentum* Mill. (Haghighi et al. 2012) and *Cucumis melo* L. (Zhang et al. 2020). In the same line, Almutairi (2016) showed that tomato seedling growth, in terms of root length and fresh weight, were significantly improved after treatment with nano-Si under salt stress.

Benmahioul et al. (2009) reported that 256.6 mM NaCl might reduce the germinated embryo survival rates from 100% to 62.9% referred to control in *Pistacia vera* L. The same was found in this study after TTC staining, suggesting that the embryonic cells of salt-stressed seeds was became unviable after 48 h of exposure to salt stress. Conversely, the embryo in Si treated seeds stayed completely viable at different times of experimentation under salinity. These results explained the observed reductions in the germination parameters of salt-stressed seeds, also suggested that the exogenous Si kept embryo viability as well as seed germination process under salt stress.

The negative effects of salt stress on seed germination could be the results of an osmotic stress, as the water content of the seed was reported to diminish (Salahuddin et al. 2017). It was also reported that this abiotic stress unregulated genes expression of gibberellic acid and abscisic acid and consequently disturbing hormonal balance in seed during germination stage (Sebei et al. 2007; Liu et al. 2019). On the other hand, several studies were correlated the deleterious effects of salt stress on seed germination with ions toxicity, which induced by higher accumulation of Na^+ and Cl^- in embryotic cells during the exposure of seeds to salt stress (Farissi et al. 2011; Li et al. 2019). In the same way, Bonilla et al. (2004) suggested that most toxic effects of NaCl can be attributed to Na^+ toxicity. Similar results were found in the present work, where salt stress caused a significant ($p < 0.001$) increase of Na^+ content comparatively to control. Also, this increase of Na^+ content was negatively correlated with all studied germination parameters exempt MGT, explaining the negative effects of salt stress on fenugreek seedlings growth. In contrast, the exogenous Si treatment reduced Na^+ accumulation by 70%, but enhanced endogenous Si content under even stressed or unstressed conditions. More than that, the result of statistical analysis showed significant positive correlations between Si content and all germination traits exempt MGT, which was negatively correlated with endogenous Si content. Therefore, in this work, the observed salt alleviating effects through Si on seed germination might be due to higher Si uptake and accumulation in Si-treated seedlings under salinity stress. Similarly, Biju et al. (2017) explained the positive effects of Si on seed germination of lentil under drought stress by deposition of Si in cell walls of lentil. In the same line, Al-Saeedi et al. (2017) reported that addition of 300 mg L^{-1} of Nanosilica significantly reduced Na^+ ion accumulation in *Phaseolus vulgaris* L. seedlings under Na^+ stress.

Under salt stress, seed reserve mobilization is inhibited by immobilization of nutrient reserve such as amino acids and sugars stored at albumen (Sebei et al. 2007; Aghaei and Komatsu 2013). Similarly, the present research proved that salt stress decreased the use of soluble proteins and sugars by embryo during seed germination. Indeed, it was reported that many important hydrolytic enzymes like amylase and glycosidase are drastically affected during seed germination under salt stress. In this context, Liu et al. (2019) reported that the activity of amylase was significantly inhibited under 200 mM NaCl in *Limonium bicolor*. Also, Hua-long et al. (2014) found that the activities of α-amylase, β-amylase and the total amylase were significantly reduced during seed germination of rice under salt stress. In our study, we noted that Si addition enhanced seed reserve mobilization under salt stress, which could explained by the decrease in soluble protein and soluble sugar contents during seed germination of Si-treated seeds under salinity stress. To understand the seed reserve mobilization enhancement by exogenous Si, Zhang et al. (2020) demonstrated that Si treatment up-regulated amylases genes expressions, which in return improved significantly its activity in melon seed under auto-toxicity stress. Also, Biju et al. (2017) found that Si supplied under drought stress significantly enhanced the activity of α and β-amylase and α-glycosidase in *Lens culinaris* L. The same was reported by Gou et al. (2020) of α-amylase activity, where the added Si significantly improved α-amylase activity in cucumber seeds under salt stress. Therefore, these findings exhibited that Si might have a crucial effect on reserve mobilization to enhance seed germination of fenugreek under salt stress.

It was reported that abiotic stresses, including salinity stress, affected seed germination by triggering the over production and accumulation of ROS such as O_2^- and H_2O_2, resulting in membrane peroxidation and even cell death (Luo et al. 2021). This is in agreement with our results, where O_2^- and H_2O_2 accumulations were markedly raised in fenugreek seedlings under salt stress, inducing in turn a significant increase in MDA content and EL ($p < 0.05$). In addition, exposure of fenugreek seeds to 200 mM NaCl caused a significant ($p < 0.001$) decrease in the activities of SOD and PPO antioxidant enzymes and the content of total polyphenol and flavonoid. In the same line, our result found a significant positive correlation between oxidative stress markers, in terms of MDA ($r = 0.81$), H_2O_2 ($r = 0.97$) and EL ($r = 0.88$), and the content of Na^+, which was negatively correlated with SOD ($r = -0.92$) and PPO ($r = -0.94$). According to these results, reduction in both enzymatic and non-enzymatic antioxidant activities could decreased the ability of fenugreek seedlings to scavenge O_2^- and H_2O_2 radicals, inducing in turn an accumulation of ROS in seed under salt stress, which might cause the membrane oxidative damages, reflected by high MDA content and EL. The decline in the SOD activity, during seed germination, was reported also by Sekmen et al. (2012), where the activity of SOD and CAT enzymes and the intensity of their isoenzymes were significantly decreased in *Gypsophila ablanceolat* (Bark.) under 50 mM NaCl stress as compared to control. Also, at plant stage, Ben Taârit et al. (2012) found that, the total polyphenol and antioxidant activity of *Salvia sclarea* L. were reduced with an increase in salt stress from 50 to 75 mM NaCl. In the same way, Jaleel et al. (2007) demonstrated that 80 mM NaCl treatment significantly decreased overall growth of *Catharanthus roseus* L. and reduced the proteins content and the activities of some antioxidant enzymes like POX, SOD and PPO. Other studies confirmed these findings regarding decline in the antioxidant activity under salt stress (Abdul Jaleel et al. 2008; Perveen et al. 2011; Amraee et al. 2020). However, in this work, Si treatment significantly ($p < 0.001$) reduced O_2^- and H_2O_2 accumulations under salt stress, suggesting that Si has a crucial role on reduction of ROS production to alleviate oxidative damage induced by 200 mM NaCl stress. In addition, an important decline in MDA content and EL was also obtained after Si treatment. Thus, Si could protect membrane from salt-induced lipid peroxidation. The decrease in oxidative stress markers through exogenous Si supplementation could be explained by the improvement of antioxidant activity of fenugreek seedlings under salt stress. In fact, the present study showed that Si treatment significantly ($p \leq 0.001$) enhanced the content of total polyphenol and flavonoid and the activities of SOD and PPO, thus it improved both enzymatic and non-enzymatic antioxidant activities of fenugreek under salt stressed conditions. These finding were confirmed by the statistical analysis, which showed a significant positive correlation between Si content and SOD ($r = 0.69$), PPO ($r = 0.67$) and total polyphenol ($r = 0.66$), while there was a significant negative correlation between endogenous Si content and all of Na^+ content ($r = -0.86$), MDA ($r = -0.71$), H_2O_2 ($r = -0.85$) and EL ($r =-0.64$). Similar results were suggested by Wang et al. (2010) in *Momordica charantia.* In the same way, Zhang et al. (2015) suggested that supplemented Si enhanced seed germination of *Glycyrrhiza uralensis* by reducing MDA content and improving antioxidant enzyme activity under salt stress. Also, Shi et al. (2014) reported that Si application significantly reduced oxidative stress by enhancing the activities of antioxidant enzymes like SOD and CAT in *Solanum lycopersicum* L. under water stress.

On the other hand, several studies demonstrated that during germination phase, the seeds accumulate a variety of organic and inorganic solutes to induce an osmotic adjustment, and in order to maintain their germination under salt stress (Thakur and Sharma 2005; Farissi et al. 2011). In fact, osmotic regulation is one of the important mechanisms to increase the concentration of cell fluid to maintain the ability of cell absorption or water retention. In this context, it was reported that glycine betaine and proline are important compatible solutes (İbrahimova et al. 2021). Indeed, when the cell is dehydrated, proline acts as a chaperone and prevents the structure of macromolecules from destruction (Tang et al. 2015). Also, glycine betaine, as an organic compound, is maintains the structure of macromolecules by scavenging ROS under salt and drought stresses (Cha-Um and Kirdmanee 2010). Importantly, our results found that application of exogenous Si interestingly improved the content of proline and glycine betaine, as key compatible solutes, in salt stressed fenugreek seedlings, alleviating in turn osmotic stress induced by 200 mM NaCl stress. Similarly, Zhang et al. (2017) reported that Si addition significantly increased proline content in *Glycyrrhiza uralensis* seedlings to regulate osmotic stress induced by salinity and drought stresses. Altogether, to enhance seed germination of fenugreek under salt stressed conditions, supplemented Si reduced oxidative stress by improving antioxidant activity and accumulation of osmolyte compounds. In the same line, Wang et al. (2010) reported that exogenous Si might increase GR, GI and VI through their contribution to reducing oxidative stress and increasing antioxidant activity in *Momordica charantia* under NaCl stress.

V. Conclusion

Taken together, the treatment with exogenous Si significantly increased germination parameters, fresh weight and seedling length of fenugreek under salt stress. Furthermore, the Si treatment improved seed reserve mobilization and embryo viability under this abiotic stress. The enhancement of salt tolerance through Si during seed germination might be explained by a significant increase in the content of endogenous Si, which was correlated with a significant decrease in lipid peroxidation and ROS production. In addition to these beneficial effects, exogenous Si also significantly improved the antioxidant activity and the accumulation of osmolyte compounds like proline and glycine betaine in fenugreek seedlings under salt stress. Hence, according to above mentionned results, application of Si could alleviate the harm effects of salt stress on seed germination of fenugreek and enhance its salt tolerance by improving seed reserve mobilization, antioxidant activity and osmolytes accumulation, thus it might be a potential approach to improve seed germination of fenugreek in salt-affected soils.

Chapter 3: Beneficial role of exogenous silicon on yield, antioxidant systems, osmoregulation and oxidative stress in fenugreek (*Trigonella foenum-graecum* L.) under salinity stress

Abstract

In the Earth's crust, silicon (Si) is the most abundant element after oxygen, while, under salt stress, its role in the tolerance of aromatic and medicinal plants (AMPs) is not yet detailed. For this reason, in this study we evaluated the effect of exogenous Si on some tolerance-related parameters in salt-stressed fenugreek, as an important AMP. For this purposes, 3 mM of exogenous Si was applied to assess its impact on plant biomass and on some tolerance-related parameters in fenugreek (*Trigonella foenum-graecum* L.) grown under 150 mM NaCl stress. The results showed that salinity reduced growth parameters, relative water content, photosystem II efficiency, stomatal conductance and K^+ and Ca^{2+} contents, while it increased the Na^+ content, which could explain the obtained reduction in fenugreek growth and yield. However, Si supply reversed the depressive effects of salinity and improved fenugreek growth and yield. Adding exogenous Si also caused a significant reduction in Na^+ content and increased K^+ and Ca^{2+} concentrations. The content of malonyldialdehyd and hydrogen peroxide and the level of electrolyte leakage were significantly increased in salt-stressed fenugreek, while were significantly decreased after Si supplementation. The reduction in oxidative stress markers in Si-treated plants was correlated with a significant increase in both enzymatic and non-enzymatic antioxidant systems and an important accumulation of compatible solutes. Therefore, exogenous Si was directly involved in the central defensive mechanisms to enhance salt tolerance of fenugreek, thus its application could be a promoting strategy to alleviate the damages of salinity on fenugreek growth and yield.

Keywords: Fenugreek, Oxidative Stress, Salinity, Silicon, Tolerance, Yield

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I. Introduction

In recent years, climate change has given rise to several abiotic stresses. Soil salinization is one of the most of these environmental challenges, because approximately 7% of the world land area, 50% of the irrigated land and 20% of cultivated land are affected by high accumulation of salt ions, causing a considerable decrease in agricultural systems, in terms of production and yield (Hu and Schmidhalter 2004). In fact, salinity stress induces oxidative stress and consequently destructs most of the vital plant processes, including seed germination, photosynthesis, ions uptake, membrane permeability and cell homeostasis (Zhu 2003; Said-Al Ahl and Omer 2011; Khoshsokhan et al. 2012). Salt stress significantly reduced the content of photosynthetic pigments and the efficiency of both photosystems, thus it greatly affected photosynthetic process activity, as a vital mechanism in the plant life cycle (Sudhir and Murthy 2004). Membrane stability also drastically affected under salt stress due to high accumulation of malondialdehyde (MDA) and reactive oxygen species (ROS), like hydrogen peroxide and superoxide (El Moukhtari et al. 2021b). In this context, Luo et al. (2021) found that salinity stress actives transcription of NADPH oxidase genes like *RbohD*, leading to uncontrolled production and accumulation of ROS, and in turn disturbs cell membrane permeability. In plant rhizosphere, accumulation of salt ions like sodium (Na^+), chloride and sulfate, causes an osmotic stress and eventually reduces water and nutrients accessibility to plant root (Chrysargyris et al. 2018). In plant, excess of Na^+ ions negatively affect nutritional balance by disrupting plant nutrient uptake, explaining by reduction in the content of essential elements, like potassium, in plant tissues during exposure to salt stress (Ali et al. 2021). Following their above-mentioned injurious effects, salt stress can destruct and inhibit the growth process of various plants, including aromatic and medicinal species.

Fenugreek (*Trigonella foenum-graecum* L.), as an oldest medicinal plant, it has long been cultivated as a spice crop in the Mediterranean area, where it has been used by people as one of the ingredients in daily diet (Bashan et al. 2004; Benayad et al. 2014a,b). Regarding the medicinal and therapeutical properties, it has been reported that fenugreek seeds are used for two main pharmacological properties; hypocholesterolaemic and antidiabetic activities (Yadav and Chowdhury 2017; Jeni James and Meena Devi 2021). More than that, in a recent study, which aimed to evaluate the anticancer potential of methanolic fenugreek seed extract, Alrumaihi et al. (2021) documented that fenugreek seed extracts have many substances with significant cytotoxicity effect for cancer cells. On the other hand, fenugreek, as other legume plants, is known for its atmospheric nitrogen fixation ability by its symbiosis with rhizobia. In this context, Singh et al.(2008) estimated that fenugreek can fix 48% of its total nitrogen (N) during growing season. Thus, in addition to their medicinal properties, fenugreek can be used as a good soil renovator and a best green manure (Tsegaye et al. 2015). However, salt stress and other environment-stressed factors drastically affected growth and yield of fenugreek. Indeed, germination parameters i.e., seedling biomasses, embryo viability and seed reserve mobilization of fenugreek seeds are negatively affected under 200 mM salinity stress (Lamsaadi et al. 2022a). Nasseri et al. (2012) found that plant biomasses, chlorophyll content and membrane integrity in fenugreek were significantly reduced with addition of NaCl in the growth medium. Also, Zaghdoudi et al. (2011) demonstrated that 150 mM NaCl salt stress decreased the activities of both photosystem I and II, explaining the significant decrease in photosynthetic activity, as well as fenugreek growth. The large above-cited medicinal and agricultural advantages of this medicinal plant encouraged future researchers to develop new and ecofriendly strategies, such as treatment with exogenous nutrients like silicon Si, to enhance the growth and production of fenugreek under stressed conditions.

In terms of abundance, Si is the most abundant element after oxygen in earth (Rizwan et al. 2015). Various plant species are known for its ability to absorb and accumulate Si in their cell tissues (El Moukhtari et al. 2022b). Many studies reported that Si is a beneficial element that enhance plant growth and improve plant tolerance to several abiotic stresses like heavy metal (Fatemi et al. 2020), drought (Shi et al. 2014), phosphorus deficiency (El Moukhtari et al. 2022a) and salt stress (El Moukhtari et al. 2021a). However, in case of AMPs, the effect of exogenous Si has not yet received more attention. Nasseri et al. (2012) evaluated only the effect of Si treatment on plant growth, relative water content, electrolyte leakage and chlorophyll content in salt-stressed fenugreek plants but, their effect on antioxidant system, osmoregulation and photosystem efficiency is not yet assessed and detailed. For this reason, in the present work, the effect of exogenous Si application on photosynthetic parameters, oxidative stress markers, antioxidant systems and osmoregulation were assessed in fenugreek, as an AMP, to understand the mechanism by which Si improved plants tolerance and yield under salt-stressed conditions.

II. Materials and methods

II.1 Plant material and growth conditions

Fenugreek (*Trigonella foenum-graecum* L.) seeds were supplied by the National Institute of Agronomic Research (INRA Morocco) and used as plant material. Eight fenugreek seeds, disinfected with 5% of sodium hypochlorite solution for 5 min, were sown in plastic pot, containing 160 g of sterilized sand-peat mixture (1:4 v:v), in a growth chamber at 25 ± 1 °C, 60% - 80% relative humidity and 16h photoperiod. One week after germination, 4 fenugreek seedlings were kept and irrigated with Hoagland nutrient solution (Hoaglan and Arnon 1950), containing KH_2PO_4 (250 µM L^{-1}), KNO_3 (600 µM L^{-1}), K_2SO_4 (0.75 mM L^{-1}), $MgSO_4$ (1 mM L^{-1}), $CaCl_2$ (1.65 mM L^{-1}), Fe-EDTA (16 µmol L^{-1}), H_3BO_3 (4 µM L^{-1}), $MnSO_4$ (6 µM L^{-1}), $ZnSO_4$ (1 µM L^{-1}), $CuSO_4$ (1 µM L^{-1}) and Na_2MoO_4 (0.1 µM L^{-1}). Two weeks after sowing, plants were divided into two plots: plants treated with 0 mM NaCl and plants treated with 150 mM NaCl supplied to the nutrient solution. Each plot was divided into two subplots: plants treated with 0 mM Si and plants treated with 3 mM Si supplied to the nutrient solution in $CaSiO_3$. For each treatment, 12 pots containing 4 plants each were considered. Stress was applied for one month, and then some growth attributes, photosynthetic characteristics and other biochemical parameters associated with salt tolerance, like level of oxidative stress markers and antioxidant molecules, were evaluated.

II.2 Growth attributes

After one month of salt stress and Si treatment, some growth attributes, such as shoot and root dry weight, plant length and leaf area were assessed. Just before the harvest, plant height was determined in three random plants from each treatment using a ruler graduated to centimeters and millimeters. Plants were then harvested, and shoots were separated from the roots, oven dried at 80 °C for 48h and their dry weight was determined.

Leaf area was determined in three random leaves from three random plants according to El Moukhtari et al. (2021a). Briefly, leaves were cut and laid out on a white sheet containing a scale and scanned using a digital scanner. Leaf area was measured using Mesurum software version 3.4.4.0.

After 3 months, fenugreek plants were hand-harvested and the number of immature seeds per pod (NSP) was recorded, with three replicates for each treatment.

II.3 Relative water content

Relative water content (RWC) was determined as described in Ghoulam et al. (2002). The third fully expanded youngest leaf from top was excised from three random plants from each treatment and their fresh weight (FW) was recorded immediately. Samples were then cut and transferred to distilled water for 8h and their turgid weight (TW) was determined. Samples were then kept in an oven at 70 °C for 24h and their dry weights (DW) was measured. RWC was calculated following the formula below:

$$\mathbf{RWC= [(FW-DW)/ (TW-DW)] \times 100}$$

II.4 Photosynthetic pigment content

Photosynthetic pigments were determined following Arnon (1949) method. Fresh leaf material (0.1 g) was homogenized at 4 °C in 2 mL of acetone (80%) using mortar and pestle. Homogenate was then centrifuged at 10 000 rpm for 10 min at 4 °C, and the supernatant was used to read the optical density (OD) at 645 nm, 663 nm and 480 nm. Chlorophyll (Chl) a, Chl b, total Chl and carotenoids contents were calculated according to D’souza and Devaraj (2013), with three replicates for each treatment.

II.5 Quantum efficiency of the photochemistry of ps ii (f_v/f_m) and stomatal conductance

Regarding photosystem II efficiency, the ratio of variable fluorescence to maximum fluorescence (F_v/F_m) was determined in 9 leaves from each treatment after 20 min of darkness adaptation using a portable fluorescence meter (Handy PEA, Hansatech, England) according to Mouradi et al. (2016). For stomatal conductance (gs), a leaf porometer (SC1 Model, Decagon Devices, version 2012) was used. Five replicates for each treatment were considered. Measurement was taken between 9 and 12 p.m. and before each measurement, the instrument was calibrated to ensure an accurate reading (Mahmood et al. 2016).

II.6 Malonyldialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents and electrolyte leakage percentage (EL)

MDA content was estimated in three replicates using the thiobarbituric acid (TBA) method (Heath and Packer 1968). 100 mg fresh leaf material were homogenized in 1 mL of 0.5% TBA prepared in 20% trichloroacetic acid (TCA) and the resulted homogenate was heated at 95 °C for 30 min. After cooling down, samples were centrifuged at 14 000 rpm for 10 min, and the absorbance of supernatant was determined at 532 nm and 600 nm. MDA content was determined using its extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as µmol MDA g^{-1} FW, with three replicates for each treatment.

H_2O_2 content in fenugreek leaves was determined following the method of Brennan and Frenkel (1977). 100 mg of fresh leaf materials were ground in 2 mL of cold acetone and centrifuged at 5000 rpm for 15 min at 4 °C. Afterward, to 1350 µL of supernatant, 150 µL of 20% titanium, prepared in concentrated hydrochloric acid (HCl), (v/v), and 300 µL of concentrated ammonia were added and the mixture was centrifuged at 10 000 rpm for 10 min. Supernatant was then discarded and the precipitate was washed five times with cold acetone and recovered in 3 mL of 2N sulfuric acid to determine H_2O_2 content after absorbance measurement at 410 nm. H_2O_2 was calculated using a standard curve prepared with known concentration of H_2O_2 and expressed as mmol H_2O_2 g^{-1} FW, with three replicates for each treatment were considered.

According to Ghoulam et al. (2002), the EL was determined in three replicates per treatment. Three leaves from each treatment were cut and washed thoroughly with deionized water, to remove all surface electrolytes, and immersed in 10 mL of distilled water. After 24h of agitation at 25 °C, the initial electrical conductivity was measured (EC_1) using a conductivity meter (DDS-12DW, Benchtop Conductivity Meter). Samples were then autoclaved at 120 °C for 20 min and the finale electrical conductivity (EC_2) was measured. EL was calculated by the following formula:

$$\mathbf{EL\ (\%) = (EC_1/EC_2) * 100}$$

II.7 Enzymatic antioxidant activity

Polyphenol oxidase (PPO) was extracted by grinding 100 mg of fresh leaf material in 1 mL of 50 mM phosphate buffer (pH 6), containing 5% of polyvinylpyrrolidone (PVP). The PPO activity was determined according to Hori at al. (1997), following the oxidation of catechol for 3 min at 410 nm. One unit of PPO activity was defined as the amount of enzyme causing 0.01 absorbance increases. PPO activity was expressed as enzymatic unit (EU) min^{-1} mg^{-1} protein, with three replicates for each treatment.

For superoxide dismutase (SOD), 0.1 g of fresh material was ground in 1 mL of 50 mM phosphate buffer (pH 7.8), containing 1% of PVP and 0.1 mM ethylenediaminetetraacetic acid. The mixture was centrifuged at 12 000×*g* for 20 min at 4 °C and the resulted supernatant was used for SOD activity as reported previously (Beyer and Fridovich 1987). One enzymatic unit of SOD was defined as the amount of enzyme required to inhibit the reduction of 50% NBT. SOD activity was expressed as EU min^{-1} mg^{-1} protein, with three replicates for each treatment.

For both antioxidant enzymes, Bradford (1976) method was followed to determine the content of enzymatic proteins of the extracts.

II.8 Non-enzymatic antioxidant content

Co-extraction of total polyphenols and flavonoids was realized as reported previously by Lamsaadi et al. (2022a). 100 mg of fresh plant materials were homogenized in 1 mL of methanol (80%) at 4 °C using mortar and pestle. After 20 min of centrifugation at 12 000 ×*g* at 4 °C, the supernatant was recovered and stored at -20 °C until evaluation of the total polyphenols and flavonoids contents.

For total polyphenols, Folin-Ciocalteu (FC) method was adopted (Singleton and Rossi 1965). 50 µL of the resulted supernatant was mixed with 250 µL of FC reagent and the volume was adjusted to 5 mL with distilled water. After incubation for 3 min at room temperature, the volume was adjusted to 6.5 mL with Na_2CO_3 (20%) and the resulted mixture was incubated at the dark for 1h at room temperature. The OD was then read at 725 nm and the content of total polyphenols was determined and expressed as mg gallic acid equivalents g^{-1} FW, with three replicates for each treatment.

Flavonoids content was assessed following the method of Chang et al. (2002). Briefly, 300 µL of methanol (95%), 20 µL of 10% aluminum chloride ($AlCl_3$), 20 µL of potassium acetate (1 M) and 560 µL of distilled water were added to 100 µL of supernatant. After incubation for 30 min at room temperature, the absorbance of the resulted mixture was read at 415 nm and the flavonoids content was calculated referring to a standard curve prepared from different concentrations of quercetin. Flavonoid content was expressed as mg quercetin g^{-1} FW, with three replicates for each treatment.

II.9 Compatible solutes accumulation

The proline content was determined by homogenizing 100 mg of fresh materials in 1 mL of aqueous sulfosalicylic acid (3%) according to Bates et al. (1973), with three replicates for each treatment. The homogenate was centrifuged at 14 000 rpm for 10 min at 4 °C and to 400 µL of the resulted supernatant, an equal volume of ninhydrin reagent and concentrated acetic acid were added. After 1h of incubation at 95 °C, the reaction was stopped using an ice bath. Afterward, 800 µL of toluene was added and the absorbance of the pink phase was read at 520 nm. The content of proline was determined using a standard curve prepared with known concentrations of proline and expressed as mmol proline g^{-1} FW.

Glycine betaine content was measured according to Grieve and Grattan (1983). 250 mg of dried plant materials were mechanically shaken with 7.5 mL of distilled water for 48h at 25 °C, and the resulted filtrate was diluted 1:1 with 2N sulfuric acid. After incubation in an ice bath under agitation for 1h, 0.2 mL of cooled potassium iodide-iodine (KI-I_2) reagent was added to 0.5 mL of mixture and incubated at 4 °C for 16 h. Then, after centrifugation at 10 000 rpm for 15 min at 0 °C, the supernatant was carefully recovered and the precipitate was dissolved in 3 mL of 1.2 dichloroethane. The OD of the dichloroethanic phase was measured at 365 nm and the content of glycine betaine was determined from a standard curve and expressed as mmol glycine betaine g^{-1} DW, with three replicates for each treatment were considered.

As described by Dubois et al. (2002), the content of soluble sugars was determined by homogenizing 100 mg of fresh leaf samples in 4 mL of 80% ethanol (v/v), with three replicates for each treatment. After 15 min of centrifugation at 5000 rpm at 4 °C, 1 mL of 5% phenol and 5 mL of concentrated sulfuric acid were added to 1 mL of supernatant. The mixture was left to cool down, and then the absorbance was measured at 485 nm. The content of soluble sugars was calculated from a standard curve prepared with glucose solutions and expressed as mg glucose g^{-1} FW.

II.10 Sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}) determination

Na^+, K^+ and Ca^{2+} contents in fenugreek plants were determined according to Oukaltouma et al. (2021). 0.5 g of dry fenugreek plants were incinerated for 6h at 600 °C in a Protherm Furnaces (PLF 120/12). The resulted ashes were recovered in 3 mL of 10N HCl and the volume was adjusted to 50 mL using deionized water. The amount of Na^+, K^+ and Ca^{2+} was determined using a flame emission photometer (AFP100 Model, Biotech Management Engineering Co. Ltd., UK).

II.11 Statistical analysis

Data were analyzed using two-way analysis of variance (ANOVA II), where Si and salinity were the independent variables. Means were compared using Tukey’s test at 95% confidence level. Pearson's correlation matrix was realized by using XLSTAT statistical software, version 2014.5.03 at $p < 0.05$.

III. Results

III.1 Growth attributes

Results indicated that salt stress significantly ($p < 0.05$) reduced shoot dry weight (SDW), root dry weight (RDW), plant height (PH) and leaf area (LA) by 60%, 66%, 145% and 89%, respectively, as compared to control (Table 9; Figure 13a-b). However, 3 mM of Si supplementation to the growth medium of salt-stressed fenugreek plants alleviated the negative impact of salt and increased SDW, RDW, PH and LA by 100%, 100%, 60% and 40%, respectively, relative to Si-untreated salt-stressed plants. Under normal conditions, Si supply increased SDW by 20%. Regarding water status of fenugreek plants, relative water content (RWC) was significantly ($p < 0.05$) decreased from 64% to 42% under salt stress. Si supplementation improved leaf RWC of fenugreek plants under either stressed or unstressed conditions. Indeed, RWC of stressed and unstressed plants was 1.6 and 1.2-fold higher under Si treatment relative to their respective Si-untreated control.

Under salinity conditions, number of seed per pod (NSP) was 1.72-fold higher in Si-treated fenugreek plants as compared to plants without Si. Moreover, under unstressed conditions, Si improved NSP by 14% relative to Si-untreated control.

Table 9: Effect of exogenous silicon (3 mM Si) treatment on SDW, RDW, PH, LA, NSP and RWC of fenugreek plants grown under unstressed (0 mM NaCl) and stressed (150 mM NaCl) conditions. Data are the mean of three replicates ± standard error, and the different letters show a significant difference at $p < 0.05$

Treatments	SDW (mg plant^{-1})	RDW (mg plant^{-1})	PH (cm plant^{-1})	LA (cm^{2})	RWC (%)	NSP
C	153.33±5.13b	27.33±0.64a	35.66±0.20a	4.89±0.29a	63.88±0.82b	14.00±0.76b
Si	180.00±7.69a	26.35±0.51a	36.80±0.15a	4.76±0.22a	73.45±3.01a	16.00±0.38a
NaCl	60.00±3.84d	10.00±0.48c	14.56±0.29c	2.59±0.09c	42.40±1.97c	6.00±0.38d
NaCl+Si	123.33±6.41c	19.66±0.25b	23.46±0.24b	3.62±0.17b	68.51±2.71ab	10.33±0.64c

C, control; Si, 3 mM Si; NaCl, 150 mM NaCl; SDW, shoot dry weight; RDW, root dry weight; PH, plant height; LA, leaf area; NSP, number of seeds per pod; RWC, relative water content.

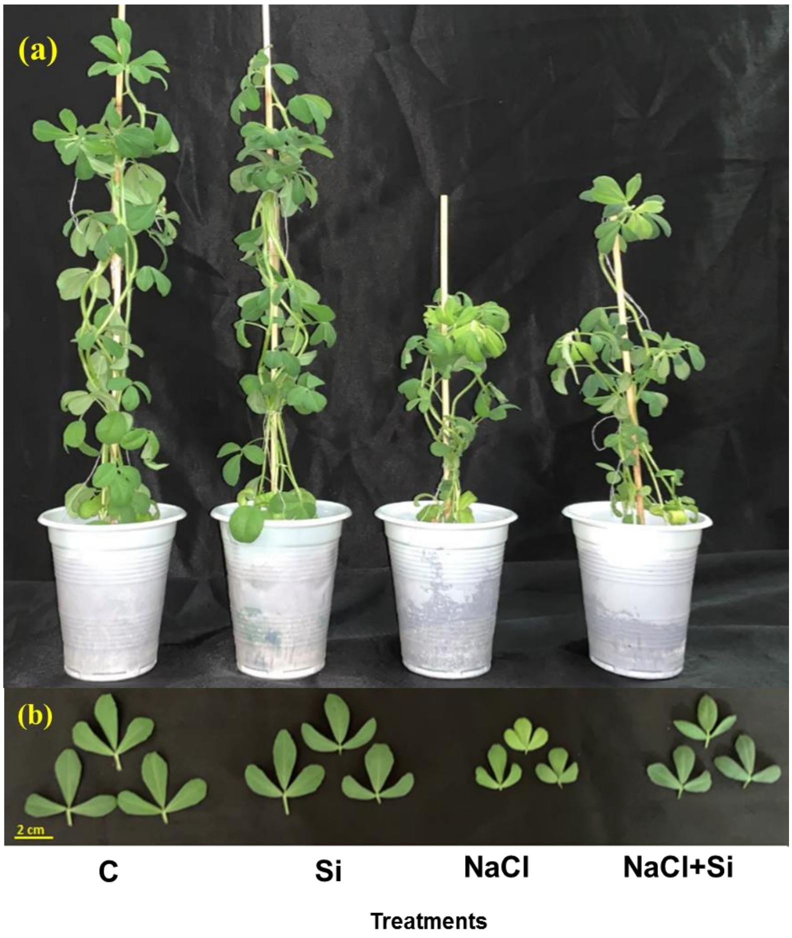

Figure 13: Effect of exogenous silicon (3 mM Si) treatment on plant phenotype (a) and leaf area (b) of fenugreek plants grown under unstressed (0 mM NaCl) and stressed (150 mM NaCl) conditions. C, control; Si, 3 mM Si; NaCl, 150 mM NaCl; NaCl+Si, combination of NaCl and Si

III.2 Photosynthetic pigments

Results presented in figure 14 (a-d) showed that photosynthetic pigments were significantly ($p < 0.05$) reduced upon salt stress. Indeed, chlorophyll (Chl) a, Chl b, total Chl and carotenoids were 2.59, 1.79, 2.32 and 2.20-times lower under 150 mM NaCl treatment as compared to the salt-untreated control. However, Si supply to the growth medium of salt-stressed fenugreek plants significantly improved Chl a, Chl b, total Chl, and carotenoids, respectively, by 40%, 33%, 38% and 37% as compared to Si-untreated salt-stressed plants. Under normal conditions, Si treatment has no significant ($p > 0.05$) effect on Chl a, Chl b and total Chl.

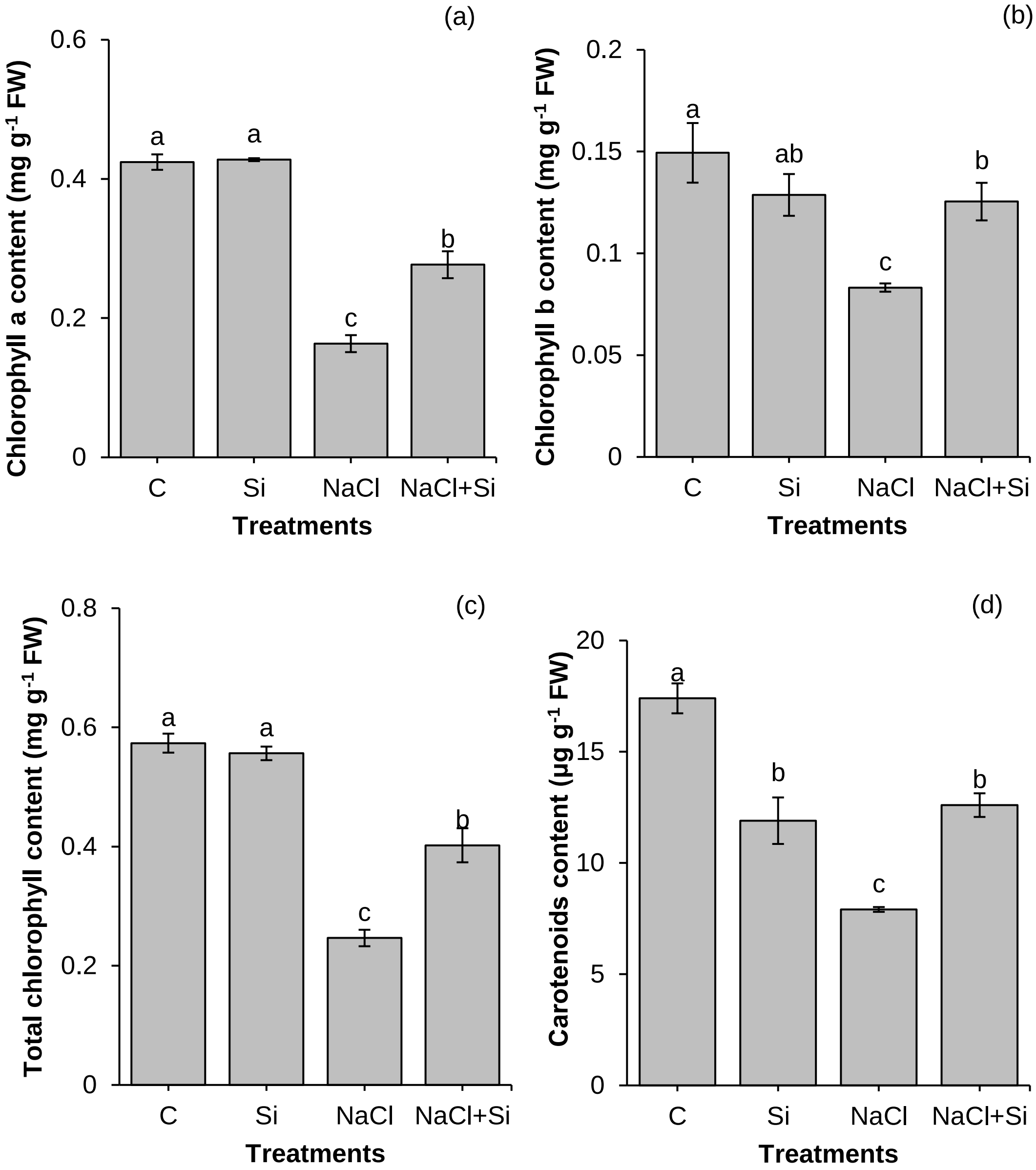

Figure 14: Effect of exogenous silicon (3 mM Si) treatment on chlorophyll a (a), chlorophyll b (b), total chlorophyll (c) and carotenoids (d) contents of fenugreek plants grown under unstressed (0 mM NaCl) and stressed (150 mM NaCl) conditions. Bars represent standard errors of three replicates and the values followed by different letters show a significant difference at $p < 0.05$. C, control; Si, 3 mM Si; NaCl, 150 mM NaCl; NaCl+Si, combination of NaCl and Si

III.3 Stomatal conductance and photosystem II efficiency

Stomatal conductance (Figure 15a) and the photosystem II efficiency (F_v/F_m; Figure 15b) were decreased respectively from 66.8 to 24.4 mmol H_2O m^{-2} s^{-1} and from 0.89 to 0.64 in response to 150 mM NaCl. However, the treatment of salt-stressed plants with 3 mM Si significantly ($p < 0.05$) improved the stomatal conductance and the photosystem II efficiency by 43% and 19%

relative to Si-untreated salt-stressed plants. In non-stressed plants, there was no significant ($p > 0.05$) difference between Si-treated and untreated fenugreek plants (Figure 15a-b).

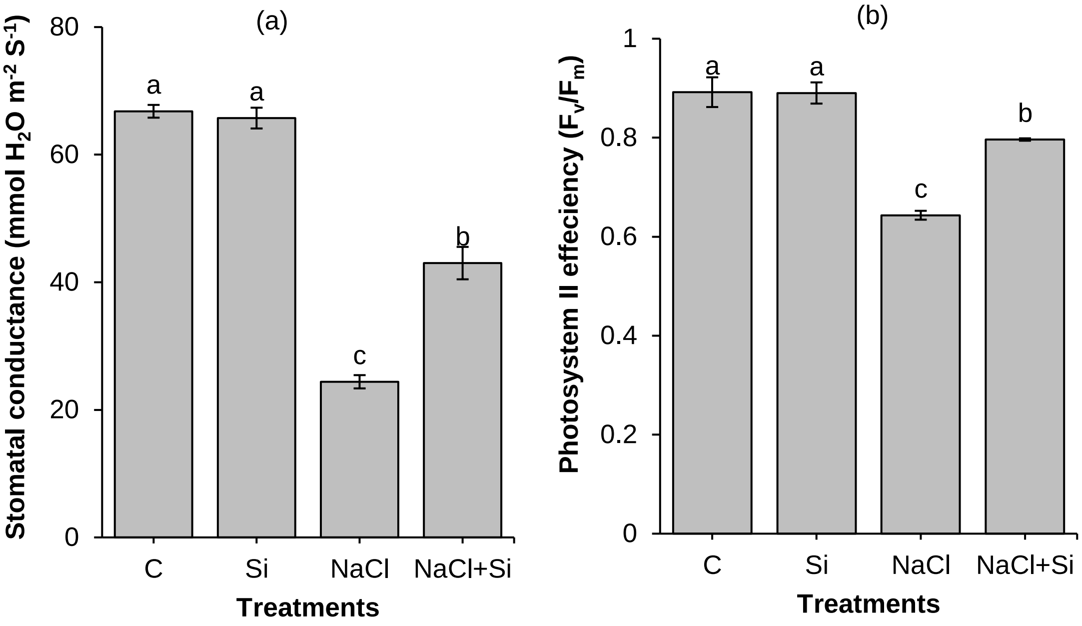

Figure 15: Effect of exogenous silicon (3 mM Si) treatment on stomatal conductance (a) and photosystem II efficiency (b) of fenugreek plants grown under unstressed (0 mM NaCl) and stressed (150 mM NaCl) conditions. Bars represent standard errors of three replicates and the values followed by different letters show a significant difference at $p < 0.05$. C, control; Si, 3 mM Si; NaCl, 150 mM NaCl; NaCl+Si, combination of NaCl and Si

III.4 Oxidative stress markers and membrane cell integrity

Exposure of fenugreek plants to 150 mM NaCl significantly ($p < 0.001$) increased the oxidative stress markers such as malonyldialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents and electrolyte leakage (EL) as compared to the control (Figure 16a-c). In fact, when compared to the unstressed control, MDA and H_2O_2 contents and EL (%) were 18.1, 1.87 and 3.4-fold higher in fenugreek plants exposed to salinity stress. However, when salt-stressed fenugreek plants were supplied with 3 mM of exogenous Si, MDA and H_2O_2 contents and EL were reduced by 40%, 18% and 40%, respectively relative to Si-untreated salt-stressed plants. No significant difference was observed between Si-treated and untreated fenugreek plants under normal conditions for all investigated oxidative stress markers (Figure 16a-c).

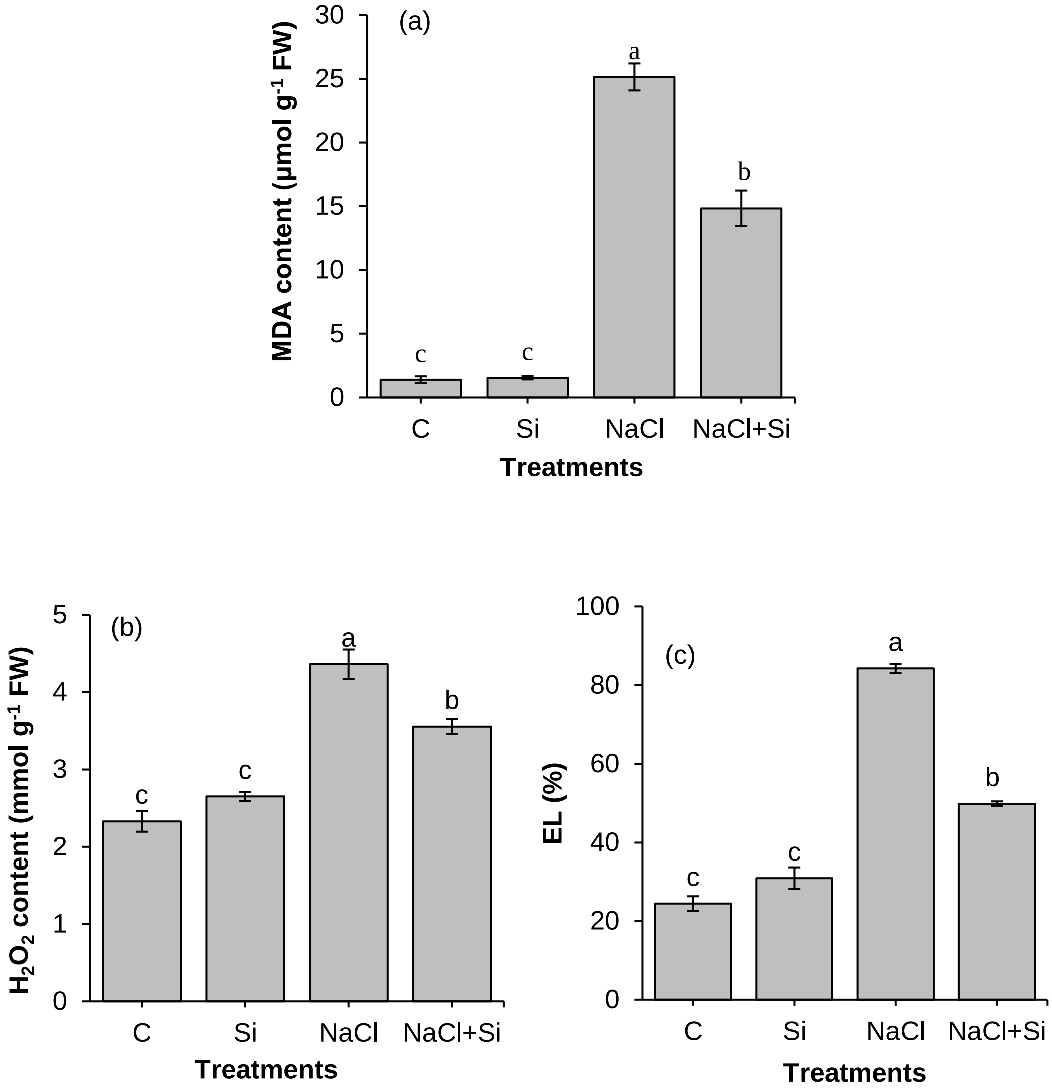

Figure 16: Effect of exogenous silicon (3 mM Si) treatment on malondialdehyde (MDA) (a) and hydrogen peroxide (H_2O_2) (b) contents and electrolyte leakage (EL) (c) in fenugreek plants grown under unstressed (0 mM NaCl) and stressed (150 mM NaCl) conditions. Bars represent standard errors of three replicates and the values followed by different letters show a significant difference at $p < 0.05$. C, control; Si, 3 mM Si; NaCl, 150 mM NaCl; NaCl+Si, combination of NaCl and Si

III.5 Enzymatic and non-enzymatic antioxidant activity

Results illustrated in Table 10 revealed that the contents of total polyphenols and flavonoids were drastically reduced upon salt stress. Indeed, 150 mM NaCl stress significantly ($p \leq 0.001$) decreased total polyphenols from 9.72 to 4.32 mg gallic acid g^{-1} FW and flavonoids from 20.50 to 6.36 mg quercetin g^{-1} FW reflected 56% and 69% of reduction rates, respectively, as compared to the control. However, Si supplementation markedly alleviated the negative impacts of salt stress and significantly ($p < 0.001$) improved total polyphenols and flavonoids contents.

Table 10: Effect of exogenous silicon (3 mM Si) treatment on total polyphenols and flavonoids contents and SOD and PPO activities in fenugreek plants grown under unstressed (0 mM NaCl) and stressed (150 mM NaCl) conditions. The represented data are the mean of three replicates ± standard error, and the different letters show a significant difference at $p < 0.05$

Treatments	Non-enzymatic antioxidant content		Enzymatic antioxidant activity	
	Total polyphenols (mg gallic acid g^{-1} FW)	Flavonoids (mg quercetin g^{-1} FW)	SOD activity (EU min^{-1} mg^{-1} protein)	PPO activity (EU min^{-1} mg^{-1} protein)
C	9.72 ± 0.24b	20.50±0.66a	33.32±3.13c	0.40±0.03c
Si	16.38 ± 1.14a	19.46±0.04a	31.81±7.11c	0.38±0.05c
NaCl	4.32 ± 0.66d	6.36±0.40c	88.84±12.82b	0.69±0.01b
NaCl+Si	7.64±0.31c	14.51±0.40b	199.03±5.97a	0.79±0.01a

C, control; Si, 3 mM Si; NaCl, 150 mM NaCl; NaCl+Si, combination of NaCl and Si; SOD, superoxide dismutase; PPO, polyphenol oxidase; EU, enzymatic unit; FW, fresh weight.

Regarding enzymatic antioxidant activity (Table 10), the activity of superoxide dismutase (SOD) and polyphenol oxidase (PPO) was significantly ($p < 0.05$) increased by 166% and 72%, respectively under salt stress as compared to the control. Additionally, the activity of both SOD and PPO was further increased in salt-stressed fenugreek plants when supplied with exogenous Si. In fact, under combined treatment of 150 mM NaCl and 3 mM Si, the activity of SOD and PPO was increased by 124% and 14%, respectively as compared to salt stress alone. When applied to the unstressed plants, Si has no significant effect ($p > 0.05$) on SOD and PPO activities.

III.6 Compatible solutes accumulation

Salt stress caused a significant increase in the content of compatible solutes, in terms of soluble sugars, proline and glycine betaine, and this increase was more furthered ($p < 0.01$) when salt stressed fenugreek plants were treated exogenously with 3 mM Si (Figure 17). Indeed, in Si-treated salt-stressed plants, soluble sugars, proline and glycine betaine contents were significantly increased by 42%, 56% and 14%, respectively, relative to plants treated with NaCl alone (Figure 17). Si treatment had no significant ($p > 0.05$) effect on compatible solutes under unstressed conditions.

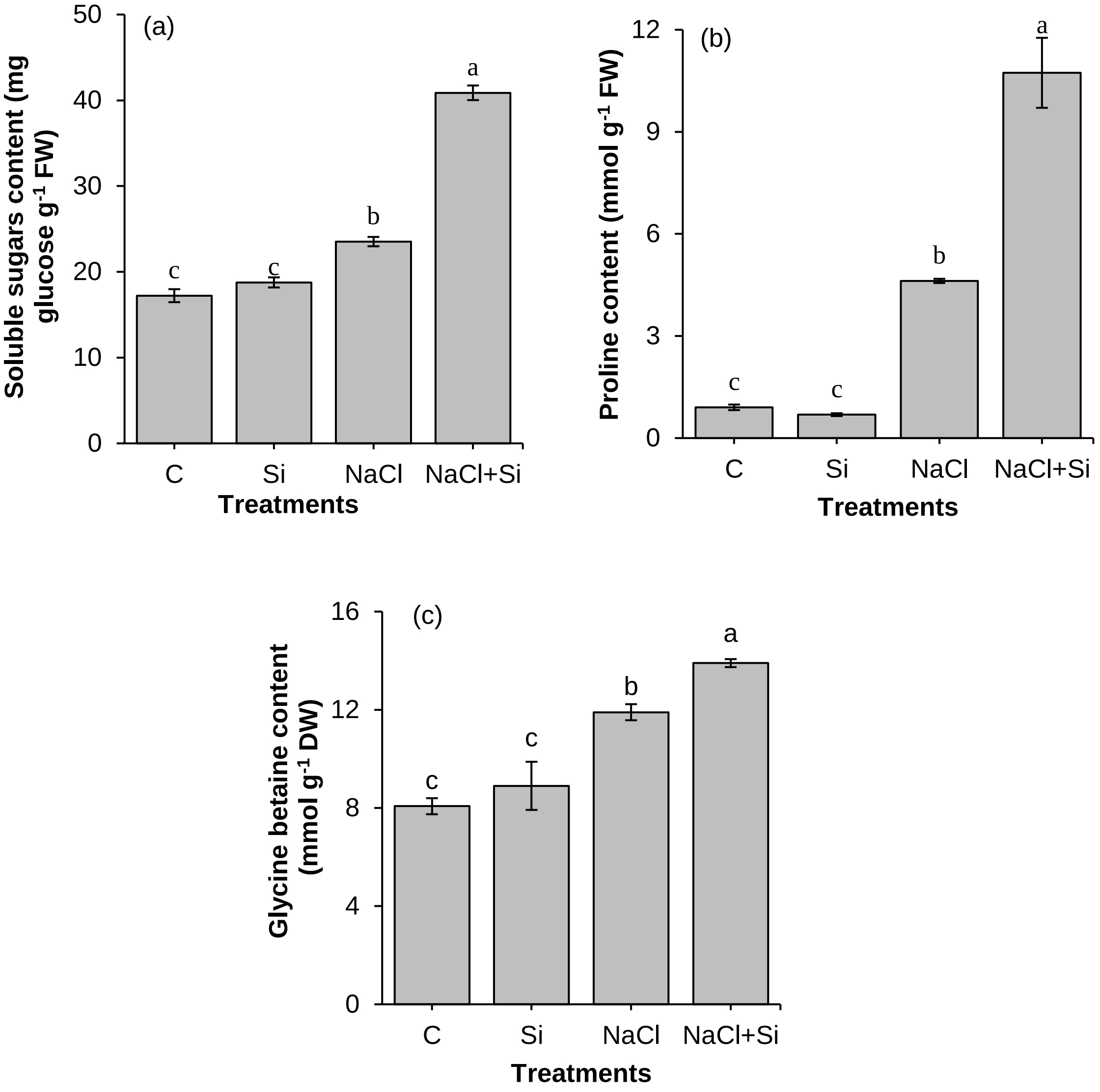

Figure 17: Effect of exogenous silicon (3 mM Si) treatment on the content of soluble sugars (a), proline (b) and glycine betaine (c) in fenugreek plants grown under unstressed (0 mM NaCl) and stressed (150 mM NaCl) conditions. Bars represent standard errors of three replicates and the values followed by different letters show a significant difference at $p < 0.05$. C, control; Si, 3 mM Si; NaCl, 150 mM NaCl; NaCl+Si, combination of NaCl and Si

III.7 Mineral nutrition

Results in Table 11 showed that salt stress imposition elevated the content of sodium (Na^+) by 217%, while it significantly ($p < 0.05$) reduced potassium (K^+) and calcium (Ca^{2+}) by 53% and 29%, respectively relative to the untreated control. In addition, the increase in Na^+ and the decrease in K^+ reduced K^+/Na^+ ratio from 0.61 to 0.12 reflected 80% of reduction rate (Table 11). However, treatment with Si significantly ($p < 0.05$) increased K^+, while it significantly decreased Na^+ content leading to a higher K^+/Na^+ ratio (0.26) compared to Si-untreated salt-stressed fenugreek plants (0.12). Moreover, Si treatment alleviated the negative effect of salinity and significantly ($p < 0.05$) improved Ca^{2+} content. Under normal conditions, Si application caused a significant increase in the content of Na^+, K^+ and Ca^{2+}, but it remarkably decreased K^+/Na^+ ratio.

Table 11: Effect of exogenous silicon (3 mM Si) treatment on Na^+, K^+ and Ca^{2+} contents and K^+/Na^+ ratio in fenugreek plants grown under unstressed (0 mM NaCl) and stressed (150 mM NaCl) conditions. The different letters show a significant difference at $p < 0.05$

Treatments	Na^+ (mg g^{-1} DW)	K^+ (mg g^{-1} DW)	Ca^{2+} (mg g^{-1} DW)	K^+/Na^+
C	35.33d	48.35c	44.69c	0.61a
Si	70.43c	60.48a	52.08b	0.42b
NaCl	112.04a	31.55d	31.50d	0.12d
NaCl+Si	107.70b	59.80b	73.05a	0.26c

Na^+, sodium ; K^+, potassium ; Ca^{2+}, calcium ; C, control; Si, 3 mM Si; NaCl, 150 mM NaCl; NaCl+Si, combination of NaCl and Si.

IV. Discussion

Reduction in plant growth is a common response to salt stress, which might be due to a reduction in cell division and cell elongation (Latef et al. 2017). Similarly, our findings revealed that under salt stress conditions, fenugreek plants growth was severely reduced as reflected by a significant decrease in shoot and root dry weight, plant height and leaf area. Salinity also remarkably reduced the number of seeds per pod by 57% relative to the unstressed control. Therefore, exposure to 150 mM NaCl interestingly reduced both fenugreek growth and yield. However, exogenous Si supply to the growth medium significantly alleviated the harmful effects of 150 mM NaCl stress on all the above studied growth and yield parameters. These findings are in agreement with those of El Moukhtari et al. (2021a), who reported that 3 mM Si significantly improved plant biomass, plant height, leaf number and leaf area in *Medicago sativa* L. under 120 mM NaCl stress. Likewise, Ali et al. (2021) found that Si was able to improve plant biomass and RWC in exposed maize to salinity. According to Bayat et al. (2013), when applied under salt stress, Si caused a significant increase in calendula growth traits including shoot and root dry weight, plant height and leaf area. Similar findings were reported in purslane (Kafi and Rahimi 2011a), basil (Robatjazi et al. 2020) and honeysuckle (Gengmao et al. 2015a). Several studies demonstrated that, under stressed conditions, the plants biomass reduction could be the results of photosynthesis capacity reduction. Indeed, the decrease in plants growth parameters is significantly correlated with a decrease in chlorophyll synthesis and in other photosynthetic parameters in various plants species like lavender (Chrysargyris et al. 2018), *Vigna angularis* (Ahanger et al. 2019) and Cucumber (Mousavi et al. 2022). In the present study, salt-stressed fenugreek plants had reduced photosynthetic pigments (Chl a, Chl b, total Chl and carotenoids) as compared to controls. This effect is often attributed to the toxic effect of Na^+ and Cl^- on chlorophyll synthesis machinery, where there is a close negative correlation between shoot Na^+ content and photosynthetic pigments, like total chl ($r = -0.89$; $p \leq 0.05$; Figure 18). Another explanation of the decreased Chl content under salt stress is the increase in the activities of Chl degrading enzymes such as chlorophyllase, Chl-degrading peroxidase and pheophytinase (Alamri et al. 2020). Yang et al. (2020) reported that photosynthesis inhibition is one, among others, factors that will minimize growth under salt stress. This was clearly observed in our study as indicated by the highly significant correlation (Figure 18) observed between root dry weight and Ch a ($r = 95$; $p \leq 0.05$), Chl b ($r = 69$; $p \leq 0.05$), total Chl ($r = 93$; $p \leq 0.05$) and carotenoids ($r = 71$; $p \leq 0.05$). However, supply of Si in salt-stressed fenugreek plants led to significant increases of Chl a, Chl b, total Chl and carotenoids. Another consequence of salt stress on photosynthesis is the decrease of Chl fluorescence, especially the photosystem II parameter; F_v/F_m ratio (Farissi et al. 2018). This might be a consequence of Chl reduction in response to salt. Our results revealed that F_v/F_m ratio was remarkably reduced upon salt stress and this negative effect was reversed by Si treatment. Interestingly, F_v/F_m ratio was positively correlated with Chl content (r = 0.89, $p \leq 0.05$ for total chl), confirming the finding of Ganieva et al. (1998). Previous studies indicated that Si supplementation significantly improved photosynthetic pigments due to its ability to increase the activities of some Chl synthesis enzymes, including δ-aminolevulinic acid dehydratase and porphobilinogen deaminase, under salt stress (Alamri et al. 2020).

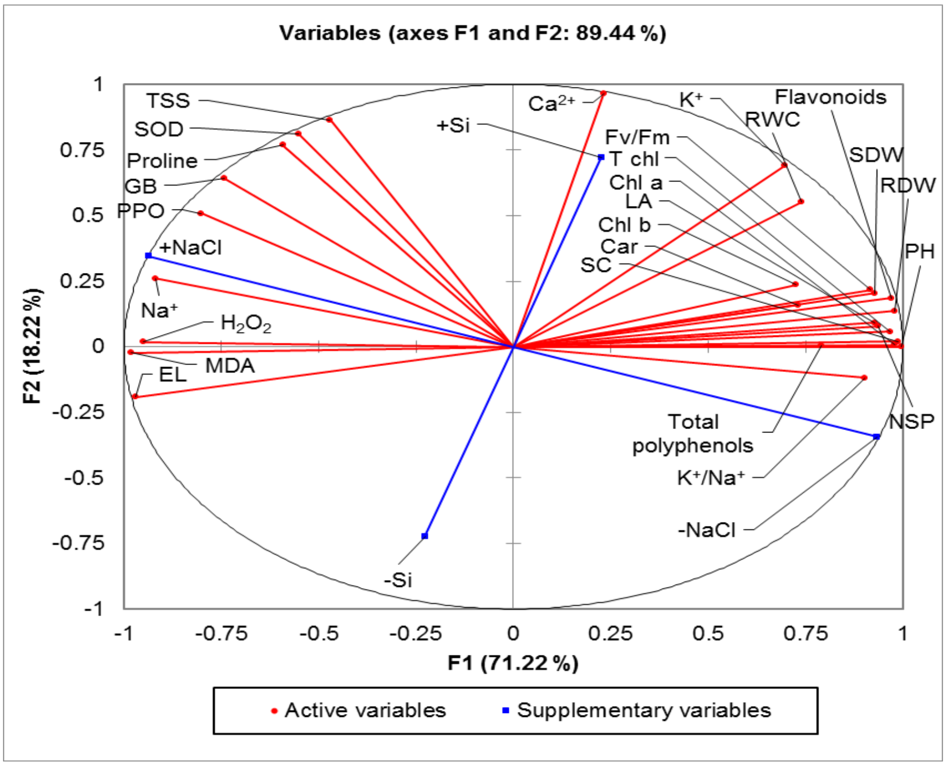

Figure 18: Principal component analysis (PCA) of all studied parameters related to response of fenugreek to salt stress and Si supplementation in the growth medium. The most variables (arrows), Si treatment and 150 mM NaCl treatment are projected onto the F1-F2 principal factorial plane that explains 89.44 % of the variation. SDW: Shoot dry weight; RDW: Root dry weight; PH: Plant height; LA: Leaf area; NSP: Number of seed per pod; T Chl: Total chlorophyll; Chl a: Chlorophyll a; Chl b: Chlorophyll b; Car: Carotenoids; F_v/F_m: Photosystem II efficiency; SC: Stomatal conductance; RWC: Relative water content; MDA: Malonyldialdehyde; H_2O_2: Hydrogen peroxide; EL: Electrolyte leakage; SOD: Superoxide dismutase; PPO: Polyphenol oxidase; TSS: total soluble sugars; GB: Glycine betaine; Si: Silicon; Ca^{2+}: Calcium; Na^+: Sodium; K^+: Potassium.

Added exogenous Si also contracted the inimical effects of 150 mM NaCl constraint on nutrition balance by enhancing fenugreek nutrition, in terms of high content of K^+ and Ca^{2+} and a significant decrease of Na^+ accumulation, which might be the reason of improved biomasses and phenotype of fenugreek plants under salt stress. In agreement to the above findings, Shekari et al. (2017) found that application of Si decreased Na^+ concentration and increased K^+ concentration in roots and shoots of *Anethum graveolens* L. plants, correlating with a significant amelioration in chlorophyll content and plant biomasses under saline condition. Therefore, the positive effect of Si on photosynthetic pigment can also be attributed to the involvement of Si in reducing Na^+ uptake by salt-stressed plants. Shen et al. (2022) demonstrated that Si supplementation resulted in reduced Na^+ content and improved K^+ content, photosynthetic pigments content and gas exchange parameters, which in return enhanced *Glycyrrhiza uralensis* and *G. inflata* growth under salinity stress. Previously, the significant improvement of plant growth, photosynthetic activity and nutrition balance in response to adding exogenous Si under salt stress has been reported, also, in several plant species such as wheat (Hajiboland et al. 2017), and *Crocus sativus* L. (Fahimi et al. 2017). In salt-stressed okra plant, Abbas et al. (2015) reported that foliar spray of Si enhanced stomatal conductance, photosynthetic rate, transpiration rate and number and size of stomata. More than that, Gou et al. (2020) showed that added Si could significantly decrease chlorophyll degradation and tomato plant senescence under salt stress. Based on above cited positive effects, Si-mediated increase in growth, yield and photosynthetic activity of fenugreek plants might be partly attributed to different mechanisms including decrease in salt ions uptake, like Na^+, increase in mineral nutrition (K^+ and Ca^{2+}), modification in gas exchange and photosystems performance under salt stress.

Closing stomatal pores is a common response of plants to overcome water loss by transpiration especially under osmotic stress conditions (Bertolino et al. 2019). However, this led to reduction of CO_2 assimilation and to perturbation of photosynthetic activities. In the present study, salt-stressed fenugreek plants showed a lower RWC as compared to control, indicating an osmotic stress. The decrease in RWC in salinity conditions was positively and significantly correlated to a decrease in stomatal conductance ($r = 0.78$, $p \leq 0.05$, Figure 18). However, as previously reported by Siddiqui et al. (2014) and Avestan et al. (2019), Si supply along with NaCl significantly improved both RWC and stomatal conductance. Si improved RWC under salt stress has been reported in several plant species including maize (Rohanipoor et al. 2013), wheat (Hajiboland et al. 2017), cucumber (Mousavi et al. 2022) and turfgrass (Esmaeili et al. 2015). On the one hand, it was reported that, after the uptake, Si accumulates on the epidermis of various plant tissues mainly as a polymer of hydrated amorphous silica, and consequently raised the wax content of the plant epidermis to regulate the water use efficiency and water evaporation (Epstein 1994; Feng Ma 2004; Wang et al. 2022), which could explain the enhanced RWC in Si-treated salt-stressed plants.

Compatible osmolytes are small molecules that can act as osmoprotectant, alleviating salt stress by regulating cellular osmotic pressure (Meena et al. 2019; El Moukhtari et al. 2020). Furthermore, the ability of stressed plant to accumulate compatible osmolytes may define their tolerance capability (Suprasanna et al. 2016). In the present study, salt-stressed fenugreek plants have accumulated numerous compatible solutes, including organic (proline, glycine betaine and soluble sugars) and inorganic (K^+) compounds. More interestingly, the increased compatible solutes in salt-stressed plants were further enhanced when they were treated with 3 mM Si, which could explain the RWC amelioration under salt-mediated osmotic stress and in return enhanced morphological aspect and growth of stressed plants. Previous research indicated that Si increased salt tolerance of plants by regulating osmolytes accumulation, allowing osmotic potential adjustment. For example, in a study conducted on salt-stressed Cucumber by Mousavi et al. (2022), Si incorporation in cultured media resulted in a significant increase in the content of proline and soluble sugars. The same has been reported in other plant species such as wheat (Hajiboland et al. 2017) and okra (Abbas et al. 2015). According to Zhu et al. (2020), exogenous Si was involved directly in proline biosynthesis by inhibiting the activity of proline dehydrogenase and enhancing that of pyrroline5-carboxylase synthase, which resulted in an increase in plant proline content.

The effect of salt stress in plants can also be seen in the form of oxidative stress. Elevated Na^+ content particularly in the aerial parts led to a dramatic accumulation of H_2O_2 ($r = 0.90$, $p < 0.05$) in the leaves. If not metabolized, H_2O_2 could induce membrane damages (Slimen et al. 2014). In our study, the increase in H_2O_2 content was significantly correlated with MDA content ($r = 0.94$, $p < 0.05$) and electrolyte leakage percentage ($r = 0.90$, $p < 0.05$), indicating an oxidative damage. Similar findings were obtained by Ahanger et al. (2019), who found that, under salinity, the accumulation of ROS was positively correlated with an increase in lipid peroxidation. However, in the current study, incorporation of Si to the growth medium significantly mitigated the adverse effects of salt stress on membrane integrity by decreasing H_2O_2 and MDA contents and electrolyte leakage value. Similarly, several studies reported that ROS generation and membrane cell instability were significantly declined in response to exogenous Si application under salt stressed conditions (Al-aghabary et al. 2007; Avestan et al.

2019; Robatjazi et al. 2020). To overcome salinity-mediated oxidative stress, tolerant plants adopt some tolerant strategies. This includes the induction of the enzymatic and non-enzymatic antioxidant pathways (Aghaei and Komatsu 2013). Importantly, in the current study, supply of Si to salt-stressed plants furthered the increase in the activity of SOD and PPO, together with an increase in the content of non-enzymatic antioxidant compounds (total polyphenols and flavonoids). Thus, Si treatment induced ROS detoxification by promoting the activities of antioxidant enzymes, and enhancing the content of non-enzymatic antioxidant compounds such as total polyphenol and flavonoids. Likewise, in salt-stressed tomato, Al-aghabary et al. (2007) reported that Si treatment decreased lipid peroxidation and H_2O_2 content, while it increased SOD and catalase activities. Si-mediated reduction in oxidative stress under salinity stress was also reported in rice (Kim et al. 2014), okra (Hajiboland et al. 2017), *Anethum graveolens* L. (Shekari et al. 2017) and *Glycyrrhiza uralensis* (Zhang et al. 2017) during response to salinity constraint. Thus, in addition to the enhancement of photosynthetic process performance and accumulation of osmoprotectant compounds, incorporation of exogenous Si to the stressed growth medium was also able to alleviate the harmful effect of 150 mM NaCl on fenugreek plants by activating both enzymatic and non-enzymatic antioxidant systems, as well as detoxification of oxidative stress markers.

V. Conclusion

Overall, salt stress significantly reduced fenugreek growth and yield, due to reduction in relative water content, photosystem II efficiency and chlorophyll content. Also, Salinity caused a significant induction of oxidative stress, reflecting by high accumulation of MDA and ROS in salt stressed fenugreek plants. However, Si addition alleviated salt-induced reduction in plant growth and yield by enhancing photosynthesis, relative water content and the uptake of indispensable nutrients like K^+ and Ca^{2+}. Exogenous Si also decreased Na^+ accumulation and saved the membrane permeability, due to a decrease in oxidative stress markers. In addition, adding Si induced defence-related mechanisms *via* activation of both enzymatic and non-enzymatic antioxidant systems and accumulation of organic compounds, in terms of proline, glycine betaine and soluble sugars. Thus, under salt stressed conditions, Si treatment might be a useful method for improving fenugreek tolerance and yield.

Chapter 4: Exogenous silicon alleviates salinity stress in fenugreek (*Trigonella foenum graecum* L.) by enhancing photosystem activities, biological nitrogen fixation and antioxidant defence system

Abstract

Currently, many studies are conducted on the beneficial roles of exogenous application of silicon (Si) under stressed conditions. However, the mechanisms for Si alleviated salt damage especially on photosynthetic machinery and biological nitrogen fixation in legume plants are still not very clear. Therefore, the present study was carried out to investigate the beneficial role of exogenous application of Si in alleviating the salt (150 mM NaCl) damage to fenugreek (*Trigonella foenum graecum* L.), as an important both legume and aromatic and medicinal plant. Our results indicated that salinity stress significantly decreased growth parameters, chlorophyll content and all assessed photosynthetic traits. However, these depressive effects were markedly mitigated by Si supplementation in the growth medium. In fact, Si treatment alleviated the salinity effect on the plant growth parameters, relative water content, photosynthetic pigments, and photosystem (PS) I and II functions, in terms of maximal quantum yield, energy conversion and regulation and electron transporter rate. Interestingly, Si had a great beneficial effect on the function of PSII in comparison to PSI. Nodule number and nitrogen content, as symbiosis-related parameters, were significantly improved in salt-stressed plants by Si treatment compared to salt stress without Si. The ameliorative effects of Si could be explain by the significant decrease in oxidative stress markers and the high obtained antioxidant activity and endogenous Si content in both shoot and root of Si-treated fenugreek plants under salt stress. Taken together, Si mediated salt tolerance of fenugreek plants by enhancing photosynthesis and biological nitrogen fixation, which are among the vital processes for legume development, thus in return, their application could be a promoting way to overcome the harmful effects of salinity stress on agricultural ecosystems production.

Keywords: Antioxidant activity, Biological nitrogen fixation, Fenugreek, Photosynthesis, Salinity, Silicon, Tolerance.

I. Introduction

Crop legume family (Fabaceae) are cultivated in up to 15% of arable land in the world, where they represent 27% of the primary crop production worldwide (Graham and Vance 2003; Choi et al. 2004). In addition to their importance as a source of proteins in human and animal diet, legume species are able to transform, via a symbiotic process, atmospheric nitrogen into ammoniac in order to make it available to plant roots. For these reasons, plant legumes are recommended and cultivated as a good soil renovator and widely used as green manure in many agricultural ecosystems (Tsegaye et al. 2015). Besides to atmospheric nitrogen fixation, many legume species are known for other advantages. For instance, fenugreek (*Trigonella foenumgraecum* L.) has been reported to have many medicinal and therapeutical properties in addition to its capacity to fix atmospheric nitrogen (N_2) in symbiosis with rhizobia (Singh et al. 2008). For example, among the major fenugreek producer countries of which Morocco, it is documented that this aromatic and medicinal plant has been used for a long time as a spice crop, a remedy against fever and for stimulation of human appetite (Haddad et al. 2003; Benayad et al. 2014a,b). Moreover, recently Alrumaihi et al. (2021) found that fenugreek seed extract has a significant cytotoxic effect against cancer cells. However, despite the above-cited considerable agricultural and medicinal advantages, the research on the development of new approaches and strategies to keep fenugreek growth and yield under stressed conditions remains less developed.

Salt stress is one of the most important abiotic stresses that limits plant growth by causing osmotic stress and ion toxicity that trigger oxidative stress reflected by an over synthesis of reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2) (Rasool et al. 2012). Induction of oxidative stress, under salt-stressed conditions, perturbs many vital processes, like photosynthesis, by destructing membrane permeability and chloroplast structure (Dos et al. 2021). In *Vigna angularis*, various photosynthetic attributes, including chlorophyll content, net photosynthetic rate, photosystem II efficiency and stomatal conductance were remarkably decreased after an increase in ROS accumulation and lipid peroxidation in response to salt stress (Ahanger et al. 2019). Similar results have been found in *Trigonellafoenum-graecum* (Latef et al. 2017) and *Medicago sativa* (Meng et al. 2020).

Additionally, inhibition of biological N fixation is a common destructive impact of salinity in legume species (Manchanda and Garg 2008). Indeed, before symbiosis establishment, salt stress could reduce rhizobial survival and their ability to produce Nod factors and lateral root development, which in results inhibiting nodule formation (Zahran 1999, Dardanelli et al. 2008). Salinity can affect symbiosis even after the establishment, by reducing nodule number, nodule function and N content in many legume species, including faba bean (Fahmi et al. 2011), alfalfa (El Moukhtari et al. 2021a), *Phaseolus vulgaris* and *Sesbania aculeata* (Ashraf and Bashir 2003). Moreover, in salt-stressed chickpea, Abd-Alla et al. (2019) found that the reduction in N fixation was linked to decreasing reduction in leghemoglobin content and nitrogenase activity. Another common harmful impact of salinity on plant growth is a high accumulation of sodium (Na^+) and chloride (Cl^-) in different plant organs, generating an ionic toxicity (Yang and Guo 2018).

Recently, many exogenous molecules, such as nutrient elements, are recommended as bio-stimulants to mitigate salt-induced plant growth inhibition (D’souza and Devaraj 2013; Askary et al. 2017). Among these nutrient elements, silicon (Si) has been reported to improve abiotic stress tolerance, including salinity in many plant species (Nasseri et al. 2012; Xu et al. 2015; Liu et al. 2015; Din et al. 2021). However, the mechanisms by which Si improved plant abiotic stress tolerance is still ambiguous. For instance, exogenous Si treatment has been shown to improve photosynthesis-related parameters, including chlorophyll content and PSII efficiency under salt-stressed conditions (Gengmao et al. 2015a; Liu et al. 2015; Ju et al. 2019; Zhang et al. 2022). However, its effect on two photosystem performance, on electron transporter rate in PSI and PSII, on energy conversion in PSI and PSII and on the regulation of lost energy during two PS functioning in salt-stressed plants has not been investigated so far. In this regard, the present study was conducted to evaluate the role of exogenous Si on photosynthesis, in terms of chlorophyll content, chlorophyll fluorescence, electron transporter rate, quantum yields and energy conversion in PSI and PSII and the quantum yield of regulated and non-regulated non-photochemical energy in both photosystems of salt-stressed fenugreek plants. Likewise, the effect of combined treatment of salt stress and Si on growth parameters, symbiosis-related parameters, oxidative stress and antioxidant activity was also assessed in exposed fenugreek plants to salinity stress.

II. Materials and methods

II.1 Plant material and growth conditions

In the present study, fenugreek (*Trigonella foenum graceum* L.) seeds were supplied by the National Institute of Agronomic Research (INRA, Morocco). After disinfection by immersing seeds in 5% of sodium hypochlorite solution for 5 min, seeds of fenugreek were rinsed thoroughly with distilled water and allocated to germinate in a plastic pot (ten seeds per pot), containing a sterilized sand-peat mixture (1:4 v:v). The experiment was conducted in a greenhouse under sunlight conditions (23 - 25 °C). Two weeks after sowing, four homogenous fenugreek seedlings were selected per pot and inoculated three times with an *Ensifer meliloti* Rm41 strain and watered with a N-free nutrient solution once a week (Hoaglan and Arnon 1950). Plants were then divided into four lots: (i) plants irrigated with N-free nutrient solution alone (C treatment), (ii) plants watered with N-free nutrient solution supplemented with 3 mM silicon in $CaSiO_3$ (Si treatment), (iii) plants watered with N-free nutrient solution containing 150 mM NaCl (NaCl treatment), and (iiii) plants watered with N-free nutrient solution supplemented with 3 mM Si and 150 mM NaCl (NaCl + Si treatment). For each lot, 16 pots containing four plants each were considered. 150 mM NaCl and 3 mM Si were selected according to our previous works (Lamsaadi et al. 2022a, b). Stress was applied for 25 days, then plant growth attributes, nodulation parameters, photosynthetic activity and other salt tolerance-related parameters were assessed.

II.2 Rhizobia strain isolation, characterization, identification and inoculum preparation

The used rhizobial strain *Ensifer meliloti* Rm41 in the current study was isolated and purified from *Medicago sativa* L. root nodules grown in the Beni Mellal region, Morocco (32°26'24.9"N 6°19'44.8"W). The rhizobial isolate was identified as *E. meliloti* strain Rm41 (accession number: CP021808.1) using the housekeeping gene *gyrB* (Farssi et al. 2021). Its aptitude to grow under 1.02 M NaCl or 20% polyethylene glycol (PEG-6000) and its ability to promote N_2 fixing, to solubilize tricalcium phosphate, to produce exopolysaccharides and indole acetic acid, was the key reasons to select this strain for plant inoculation (El Moukhtari et al. 2021a). The rhizobial strain was grown in liquid yeast extract-mannitol medium for 3 days at 28 °C to reach an approximative cell density of 10^8 bacteria per mL and was further used to inoculate the plants.

II.3 Growth, nodulation and physiological parameters

After 25 days of treatment, plant length was determined using a ruler. The leaf area was measured using Mesurum software version 3.4.4.0. according to Lamsaadi et al. (2022b). Plants were then harvested and the shoots were separated from roots and their fresh biomass was determined. Shoots and roots parts were then dried in an oven at 80 C and their dry weight was determined. During harvest, the number of nodules per root was also determined to evaluate the effect of the applied treatments on nodule formation. All of these growth attributes were determined in five individual plants per treatment.

Barrs and Weatherley's method (1962) was used to determine relative water content (RWC). For this purpose, the fresh weight (FW) of three leaves of five plants from each treatment was determined. Leaves were then immersed in distilled water for 24h at room temperature and their turgid weight (TW) was measured. After an oven drying at 70 °C for 24h, dry weight (DW) of the leaves was taken and the RWC was determined using the below formula.

$$\mathbf{RWC\ (\%) = [(FW-DW) / (TW-DW)] \times 100}$$

II.4 Photosynthetic pigment contents

Chlorophyll (Chl) a, Chl b and carotenoids were extracted by incubating healthy leaves (100 mg) in 5 mL of 80% acetone. After 72h of incubation at 4 °C in darkness, the absorbance of the extract was measured at 470, 646 and 663 nm using a UV-visible spectrophotometer (Specord 210 Plus, Analytik Jena, Germany). The content of photosynthetic pigment was determined according to Lichtenthaler (1987).

II.5 Simultaneous measurement of quantum yield, energy conversion, Chl fluorescence and P700 redox state in both photosystems I and II

To study the machinery and functioning of both photosystems I and II, five leaves from each treatment were adapted in the dark for 30 min and then, the minimal, the variable and the maximal Chl fluorescence at opened PSII centers were calculated according to Baker (2008), using a Dual-PAM-100 fluorimeter (Heinz Walz, Effeltrich, Germany). Additionally, the measurement of quantum yield and energy conversion in both photosystems was also recorded under different light intensities (6–1017 µmol photons m^{-2} s^{-1}) (Klughammer and Schreiber 2008a, b). According to Klughammer and Schreiber (2008a), Dual-PAM-100 Fluorimeter is also equipped to determine the oxidized PSI (P700ox) and the maximal fluorescence yield (P700m) of dark-adapted leaves with all PSI centers closed.

II.6 Hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents

The method of Brennan and Frenkel (1977) was followed to determine the H_2O_2 content in leaves and roots of five individual fenugreek plants per treatment. Briefly, 100 mg of fresh weight were homogenized in 2 mL of cold acetone and centrifuged at 6000 rpm for 15 min at 4 °C. Then, 150 µL of 20% titanium (prepared in concentrated hydrochloric acid; v/v) and 300 µL of concentrated ammonia were added to 1350 µL of the resulted supernatant and the mixture was centrifuged at 10 000 rpm for 10 min at 4 °C. The supernatant was then discarded and the pellet was washed five times with cold acetone and solubilized in 3 mL of 2N sulfuric acid. The absorbance of the resulted solution was measured at 410 nm and the H_2O_2 content was calculated and expressed as mmol H_2O_2 g^{-1} FW.

MDA content was estimated in five individual plants for each treatment using the thiobarbituric acid (TBA) method (Heath and Packer 1968). After homogenization of 100 mg of fresh leaf and root materials in 1 mL of 0.5% TBA (prepared in 20% trichloroacetic acid), the resulted homogenate was heated at 95 °C for 30 min. After cooling down, the homogenate was centrifuged at 14 000 rcf for 10 min and the absorbance of the resulted supernatant was determined at 532 nm and 600 nm. MDA content was estimated by using its extinction coefficient of 155 mM^{-1} cm^{-1}. Thereafter, the content of MDA was expressed as µmol MDA g^{-1} FW.

II.7 Enzymatic antioxidant activities

To evaluate the enzymatic antioxidant activities of fenugreek plants under the applied treatments, the activity of superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (GPX) were estimated by grounding 500 mg of fresh leaf and root materials in 5 ml of 50 mM phosphate tampon (pH 7.8), containing 1% PVP and 0.1 mM EDTA. After 20 min of centrifugation at 15 000 rcf at 4 °C, the resulting supernatants were stored at -20 °C until enzyme activity and enzymatic protein content assessments. SOD activity was evaluated by measuring its ability to inhibit the photoreduction of nitro blue tetrazolium (NBT) using the method of Giannopolitis and Ries (1977). GPX and CAT activities were measured according to the method described by Robatjazi et al. (2020). For enzymatic protein determination, the Bradford (1976) method was used. For each enzyme, five technical replicates per treatment were considered.

II.8 Mineral contents

Following the method described in El Moukhtari et al. (2021a), the Kjeldahl method was used to determine the nitrogen content (N%) in the shoot and root of fenugreek plants. Briefly, 0.5 g of dry material was put in mattress tubes and 5 mL of concentrated sulfuric acid and 2 g of catalyst were added. Thereafter, the mixture was digested for 4h at 420 °C, and after cooling down, the resulted solution was distilled after adding 10 mL of boric acid and 20 mL of 40% NaOH. After titration by 0.019 M of sulfuric acid, the N total content was determined by the following formula:

$$\mathbf{N\ (\%) = (0.019\ (Ve - Vt) * 1.4) / g\ DW}$$

Ve: Sample titer volume

Vt: Blank titer volume

Si content was estimated by the colorimetric molybdenum blue method according to Dai et al. (2005). Briefly, to 10 mg of dry powder of leaf and root of fenugreek plants, 300 µL of 50% NaOH was added and the mixture was autoclaved at 121 °C for 21 min. Then, the volume of the resulted mixture was adjusted to 500 µL using demineralized water and centrifuged at 15 000 rcf for 15 min. In 2 mL tubes, 160 µL of supernatant was added to 1200 µL of 20% acetic acid, and after mixing by vortex, 400 µL of ammonium molybdate (43.7 mM, pH 7) was added to the resulted mixture. After 5 min of incubation, 200 µL of 20% tartaric acid and 40 µL of reducing solution, containing 8 g L^{-1} sodium sulfite (Na_2SO_3), 1.6 g L^{-1} 1-amino-2-naphthol-4-sulfonic acid and 100 g L^{-1} sodium bisulfite ($NaHSO_3$), were added to the mixture, which was, then, mixed and incubated for 30 min at room temperature in the dark. The absorbance was measured at 650 nm and the endogenous Si content was calculated using a standard curve prepared from SiO_2 standard solutions.

II.9 Statistical analysis

In the present study, the presented data were statistically analyzed using two-way analysis of variance (ANOVA II) and the means were compared using Tukey’s test at 5% level of significance. The XLSTAT statistical software, version 2014.5.03, was used to realize Pearson’s correlation matrix at $p < 0.05$.

III. Results

III.1 Plant growth and biomass production

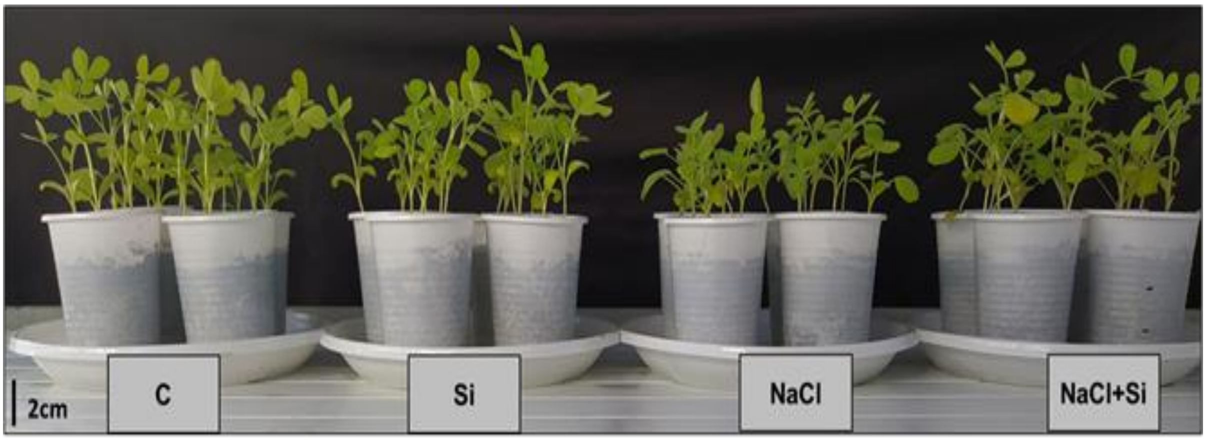

Figure 19: Effect of silicon on morphological aspect of fenugreek plants grown in the growth medium containing 0 or 150 mM NaCl. C, control; Si, 3 mM silicon application; NaCl, 150 mM NaCl stress and NaCl + Si, combined treatment of 3 mM silicon and 150 mM NaCl.

As shown in figure 19, the morphological aspect of Si-treated plants under 150 mM NaCl stress was similar to that of unstressed control plants, thus treatment with exogenous Si was able to alleviate the salt stress symptoms in fenugreek plants. To confirm this finding, when compared to control, plant biomass, in terms of SFW, RFW, SDW and RDW of fenugreek, was significantly (p <0.001) decreased by 36%, 58%, 40% and 57%, respectively, under salt stress as shown in figure 20. However, the supplementation of exogenous Si interestingly alleviated the harmful effects of salt stress on plant biomass and morphological aspect ($p < 0.05$). Indeed, Si treatment significantly increased SFW, RFW, SDW and RDW of salt-stressed plants by 23%, 75%, 33% and 58%, respectively.

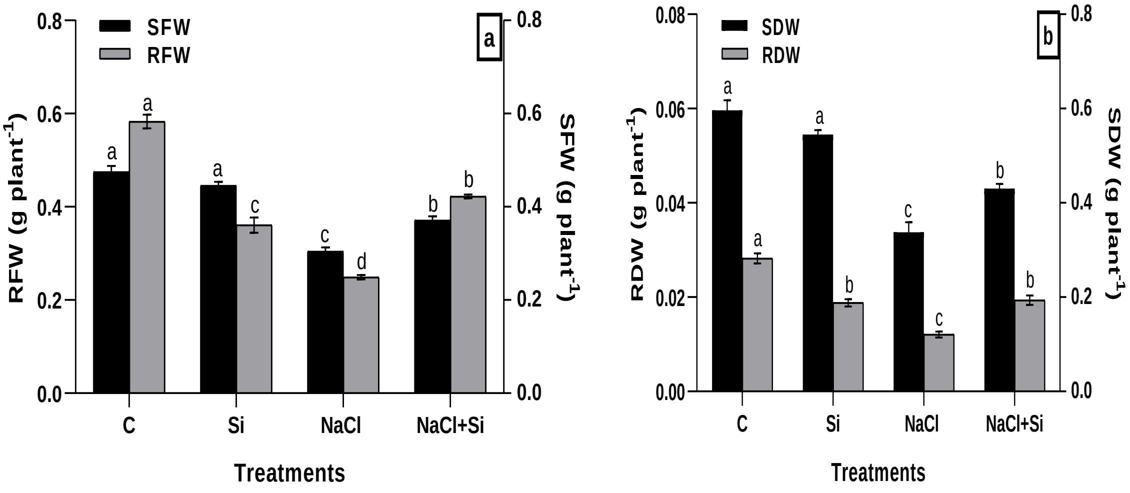

Figure 20: Effect of silicon on SFW (a), RFW (a), SDW (b) and RDW (b) of fenugreek grown in the growth medium containing 0 or 150 mM NaCl. Data are the mean ± S. E. of 5 individual plants and the small letters above bars mean statistically significant values at $p \leq 0.05$. SFW, shoot fresh weight; RFW, root fresh weight; SDW, shoot dry weight; RDW, root dry weight; C, control; Si, 3 mM silicon application; NaCl, 150 mM NaCl stress and NaCl + Si, combined treatment of 3 mM silicon and 150 mM NaCl.

Similar to plant biomass, the depressive effect of salt stress was also significantly ($p < 0.001$) higher in PH, LA, and RWC in fenugreek plants (Figure 21a, b). Indeed, relative to control treatment, PH, LA, and RWC were decreased by 30%, 33% and 28%, respectively, under salt stress. However, these growth and physiological attributes were importantly improved by 23%, 20% and 21% in Si-treated plants under salt-stressed conditions, respectively. Likewise, while salinity reduced the symbiosis-related parameters in terms of nodules number by 71% relative to untreated control, nodule number was 2-fold higher in the root of Si-treated plant as compared to salt-stressed plants alone (Figure 21a). Moreover, under unstressed conditions, there was no significant ($p > 0.05$) difference between Si-treated and untreated fenugreek plants regarding all the above-cited growth and physiological parameters.

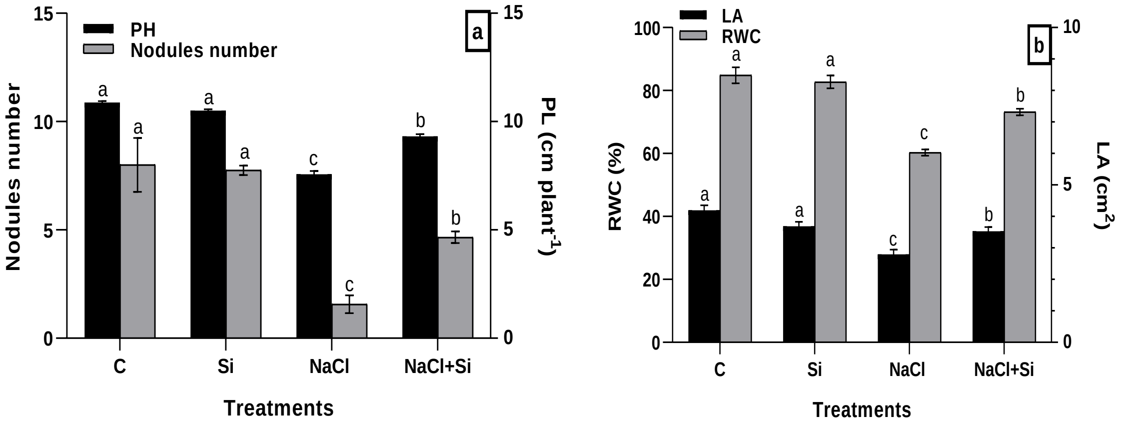

Figure 21: Effect of silicon on plant length (PL) (a), leaf area (LA) (b), nodule number (NN) (a) and relative water content (RWC) (b) of fenugreek grown in the growth medium containing 0 or 150 mM NaCl. Data are the mean ± S. E. of 5 individual plants and the small letters above bars mean statistically significant values $p \leq 0.05$. C, control; Si, 3 mM silicon application; NaCl, 150 mM NaCl stress and NaCl + Si, combined treatment of 3 mM silicon and 150 mM NaCl.

III.2 Photosynthetic pigments content

Exposure of fenugreek plants to 150 mM NaCl stress significantly ($p < 0.05$) reduced the content of Chl a and carotenoids in fenugreek leaves by 21% and 42%, respectively, with no significant difference for Chl b. Conversely, Si addition to the growth medium of salt-stressed plants increased Chl a and carotenoids contents by 15% and 50%, respectively. Regarding the carotenoids/(Chl a + Chl b) ratio, the presented results in figure 22 (b) showed that carotenoids were the most affected photosynthetic pigment in salt-exposed fenugreek plants. However, when Si and NaCl were applied together, this effect was reversed and the mentioned ratio was 1.31-fold higher relative to salt treatment alone.

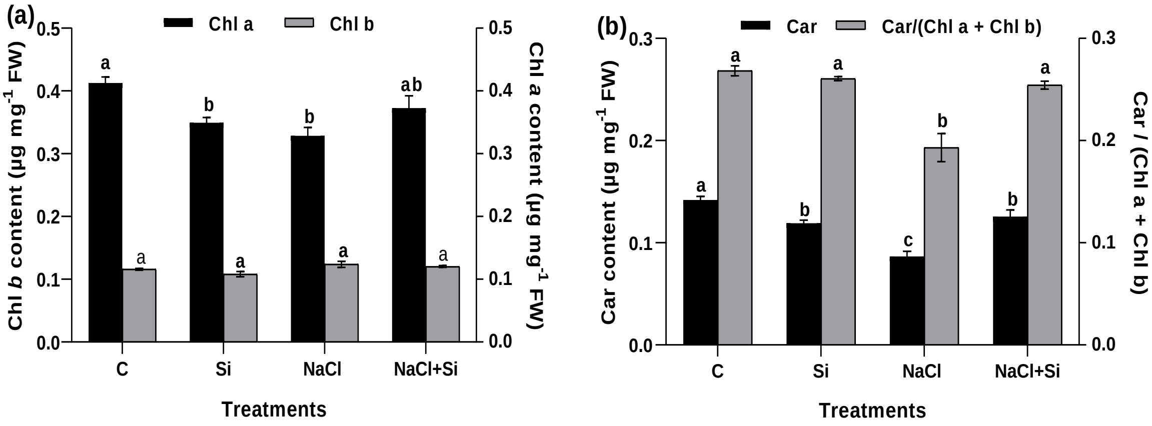

Figure 22: Effect of silicon on photosynthetic pigments (a and b) content in fenugreek grown in the growth medium containing 0 or 150 mM NaCl. Data are the mean ± S.E. of 5 individual plants and the small letters above bars mean statistically significant values $p \leq 0.05$. Chl, chlorophyll; C, control; Si, 3 mM silicon application; NaCl, 150 mM NaCl stress and NaCl + Si, combined treatment of 3 mM silicon and 150 mM NaCl.

III.3 Chlorophyll fluorescence parameters

According to the results of Chl fluorescence parameters of dark-adapted fenugreek leaves, salt stress significantly ($p < 0.01$) increased the minimal fluorescence (F_0) of PSII by 33% when compared to the unstressed control (Figure 23a). In contrast, a significant ($p < 0.01$) reduction of 10% and 11% was observed for F_v and F_v/F_m, respectively, in salt-stressed leaves relative to the control treatment. Interestingly, treatment of salt-stressed plants with exogenous Si caused a significant ($p < 0.01$) increase in both F_v (11%) and F_v/F_m (12%) of PSII when compared to salt stress alone. Regarding the maximal chlorophyll fluorescence (F_m), there was no significant difference between all the applied treatments. Additionally, under unstressed conditions, the addition of Si in the growth medium has no significant effect on all Chl fluorescence parameters (F_0, F_m, F_v/F_m) when compared with the control plants (Figure 23).

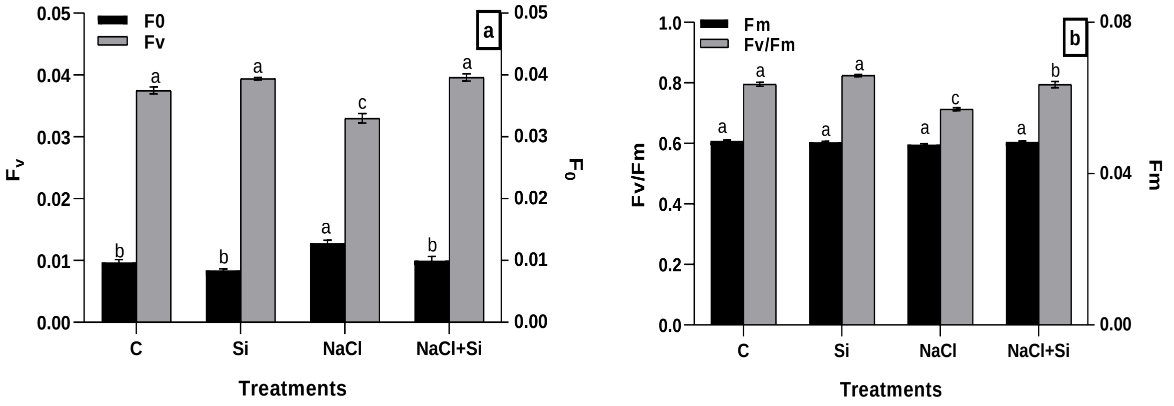

Figure 23: Effect of silicon on basic photosynthetic parameters in dark-adapted leaves of fenugreek grown in the growth medium containing 0 or 150 mM NaCl. Data are the mean ± S. E. of 5 individual plants and the small letters above bars mean statistically significant values $p \leq 0.05$. F0, Minimal fluorescence yield of dark-adapted samples with all PSII centers open; Fm, Maximal fluorescence yield of dark-adapted sample with all PSII centers closed; Fv, Variable fluorescence of dark-adapted sample (Fm - F0); Fv/Fm, Maximal photosystem II (PSII) quantum yield of dark-adapted samples ((Fm - F0)/Fm); C, control; Si, 3 mM silicon application; NaCl, 150 mM NaCl stress and NaCl + Si, combined treatment of 3 mM silicon and 150 mM NaCl.

III.4 Quantum yield and energy conversion in photosystem II

As presented in figure 24, the quantum yield of photochemical energy conversion Y (II) in PSII showed the same trend in all applied treatments, which was significantly decreased when increasing the PAR values. Compared to the control treatment, a pronounced decrease of Y (II) was observed in the leaves of stressed plants. However, this decrease was slightly alleviated by Si treatment until 150 to 600 μmol photons m^{-2} s^{-1} as compared with stress alone (Figure 24a). In contrast of the trend of Y (II), the quantum yield of regulated non-photochemical energy loss Y (NPQ) in PSII and non-photochemical quenching (NPQ) was increased when the PAR values are increased in all applied treatments (Figure 24b, c). When compared to the plant control, the Y (NPO) and NPQ were importantly reduced in salt-stressed plants. While, within all light intensity values, these latter were interestingly improved in Si-treated plants under salt stress. Regarding the quantum yield of non-regulated non-photochemical energy Y(NO) loss in PSII (Figure 24d), the presented results showed that the non-regulated energy was more pronounced in the leaves of stressed plants as compared to the control. However, this depressive effect was reversed and slightly alleviated after Si incorporation in the growth medium under salt stress.

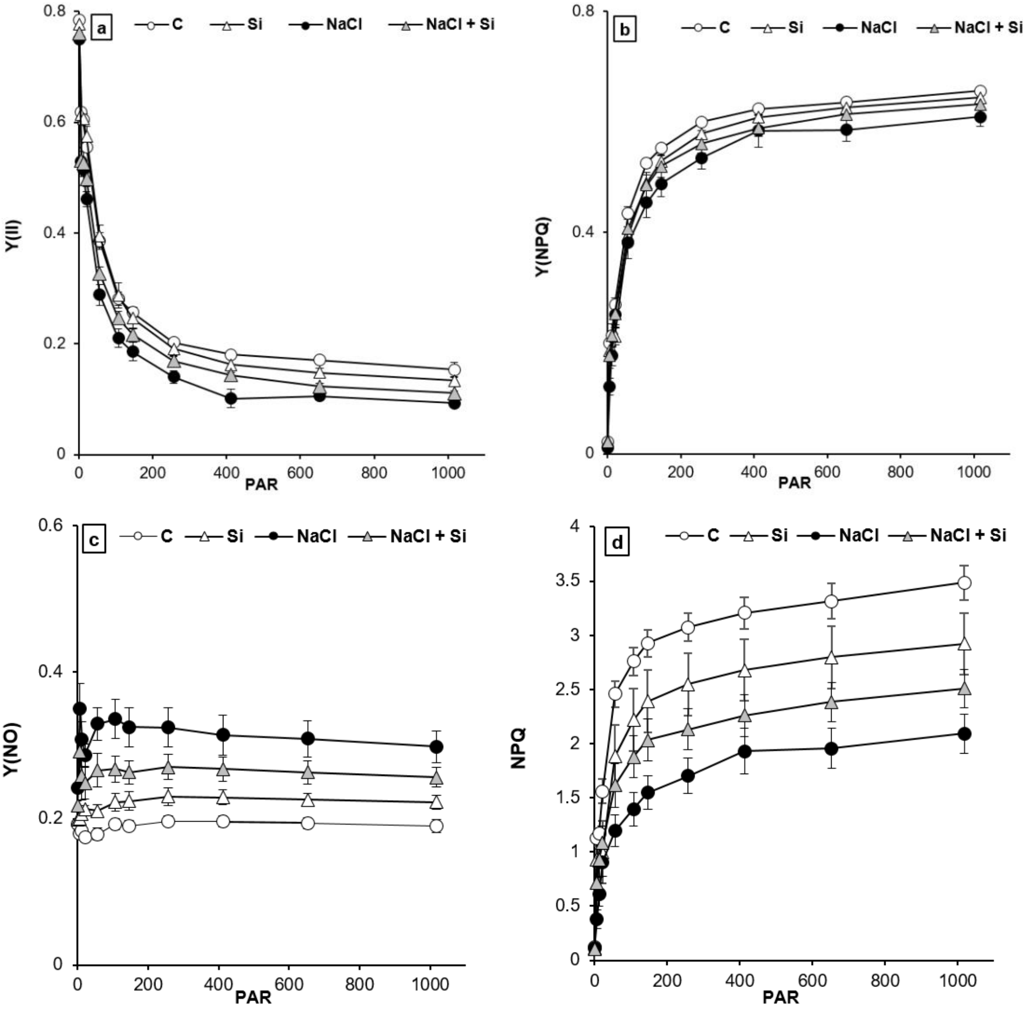

Figure 24: Effect of silicon on quantum yield of photochemical energy conversion in PSII Y(II) (a), on quantum yield of regulated Y(NPQ) (b) and non-regulated Y(NO) (c) non-photochemical energy loss in PSII and on Non-photochemical quenching NPQ) (d) of fenugreek grown in the growth medium containing 0 or 150 mM NaCl. Data are the mean ± S.E. of 5 individual plants. PAR: photosynthetically active radiation; C, control; Si, 3 Mm silicon application; NaCl, 150 mM NaCl stress and NaCl + Si, combined treatment of 3 mM silicon and 150 mM NaCl.

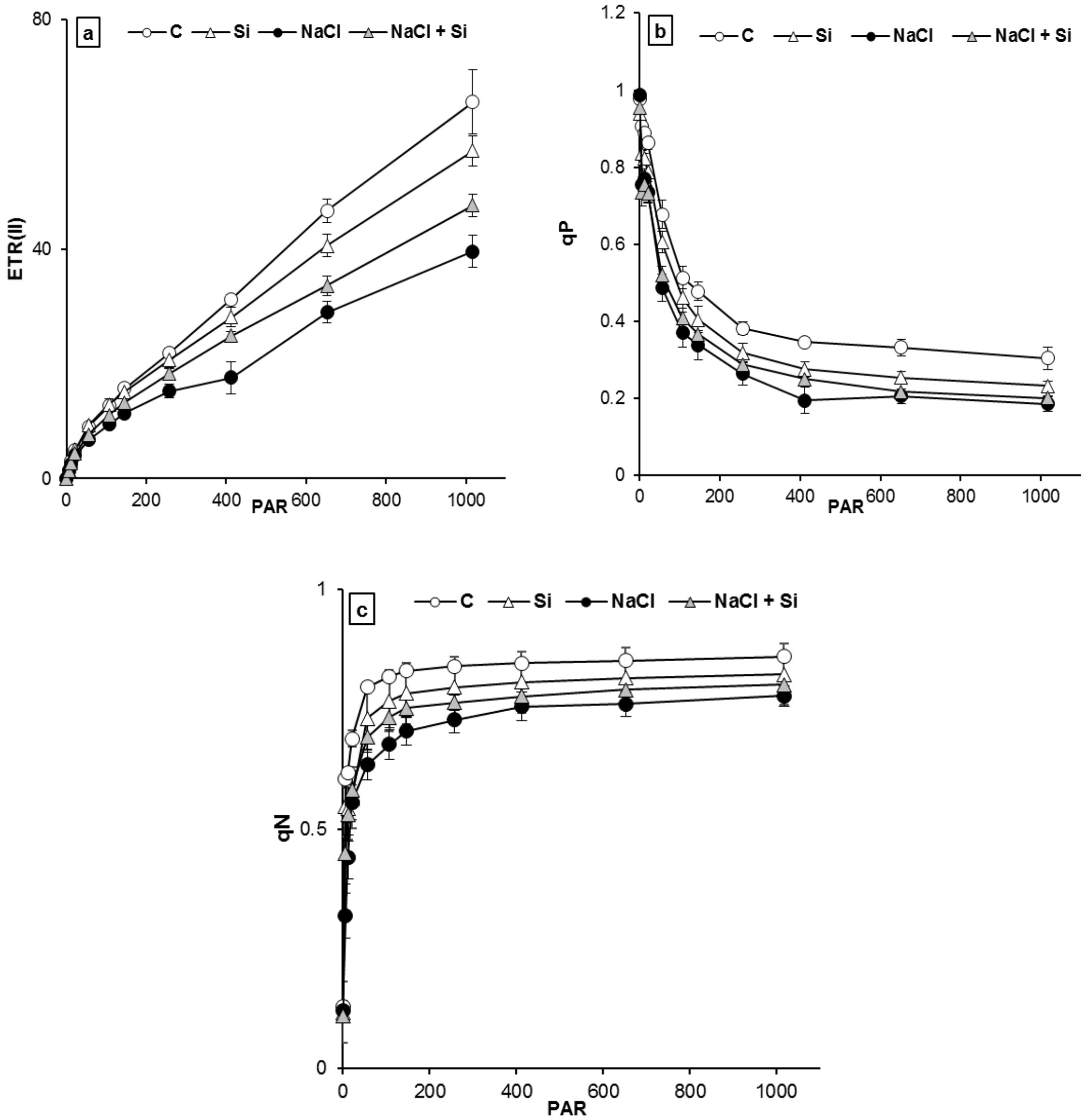

Figure 25: Effect of silicon on electron transport rate in PSII (ETR II) (a), photochemical quenching (qP) (b) and non-photochemical quenching (qN) (c) of chlorophyll fluorescence in fenugreek leaves grown in the growth medium containing 0 or 150 mM NaCl. Data are the mean ± S.E. of 5 individual plants. PAR: photosynthetically active radiation; C, control; Si, 3mM silicon application; NaCl, 150 mM NaCl stress and NaCl + Si, combined treatment of 3 mM silicon and 150 mM NaCl.

Regardless of the treatment, the electron transport rate in PSII (ETR II) and the coefficient of non-photochemical quenching (qN) were increased, but the coefficient of photochemical quenching (qP) was decreased within all the PAR values (Figure 25a, b and c). When compared to the control, the application of 150 mM NaCl stress reduced all of ETR, qN and qP in fenugreek leaves, while the addition of 3 mM Si to the growth medium alleviated these decreases in stressed plants.

III.5 Quantum yield and energy conversion in photosystems I

In contrast to Y (II), salt stress caused a significant increase in quantum yield of photochemical energy conversion Y (I) in PSI within all PAR values as compared to other treatments (Figure 26a). Under salt-stressed conditions, the functioning of PSI, in terms of Y (I), in Si-treated plants was found as the same of those of untreated control. Thus, the improving effect of Si supply was more obvious on Y (II) in comparison with Y (I). On the other hand, the effect of the applied treatments on the quantum yield of non-photochemical energy dissipation in reaction centers limited by the donor side Y(ND) and acceptor Y (NA) side of PSI was presented in figure 26 (b, c). The results showed that Y (ND) was decreased in salt-stressed leaves, while it was remarkably increased in leaves of plants treated with NaCl and Si simultaneously. In contrast, Y (NA) was higher in stressed plants than in other treatments within all PAR values.

As shown in figure 27 (a, b), a decreasing trend in the oxidized PSI (P700ox) with maximal fluorescence yield of the dark-adapted sample with all PSI centers closed (P700m) was noticed for all the applied treatments as the light of intensity values increased (Figure 27a, b). This decrease was more pronounced in the leaves of salt-stressed fenugreek plants relative to the control. However, as presented in figure 27 (a and b), the leaves of plants treated with NaCl and Si showed an intermediate response between the control and salinity treatments.

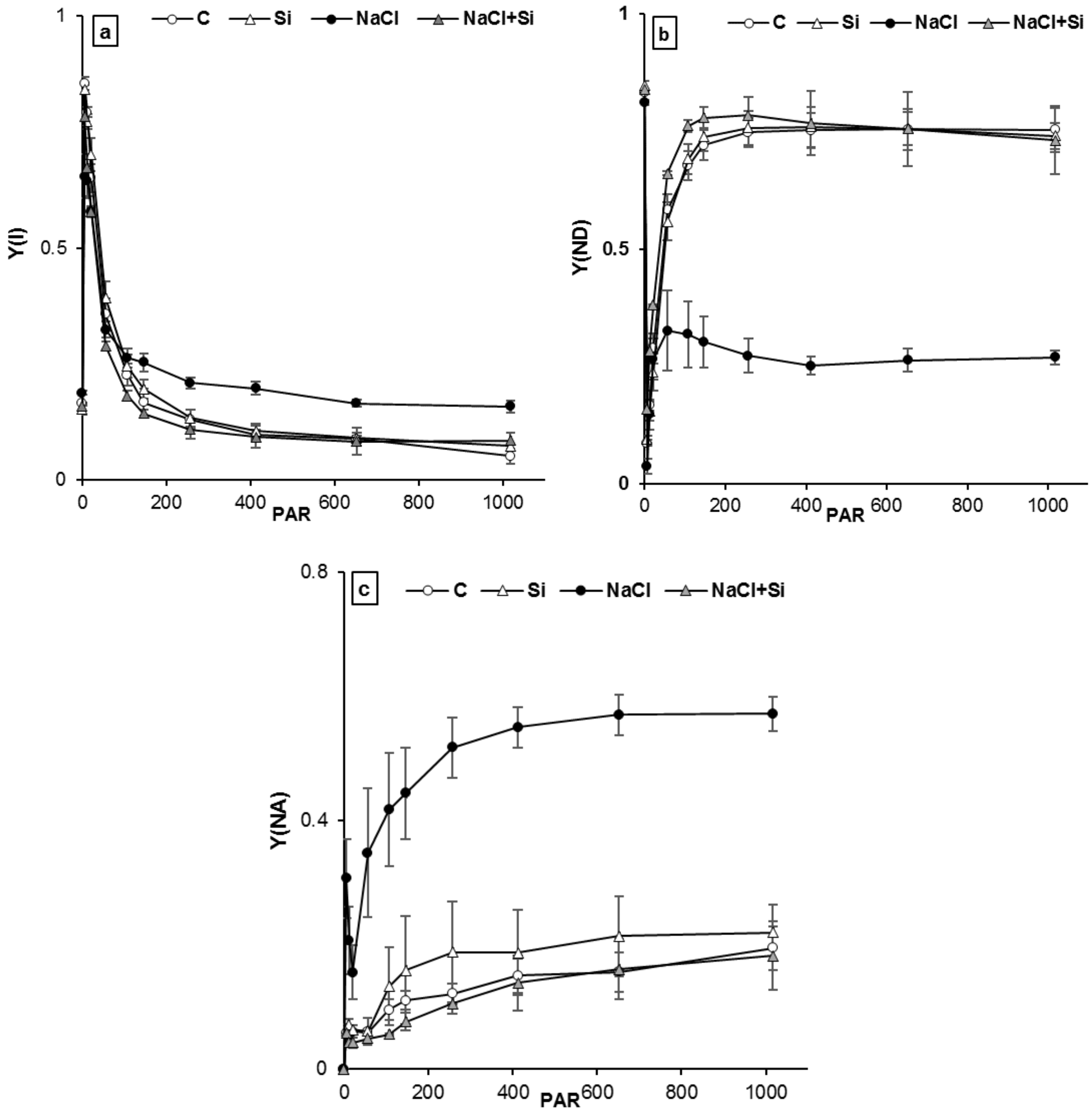

Figure 26: Effect of silicon on quantum yield of photochemical energy conversion in PSI Y (I) (a), on quantum yield of non-photochemical energy dissipation in reaction centers limited by donor side Y(ND) (b) and on quantum yield of non-photochemical energy dissipation in reaction centers limited by acceptor side Y(NA) (c) of fenugreek grown in the growth medium containing 0 or 150 mM NaCl. Data are the mean ± S. E. of 5 individual plants. PAR: photosynthetically active radiation; C, control; Si, 3 mM silicon application; NaCl, 150 mM NaCl stress and NaCl + Si, combined treatment of 3 mM silicon and 150 mM NaCl.

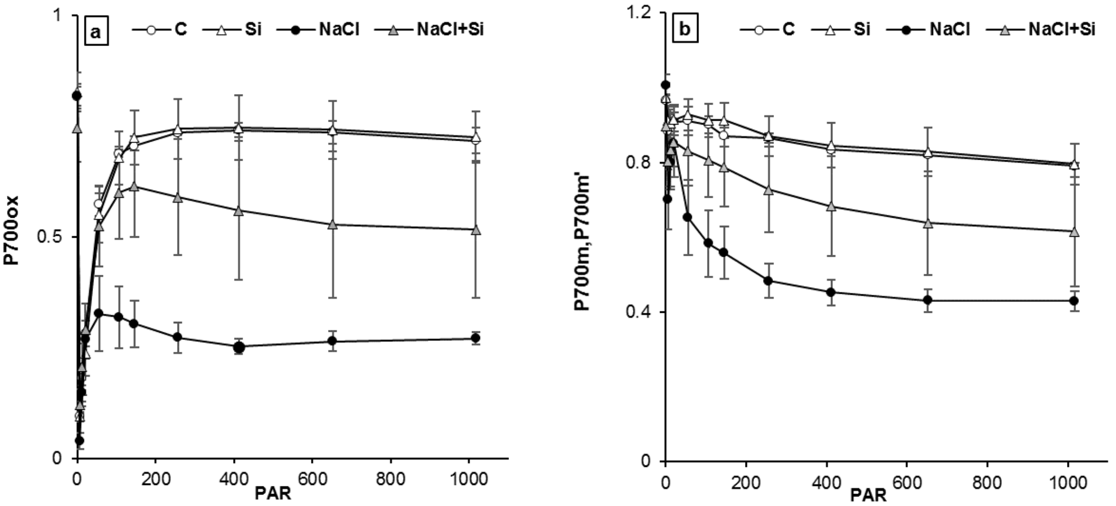

Figure 27: Effect of silicon on oxidized PSI (P700ox) (a) and on maximal fluorescence yield of dark-adapted sample with all PSI centers closed (P700m) (b) of fenugreek grown in the growth medium containing 0 or 150 mM NaCl. Data are the mean ± S. E. of 5 individual plants. PAR: photosynthetically active radiation; C, control; Si, 3 mM silicon application; NaCl, 150 mM NaCl stress and NaCl + Si, combined treatment of 3 mM silicon and 150 mM NaCl.

III.6 Oxidative stress markers

As a result of salt stress, the accumulation of oxidative stress markers, like MDA and H_2O_2, was significantly ($p < 0.05$) increased in exposed fenugreek plants to 150 mM NaCl stress (Figure 28a, b). Indeed, in both shoot and root of stressed plants, the MDA content was raised by 14% and 28%, respectively, as compared to the control plants. Although, it was significantly ($p < 0.01$) increased in the root (102%) of salt-stressed plants, the shoot H_2O_2 content was reduced significantly ($p < 0.01$) when compared to the control. However, after Si supply to the growth medium, the production of evaluated oxidative stress markers was remarkably reduced under salt-stressed conditions. The shoot and root MDA content and root H_2O_2 content of Si-treated salt-stressed plants were interesting ($p < 0.001$) decreased by 17%, 16% and 31%, respectively, with no significant ($p > 0.05$) effect of Si treatment on shoot H_2O_2 content in salt-stressed plants (Figure 28b). Under unstressed conditions, with the exception of shoot H_2O_2 content, there was no significant difference between Si-treated and untreated plants regarding the oxidative stress markers.

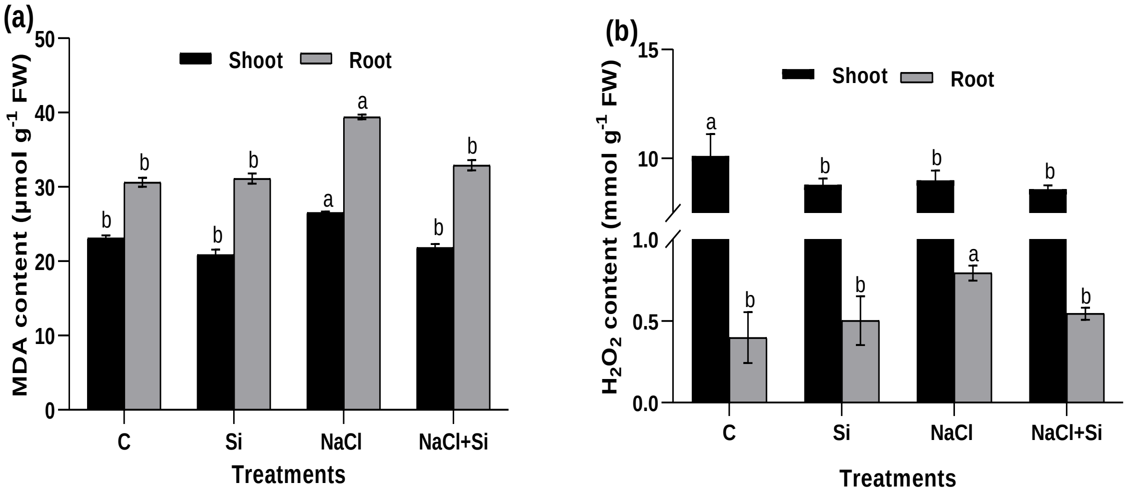

Figure 28: Effect of silicon on oxidative stress markers; malonyldialdehyde (MDA) (a) and hydrogen peroxide (H_2O_2) (b), of fenugreek grown in the growth medium containing 0 or 150 mM NaCl. Data are the mean ± S. E. of 5 individual plants and the small letters above bars mean statistically significant values at $p \leq 0.05$. C, control; Si, 3 mM silicon application; NaCl, 150 mM NaCl stress and NaCl + Si, combined treatment of 3 mM silicon and 150 mM NaCl.

III.7 Antioxidant activity

The exposure of fenugreek plants to salinity stress significantly ($p < 0.01$) increased the activity of SOD, CAT and GPX by 73%, 32% and 30%, in the shoot and by 61%, 2% and 45% in the root, respectively, relative to unstressed control. Under normal conditions, Si addition to the growth medium has no significant effect on the activity of evaluated antioxidant enzymes, exempt the shoot CAT activity, where its activity was significantly ($p <0.05$) reduced by 30% in Si-treated plants as compared to untreated control. However, under salt-stressed conditions, when compared with salt stress alone, the high activities of SOD (51%, 36%), CAT (20%, 63%) and GPX (15%, 69%) were observed, respectively in both shoot and root of Si-treated fenugreek plants.

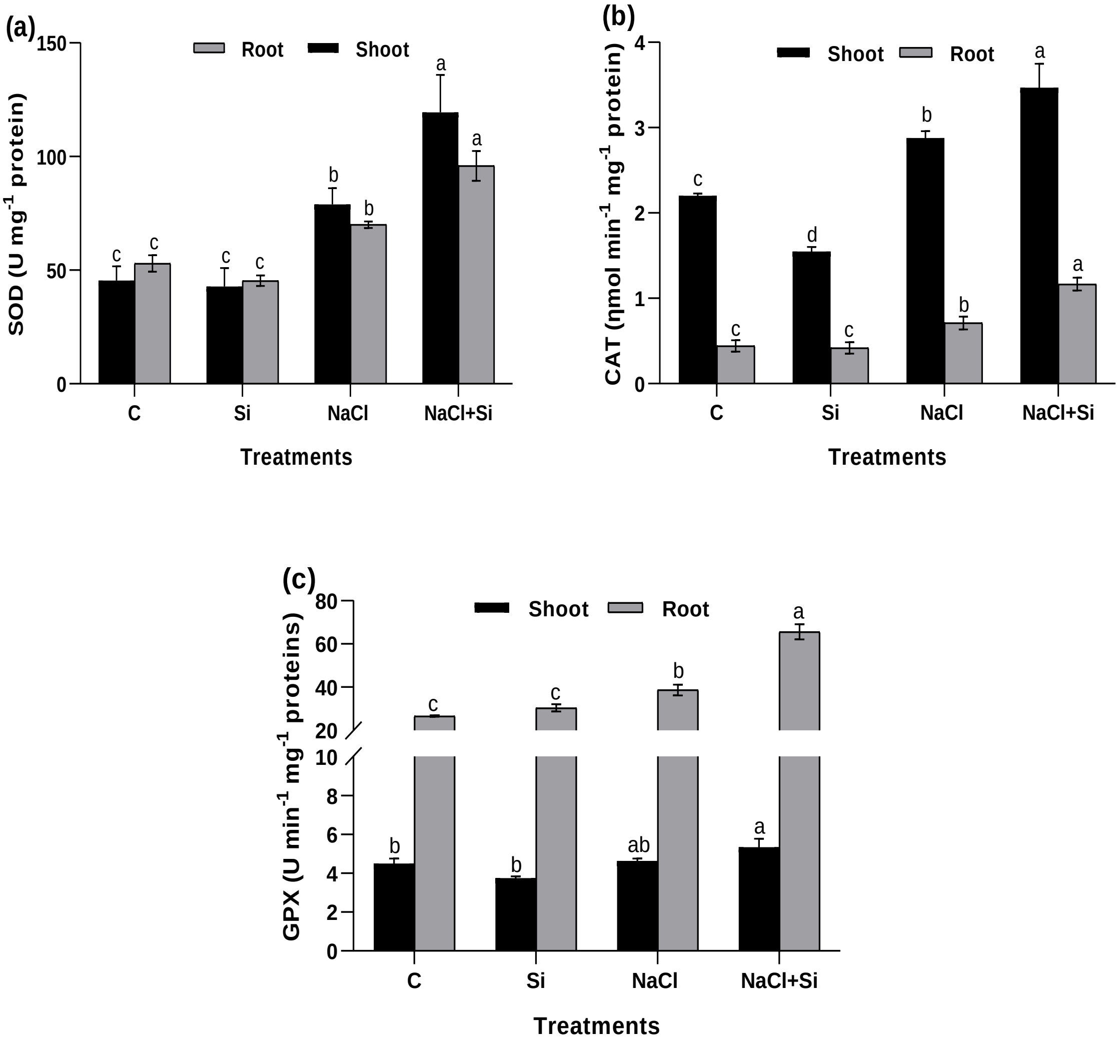

Figure 29: Effect of silicon on antioxidant enzymes; superoxide dismutase (SOD) (a), catalase (CAT) (b) and guaiacol peroxidase (GPX) (c) of fenugreek grown in the growth medium containing 0 or 150 mM NaCl. Data are the mean ± S. E. of 5 individual plants and the small letters above bars mean statistically significant values at $p \leq 0.05$. C, control; Si, 3 mM silicon application; NaCl, 150 mM NaCl stress and NaCl + Si, combined treatment of 3 mM silicon and 150 mM NaCl.

III.8 Mineral contents

Regardless of the treatment, the content of Si in the shoot was more than in the root of fenugreek plants, but there was no significant difference between shoot and root in nitrogen content as presented in table 12. However, the exposition of fenugreek plants to 150 mM NaCl stress significantly ($p < 0.01$) reduced the nitrogen content in both shoot and root by 90% and 94%, respectively, when compared with the control treatment. Conversely, Si incorporation into the growth medium importantly improved the nitrogen amount in both shoot (107%) and root (52%) of fenugreek plants under stressed conditions. The same was found about endogenous Si content, where the amount of this latter was significantly increased by 37% and 26%, respectively, in the shoot and root of Si-treated plants under salt stress. On the other hand, under unstressed conditions, the endogenous Si content in Si-treated plants was more than in untreated plants, while there was no significant ($p > 0.05$) difference in nitrogen content between these treatments.

Table 12: Effect of silicon on nitrogen (N) and silicon (Si) contents in fenugreek grown in the growth medium containing 0 or 150 mM NaCl. Regarding Si content, the data are the mean ± S.E. of three replicates. The small letters mean statistically significant values at $p \leq 0.05$. C, control; Si, 3 mM silicon application; NaCl, 150 mM NaCl stress and NaCl + Si, combined treatment of 3 mM silicon and 150 mM NaCl.

Treatment	**Mineral contents**			
	N (%)		Si (mg g^{-1} DW)	
	Shoot	Root	Shoot	Root
C	1.42^{a}	1.67^{a}	5.59 ± 0.74^{c}	1.97 ± 0.09^{d}
Si	1.5^{a}	1.59^{a}	12.99 ± 1.17^{a}	4.61 ± 0.08^{a}
NaCl	0.13^{c}	0.7^{c}	6.22 ± 0.39^{c}	2.88 ± 1.14^{c}
NaCl + Si	0.27^{b}	1.07^{b}	8.56 ± 0.12^{b}	3.65 ± 0.04^{b}

III.9 Correlation analysis

According to the PCA (Figure 30) and Pearson's correlation matrix (Figure 13), there was a significant positive correlation ($p < 0.05$) between growth parameters and photosynthetic characteristics, such as Chl a, Car and Fv/Fm, where the correlation coefficient (r) varied between 0.66 and 0.99 while a significant negative correlation was observed between plant biomass and oxidative stress markers (r between -0.68 and -0.98). Regarding symbiosis-related parameters, the shoot and root nitrogen contents were significantly correlated with nodule numbers ($P < 0.05$, $r = 0.94$, $r = 0.99$, respectively), and this latter was positively correlated with plant growth parameters (r between 0.74 and 0.99).

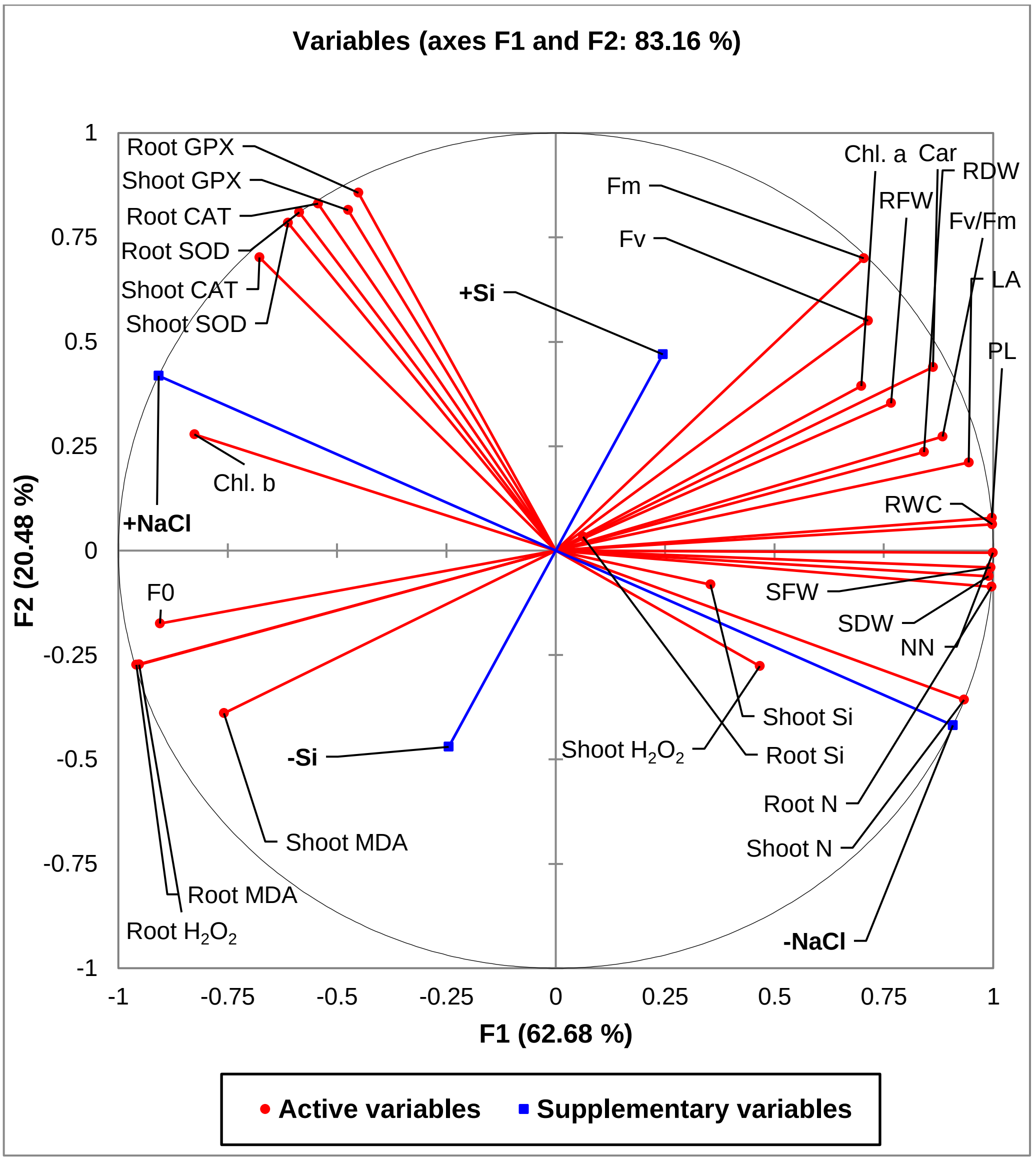

Figure 30: Principal component analysis (PCA) of studied parameters related to response of fenugreek plants to salt stress and Si treatment. The most variables (arrows), Si and 150 mM NaCl treatments are projected onto the F1-F2 principal factorial plane that explains 83.16% of the variation. SFW, shoot fresh weight; RFW, root fresh weight; SDW, shoot dry weight; RDW, root dry weight; PL, plant length; LA, leaf area; NN, nodules number; RWC, relative water content; Chl. a, chlorophyll a; Chl. b, chlorophyll b; Car, carotenoids; F0, minimal chlorophyll fluorescence; Fv, variable chlorophyll fluorescence; Fm, maximal chlorophyll fluorescence; MDA, malonyldialdehyde; H_2O_2, hydrogen peroxide; SOD, superoxide dismutase; CAT, catalase; GPX, guaiacol peroxidase; N, nitrogen; Si, silicon.

IV. Discussion

As previously reported (Sudhir and Murthy 2004), salinity stress has a depressive effect on plant growth and development. In the present study, plant biomass, plant high and leaf area of fenugreek plants were significantly reduced under 150 mM NaCl stress. Additionally, the water status and photosynthetic pigment content, in terms of chlorophyll a and carotenoids, were also impaired in the exposed fenugreek plants to salinity stress. Decreasing chlorophyll content could be attributed to the over-accumulation of Na^+ inducing the activity of chlorophyll degrading enzymes. However, treatment with exogenous Si has a significant beneficial effect on plant biomass, relative water content and photosynthetic pigments. Moreover, a positive correlation between relative water content and photosynthetic pigments and the studied growth attributes of salt-stressed fenugreek plants was observed, indicating that Si is playing and important role in salt-stressed plant growth. In a previous report, El Moukhtari et al. (2021a) showed that salt stress significantly reduced fresh and dry biomass, plant height, leaf number, leaf area, chlorophyll content and relative water content in alfalfa plants, while these latter were significantly enhanced after Si incorporation to the growth medium. Alamri et al. (2020) demonstrated that Si treatment enhanced the chlorophyll content under stressed conditions, by decreasing chlorophyll-degrading enzymes, like pheophytinase, chlorophyllase and chlorophyll degrading peroxidase, and increasing the activity of chlorophyll-metabolizing enzymes, such as δ-amino-levulinic acid dehydratase and porphobilinogen deaminase. In addition, Si supply also enhanced plant biomass and photosynthetic pigments content in maize (Ali et al. 2021), squash (Siddiqui et al. 2014) and okra (Abbas et al. 2015).

The establishment of legume-rhizobium symbiosis was also assessed in the present study through the determination of nodule number. The decrease in nodule number in the root of salt-stressed fenugreek plants could reduce the biological nitrogen fixation and nitrogen nutrition. This was confirmed in our study by the highly significant correlation between nodule number and nitrogen content (Figure 30). Importantly, the incorporation of Si to the growth medium significantly improved nodule number, which in return enhanced nitrogen content in both shoot and root of salt-stressed fenugreek plants. This is in line with the results reported by El Moukhtari et al. (2021a) on alfalfa and Steiner et al. (2018) on soybean. Si supplementation could enhance nodule formation and act on nodule functionality by increasing symbiosome number, nitrogenase activity and leghemoglobin content under salinity (El Moukhtari et al. 2021b). After the highly positive correlation between nodule number, nitrogen content and the growth traits, we can suggest that exogenous Si was involved in the key biological process in the legume family, which is the biological nitrogen fixation, to enhance fenugreek growth under salinity conditions.

In the current work, the photosynthesis process was also evaluated. In this context, it is well known that salt stress can decrease photosynthetic ability in plants (Sudhir and Murthy 2004), but there is little information about the role of Si in photosystem functioning, in terms of the performance of both photosystems I and II, under salinity stress. For this reason, chlorophyll fluorescence, quantum yield and energy conversion in both photosystems (I and II) were evaluated to investigate the key role of Si in photosynthetic machinery under salt-stressed conditions. The results indicated that salt stress significantly reduced variable chlorophyll fluorescence, but it has no significant effect on other chlorophyll fluorescence parameters. Additionally, the maximal quantum yield of PSII, assessed as F_v/F_m ratio, was also drastically reduced in salt-stressed fenugreek plants, while it was significantly improved by adding Si in addition to an improvement in the chlorophyll fluorescence parameters. Si-mediated an increase in chlorophyll fluorescence and F_v/F_m was also observed in sea barley under iron deficiency conditions (Ksiaa et al. 2021). In the same line, Zhang et al. (2022) reported that after applying Si under salinity stress, the chlorophyll content and chlorophyll fluorescence were elevated in alfalfa. In the same plant species, El Moukhtari et al. (2021a) showed that 3 mM Si significantly improved chlorophyll content and F_v/F_m ratio under salt-stressed conditions. Monitoring Chlorophyll fluorescence and F_v/F_m ratio can help to obtain information about the efficiency of the photochemical processes (Olechowicz et al. 2018). Therefore, according to the obtained results, adding exogenous Si to the growth medium under salt stress could improve the efficiency of the photochemical processes of fenugreek leaves.

Regarding the photosystem II performance, the exposition of fenugreek plants to salt stress induced a decrease in Y(II), which means the fraction of energy that is photochemically converted by PSII. According to Klughammer and Schreiber (2008a), another fraction of energy is lost during PSII functioning, this latter is divided into regulated (Y(NPQ)) and non-regulated (Y(NO)) non-photochemical energy loss in PSII. In this study, the leaves of salt-stressed fenugreek plants presented a significant reduction in Y(II), Y(NPQ) and NPQ against a high elevation in Y(NO), thus, in return salt stress drastically reduced the aptitude to use and convert light energy of PSII. However, after exogenously adding Si under salt-stressed conditions, Y(II), Y(NPQ) and NPQ, with the exception of Y(NO), were observed to be enhanced in adapted fenugreek leaves. Meanwhile, electron transport rate in PSII (ETR II), photochemical quenching (qP) and non-photochemical quenching (qN) of chlorophyll fluorescence were also improved in Si-treated salt-stressed fenugreek plants compared to stressed plants alone. The results indicated that Si could be involved in the improvement of PSII ability to convert and regulate photon energy into chemical energy, as well as photosynthesis process amelioration. These findings are consistent with a previous study conducted by Ksiaa et al. (2021) in sea barley, where the treatment with exogenous Si significantly improved PSII efficiency under iron deficiency conditions. Likewise, Zhu et al. (2020) reported that exogenous Si supplementation significantly increased photosynthetic performance. Another study conducted by Vaculík et al. (2015) showed that silicon addition could alleviate cadmium toxicity by enhancing the photosynthetic rate and electron transporter rate in PSII, which may be due to silicon-mediated enhancement of thylakoid formation in the chloroplasts of bundle sheath’s cells.

In contrast, the quantum yield of photochemical energy conversion in PSI Y(I) and quantum yield of non-photochemical energy dissipation in reaction centers limited by acceptor side Y(NA) were increased under stressed conditions, while the quantum yield of non-photochemical energy dissipation in reaction centers limited by donor side Y(ND) was decreased as compared to the control and other treatments. The Si treatment inversed these results and kept the same responses as that of the control plants regarding PSI functioning. Therefore, the PSII was the more affected photosystem in fenugreek leaves under salinity stress. On the other hand, to protect PSI to reactive oxygen species (ROS) under high light intensity, some processes were placed upstream and downstream by the P700 oxidation (Takagi et al. 2017). P700 oxidation was documented that had significant impacts on the alleviation of PSI photoinhibition (Shimakawa et al. 2018). Our results indicated that, under salt-stressed conditions, P700ox and P700m were drastically reduced when compared to the control. However, incorporation of exogenous Si to the growth medium increased these latter, thus it directly protects PSI from oxidation-induced damages. The same positive effects of Si treatment on PSI oxidation were observed in sea barley under iron deficiency conditions (Ksiaa et al. 2021). Based on these significant effects, exogenous Si could protect the photosynthesis process from abiotic stress by improving P700 oxidation and alleviating PSI photoinhibition.

Dos et al. (2021) correlated the caused damages on chloroplast ultrastructure and photosynthetic parameters with induction and accumulation of ROS in maize under salt stress. Similar to the above suggestions, the induction of oxidative stress, reflected by the high content of oxidative stress markers, was negatively correlated with chlorophyll content and other photosynthetic parameters. These findings can explain the obtained reduction in photosystem performance, P700 oxidation and the growth traits of salt-stressed fenugreek plants. Conversely, the content of oxidative stress markers, in terms of MDA and H_2O_2, was significantly reduced after Si incorporation to the stressed growth medium. Thus, Si-alleviated salt damage on photosynthesis, especially on chlorophyll content, PSI oxidation and energy conversion and regulation in PSII, could be partially attributed to the protective role of Si on the membrane structure and stability of photosynthetic apparatus. On the other hand, Si-mediated reduction in induced oxidative stress was related to a significant improvement in the enzymatic antioxidant system, in terms of SOD, CAT and GPX activities, in both shoot and root of the salt-exposed plants. Under stressed conditions, the reduction of oxidative stress and induction of antioxidant systems after Si supplementation were indicated by many previous studies (Kim et al. 2017; Hassanvand et al. 2019; Zhang et al. 2019; Kubi et al. 2021; El Moukhtari et al. 2022a). In the same way, Zhang et al. (2017) reported that Si application increased antioxidant activity and glutathione content, which decreased superoxide radicals and the content of H_2O_2 and MDA, as well as membrane permeability caused by salt and drought stresses. On the other hand, endogenous Si content was significantly increased in both shoot and root of fenugreek plants under stressed and unstressed conditions. Therefore, under salt stress, improvement in plant biomass, biological nitrogen fixation, photosynthesis efficiency and antioxidant activity in Si-treated plants could be the result of high endogenous Si accumulation. In the same line, El Moukhtari et al. (2021a) correlated the high chlorophyll, nitrogen content and plant biomass with the high content of endogenous Si in alfalfa plants under salt stress.

V. Conclusion

Exposition to salt stress caused a significant increase in the content of oxidative stress markers. In return, this induction of oxidative stress can be the reason for obtained reduction in most evaluated biological processes, including biological nitrogen fixation and photosynthesis, as well as plant growth and biomass. Interestingly, Si treatment significantly improved the growth of fenugreek plants under salt stress by enhancing photosynthesis machinery, especially PSII performance and PSI oxidation. In addition, Si supplementation also improved the symbiosis relationship, reflected by the high obtained nodule number and nitrogen content in Si-treated salt-stressed fenugreek plants. Oxidative stress was also reduced under salt stress after Si supplementation, and this decrease was significantly correlated with a significant increase in endogenous Si content and enzymatic antioxidant systems in both shoot and root of fenugreek plants. Taken together, exogenous Si was directly involved in the central and vital biological processes to ameliorate fenugreek tolerance under salt-stressed conditions. Additionally, our results could be attributed to understanding the variable mechanisms by which Si improved the tolerance of plants to abiotic stresses.

Chapter 5: Silicon seed priming mitigated salinity stress effects and improved fenugreek (*Trigonella foenum-graecum* L.) biomass and yield under field conditions

Abstract

Salt stress limits the growth and yield of valuable aromatic and medicinal plants such as fenugreek (*Trigonella foenum-graecum* L.). However, there is a lack of investigations on the role of priming with silicon (Si) on germination, growth and yield of salt stressed fenugreek under field conditions. Therefore, in the present study, fenugreek seeds were primed with different concentrations of Si (0, 10, 20, 40 and 60 mM $CaSiO_3$) and germinated in salt stressed (150 mM NaCl) and unstressed medium (0 mM NaCl). The result showed that salinity stress significantly decreased germination parameters and this reduction was significantly correlated with an important decrease in the embryo viability and a strong accumulation of oxidative stress markers in seedlings. Inversely, Si priming improved embryo viability and reduced accumulation of oxidative stress, which could explain the enhanced obtained germination parameters in Si primed seed under salt stressed conditions, but this benefic effect was in a dose-depending manner. Based on obtained results of germination parameters, we selected the beneficial Si concentration, which was 20 mM, to assess to beneficial effects of Si priming on growth and yield of salt stressed fenugreek under field conditions. The obtained results indicated that Si priming significantly improved plant biomass and yield related parameters, in terms of number of pods per plant, pod length, number of seed per pod and per plant, seed weight, pod weight, hundred seed weight and harvest index, in salt stressed fenugreek under field conditions. More interest, the evaluation of harvested seed showed that Si priming not only enhances plant and yield attributes but it was also able to improve the nutritional quality and antioxidant activity of harvested seeds under salt stress. Taken together, seed priming with Si could be a promissing and ecofriendly method to mitigate the negative effect of salinity stress on growth and yield of fenugreek under field conditions.

Key words: Fenugreek, Germination, Growth, Harvest index, Priming, Salinity, Silicon, Yield parameters.

I. Introduction

In recent years, the Earth's climate had undergone a serious and quick change, inducing inter-annual variations of precipitations and appearance of several abiotic stresses, which negatively affected the growth and yield of many agricultural systems (Porter and Semenov 2005; El-Mokhtar et al. 2022b). For example, the production and quality of many aromatic and medicinal plants (AMPs) are seriously affected under drought and salinity stresses (Aghaei and Komatsu 2013). Fenugreek (*Trigonella foenum-graecum* L.) is an oldest and important AMP, originally native to India and Northern Africa, but now is cultivated worldwide (Benayad et al. 2014b). Fenugreek seeds and leaves are known for their richness of bioactive molecules, explaining their therapeutic effect against many diseases, including cancer and diabetes (Benayad et al. 2014a; Alrumaihi et al. 2021). In addition, like other legume species, fenugreek has an ability to fix atmospheric nitrogen through a symbiosis with rhizobium (Singh et al. 2008). Thus, it can be used as a good alternative to diminish the nitrogen chemical fertilizer input. However, although their medicinal and agricultural advantages, the growth and yield of fenugreek are remarkably decreased under salt stressed conditions. Where the salt stress causes both an osmotic and oxidative stress, affecting as result plant physiology (Abdelmajeed et al. 2013; Amiri et al. 2020).

In Morocco, among the important fenugreek producing countries, the fenugreek seed are used, either in intact or powdered form, as a condiment in food preparation for its nutritive and restorative properties (Haddad et al. 2003; Benayad et al. 2014a). However, this region is subjected to desertification and soil salinization because of lowed precipitation and wrong irrigation practices (Abdelmoumen and El Idrissi 2010). This latter could be the reason of a significant decrease in crops production, including fenugreek yield. In this context, Amiri et al. (2020) reported that salinity treatment importantly reduced yield related parameters, in terms of number of pods, number of seeds per pod, thousand-seed weight and harvest index of in fenugreek. The same results was reported by Abdelmoumen and El Idrissi (2010). To overcome this agricultural problem, it is necessary to develop the tolerant plants. For this purpose, there is a conventional breeding techniques, but this approach require a long time and in cas of AMPs, there a risk to develop the tolerant plant devoid of medicinal properties (İbrahimova et al. 2021). Another strategy to alleviate the destructive effects of salt stress on fenugreek growth and yield is the application of exogenous compounds, as bio-stimulant or beneficial elements to ensure plant tolerance and grain yield stability under stressed conditions (Zrig et al. 2019; Hassanvand et al. 2019; Robatjazi et al. 2020; El Moukhtari et al. 2022b).

After the oxygen, silicon (Si) is the second most abundant element in the Earth's crust and their concentration in plant depends on species, where it is varies from 0.1% to 10% (Ma and Yamaji 2008; Ayed et al. 2021). Since, it has paradoxically been considered as a quasi-essential element, while in recent years it is recognized as a biostimulant (Laane 2016; Ayed et al. 2021). In this line, several studies reported that soil fertilization or foliar application with Si has beneficial effects on plant growth and tolerance under salt stressed conditions. Where the authors suggested that Si treatment induced salt tolerance due to its physiological, metabolic, antioxidant and molecular roles in plant (Nasseri et al. 2012; Meng et al. 2020; Alamri et al. 2020; El Moukhtari et al. 2021a). Seed priming is also an interesting alternative to mitigate the negative effect of salt stress on plant growth and yield (Azeem et al. 2015; Liu et al. 2018; Mahmoudi et al. 2019; Dos et al. 2021). Importantly, it is documented that seed priming not only used to improve seed germination, early seedling growth and yield but also to ensure extensive survivability under different biotic and abiotic stresses (Ayed et al. 2021). However, the impact of priming with Si on fenugreek germination and yield is little studied, especially, in salt stressed plants under field conditions.

Therefore, the objectives of the present study were to investigate (i) the effect of seed priming by different Si concentrations on germination parameters, embryo viability and early seedling growth under salt stressed conditions, and (ii) the effect of Si priming on plant biomass, yield related parameters and quality of harvested seeds in salt stressed fenugreek under field conditions.

II. Materiel and methods

II.1 Plant material and seed priming treatment

In the present study, the fenugreek (*Trigonella foenum-graecum* L.), was used as plant material. The fenugreek seeds were supplied by the National Institute of Agronomic Research (INRA, Morocco). After disinfection with 5% of sodium hypochlorite solution, the fenugreek seeds were rinsed thoroughly with sterile distilled water and immersed in different concentrations of $CaSiO_3$ (0, 10, 20, 40 and 60 mM) for 8 hours. Then, primed seeds with Si were washed three times with distilled water, labeled and air dried on filter paper at room temperature (25°C) for 2 days.

II.2 Germination test

In Petri dishes, containing sterile filter paper, 30 primed or unprimed seeds (P and NP, respectively) were allowed to germinate and, then subjected to stressed or unstressed treatments for one week. For salt stress treatment, fenugreek seeds were irrigated every day with 3 mL of 150 mM NaCl solution. While, control treatment consisted of irrigating the primed or unprimed seeds with 3 mL of distilled water. The germination experiment was conducted under a 16 h light/8 h dark regime in a constant environment chamber (temperature: 25 °C), with 3 replicates per treatment.

II.3 Germination and seedling development parameters

To evaluate the effect of salt stress and Si seed priming on fenugreek seed germination, some germination parameters, including final germination percentage (FG%), germination speed, time to reach 50% of germination (T_{50}) and germination value, were determined according to the our previous work (Lamsaadi et al. 2022a). In addition, in each Petri dishes, the length of radicle and hypocotyl of three germinated seed was measured daily up to seven day of germination.

II.4 Embryo viability

To assess the embryo viability of treated and untreated seed, after each 24h of germination, 10 non-germinated seeds from each treatment were taken, immersed in 2,3,5 triphenyltetrazolium chloride (TTC) solution and incubated for 24h in the dark at 30°C. After incubation, fenugreek seeds were washed three times with distilled water and the embryos were isolated under a binocular magnifying glass with 10 × magnifications and photographed (Lamsaadi et al.

2022a). TTC assay is based on the fact that viable embryos are red stained due to the reduction of TTC by cell respiratory activity (Verma and Majee 2013).

II.5 Hydrogen peroxide (H_2O_2) and malonyldialdehyde (MDA) contents in fenugreek seedlings

At the end of the experiment, 7-day-old fenugreek seedlings were harvested for determination of oxidative stress markers, in terms of H_2O_2 and MDA contents. Briefly, 500 mg of harvested seedlings were homogenized in 1.5 mL of 5% trichloroacetic acid, and the mixture was centrifuged at 12 000 rpm for 15 min. Afterward, the resulted supernatants were stored at -20 °C until determination of H_2O_2 and MDA contents. The MDA content was estimated using the thiobarbituric acid method as described previously by Hodges et al. (1999). While, the content of H_2O_2 was measured according to Junglee et al. (2014), where the H_2O_2 amount was calculated with a calibration curve prepared with H_2O_2 standard solutions.

II.6 Plant growth conditions

In the first part on this study, the results of germination traits suggested that, the most beneficial concentration of Si to prime fenugreek seed was 20 mM of $CaSiO_3$. Thus, this finding was the reason to select primed seed with 20 mM to investigate the effect of salt stress and Si seed priming on fenugreek yield under field conditions. For this purposes, unprimed and primed seeds with 20 mM Si were sown in plastic pots, containing 160 g of sterilized sand-peat mixture (1:4 v:v), in a growth chamber at 25 ± 1 °C, 60%—80% relative humidity and 16 h photoperiod. Subsequently, 15-day-old homogenized fenugreek seedlings were inoculated with rhizobial strain *Ensifer meliloti* Rm41 and transplanted into 44 cm * 15 cm pots, containing agricultural soil (the result of soil analysis was shown in Table 13). One week after transplantation, each fenugreek plants group (primed or unprimed) were divided into two plots: plants treated with 0 mM NaCl and plants treated with 100 mM NaCl. For each treatment, 3 pots containing 30 plants each were considered, and the experiment was conducted under field conditions at Polydisciplinary Faculty of Beni- Mellal, Sultan Moulay Slimane University, Morocco. Stress was applied for four months, and then some growth and yield related parameters were assessed. The quality of harvested seeds, in terms of protein, carbohydrate, flavonoids, polyphenols and mineral contents, was also evaluated.

Table 13. Soil analysis

Organic matter (%)	Salinity (mmhos/cm)	pH	N (%)	P (mg g^{-1} soil)
3	0.104±0.007	7.38 ± 0.03	0.32 ± 0.04	0.267 ± 0.01

II.7 Plant biomass and yield related parameters

After four months of growing under field conditions, 10 fenugreek plants from each treatment were harvested and their dry biomass was measured after oven drying at 80 °C for 48h. In addition, number of pods per plant, pod length, number of seed per pod and per plant, seed weight, pod weight and hundred seed weight were determined, as yield related parameters, to investigate the effect salt stress and Si priming on fenugreek yield under field conditions. The harvest index (HI) was also calculated as the ratio of seed weight to total plant weight (Moe et al. 2019).

II.8 Protein and sugars contents in seeds extract

After growth analysis, the seeds from each treatment was grounded to powder and stored for subsequent analysis. The protein content in harvested seed was determined from nitrogen content (N* 6.25), where the nitrogen percentage in seed powder was determined using the standard Kjeldahl method (Nergiz and Ötleş 1993).

Regarding carbohydrate content, 250 mg of ground seed was homogenized in 80% ethanol for 90 min. After cooling and decantation, the mixture was centrifuged at 15 000 rpm for 15 min and made up to 10 ml with deionized water (Brummer et al. 2003). Afterward, the content of ethanol soluble carbohydrate was determined according to Dubois et al. (2002).

II.9 Antioxidant activity of seeds extract

The antioxidant activity of harvested seeds was evaluated by determination of the content of total polyphenol and flavonoids in seeds extract. For this purposes, 250 mg of seed powder were dissolved in 70% aqueous ethanol for 30 min at 60 °C. After centrifugation at 15 000 for 15 min, the resulted supernatant was used to estimate the content of polyphenols and flavonoids (Shakuntala et al. 2011). The polyphenols content of these extracts was determined by Folin-Ciocalteu method (Singleton and Rossi 1965) and the content of flavonoids was assessed according to Chang et al. (2002).

II.10 Statistical analysis

The obtained data were analyzed by using two-way analyses of variance and the means were compared using Tukey’s test at 95% confidence level, using XLSTAT statistical software, version 2014.5.03.

III. Results

III.1 Germination parameters

The depressive effect of 150 mM NaCl stress was significantly appeared in all assessed germination parameters (Figure 31, $p \leq 0.05$). Indeed, the final germination percentage (FG%), germination speed and germination value were remarkably decreased by 23%, 25% and 11% respectively, while the time to reach 50% of germination (T_{50}) was significantly ($p \leq 0.01$) increased by 87% under salt stress as compared to control. However, a gradual and a significant increase was observed in Si primed seed with increasing Si concentration until 20 mM Si, while beyond this concentration, germination speed was reduced and T_{50} was increased under salt stressed conditions. Compared to Si unprimed salt stress seeds, priming with 20 mM Si significantly ($p \leq 0.01$) enhanced FG%, germination speed and germination value by 30%, 41% and 14%, respectively, whereas the decreased T_{50} (56%, $p \leq 0.001$) was observed in this treatment when compared with other treatments, including primed seeds with H_2O (P H_2O treatment). In addition, under unstressed conditions, priming with H_2O and different Si concentrations significantly ($p \leq 0.05$) enhanced germination speed, germination value and reduced T_{50} compared with unprimed control, but there was no significant ($p \geq 0.05$) difference between all priming treatments.

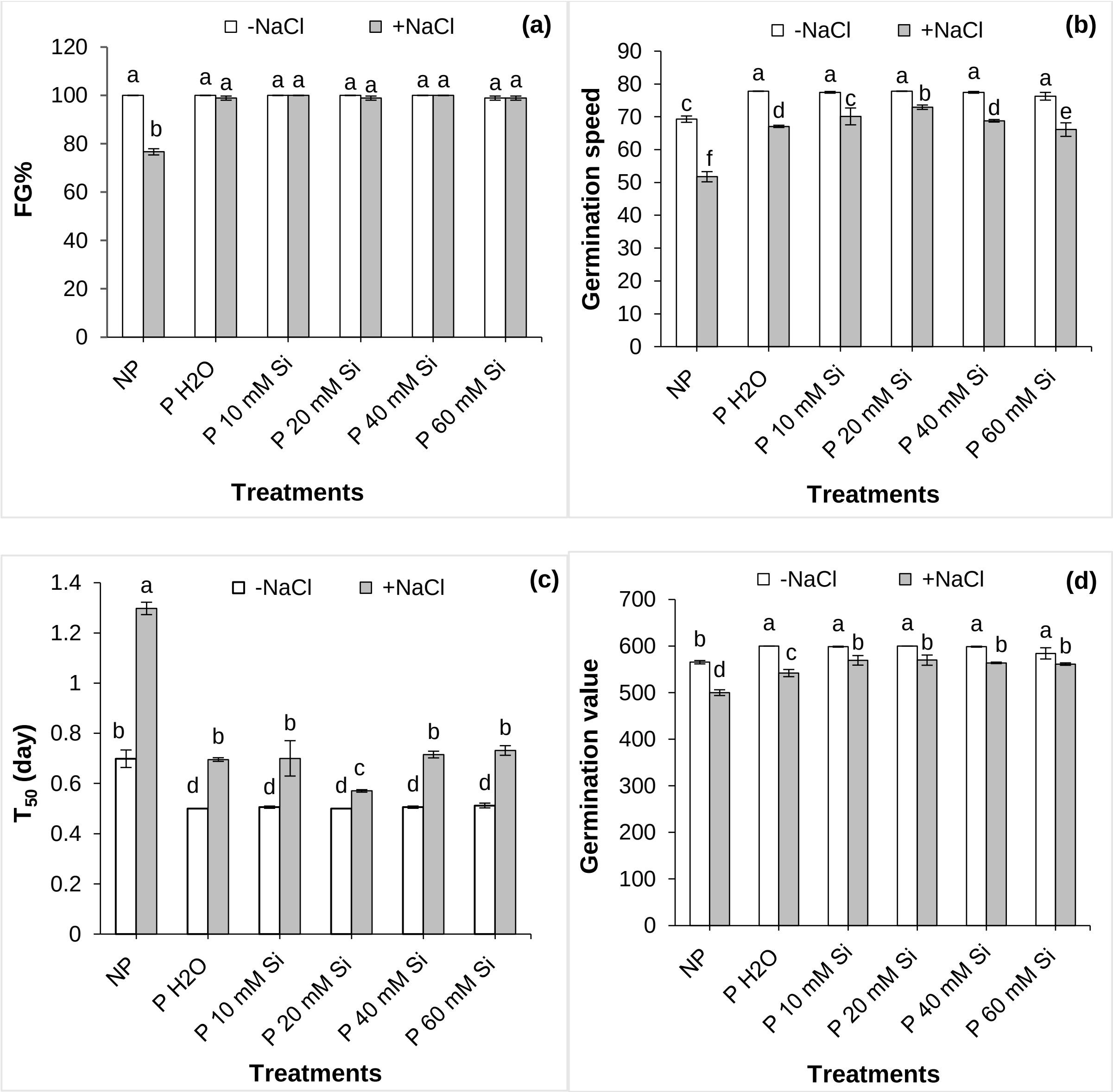

Figure 31: Effect of Si seed priming on final germination percentage (FG%, a), germination speed (b), time to reach 50% of germination (T_{50}, c) and germination value (d) of fenugreek seeds under unstressed (-150 mM NaCl) and stressed (+150 mM NaCl) conditions. Data are mean value ± SE. Bars with different letters are significantly different at $p \leq 0.05$ level. NP; unprimed seeds, P; primed seeds.

III.2 Radicle and hypocotyl development

Under normal conditions, when compared to unprimed seeds, the pretreatment of fenugreek seeds with H_2O or different concentrations of exogenous Si remarkably enhanced elongation of radicle during seven days of germination (Figure 32a). Similarly, under 150 mM NaCl stress, there was a significant increase in radicle length in H_2O and Si primed seeds relative to unprimed seeds (NP). Especially, during germination time, the maximal radicle length was observed in primed seeds with 20 mM Si (0 to 2.68 cm) compared to unprimed stressed seeds (0 to 2.04 cm), but beyond this concentration the radicle length was interestingly decreased (Figure 2b).

After figure 33 (a and b) analysis, relative to other treatments, the minimal hypocotyl length was observed in non-primed fenugreek seeds during 7 days of germination under stressed (0 to 1.17 cm) and unstressed (0 to 2.78 cm) conditions. In addition to the significant decrease in its length, a retard in hypocotyl formation was also resulted in unprimed stressed seeds, because that was appeared after 3 days of germination under stressed conditions. However, in primed seeds with H_2O or different concentrations of Si, the hypocotyl formation was appeared after 2 days of germination in both stressed and normal germination conditions, resulting an improvement in hypocotyl length when compared to unprimed stressed seeds. At the end of germination experiment, when compared to other pretreatments, the maximal hypocotyl length was observed in primed seeds with 10 (1.71 cm, 3.76 cm) and 20 (1.67 cm, 3.56 cm) mM of Si under stressed and unstressed conditions, respectively.

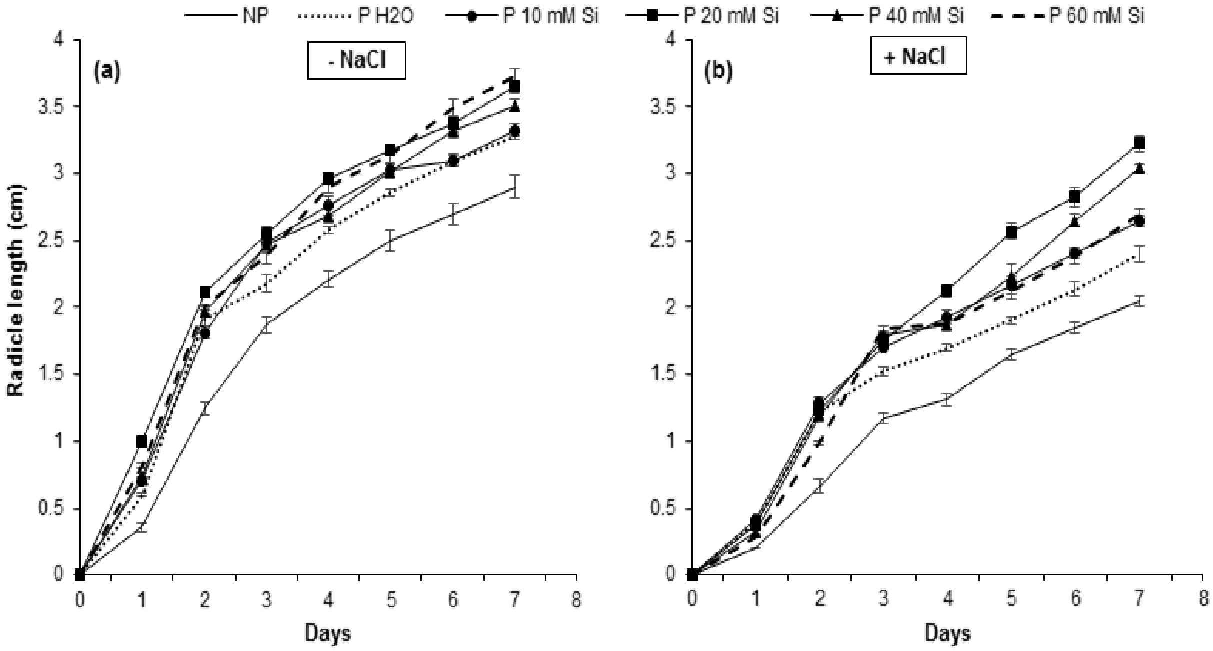

Figure 32: Effect of Si seed priming on radicle length of fenugreek seeds during 7 days of germination under unstressed (-150 mM NaCl; a) and stressed (+150 mM NaCl; b) conditions. NP; unprimed seeds, P; primed seeds.

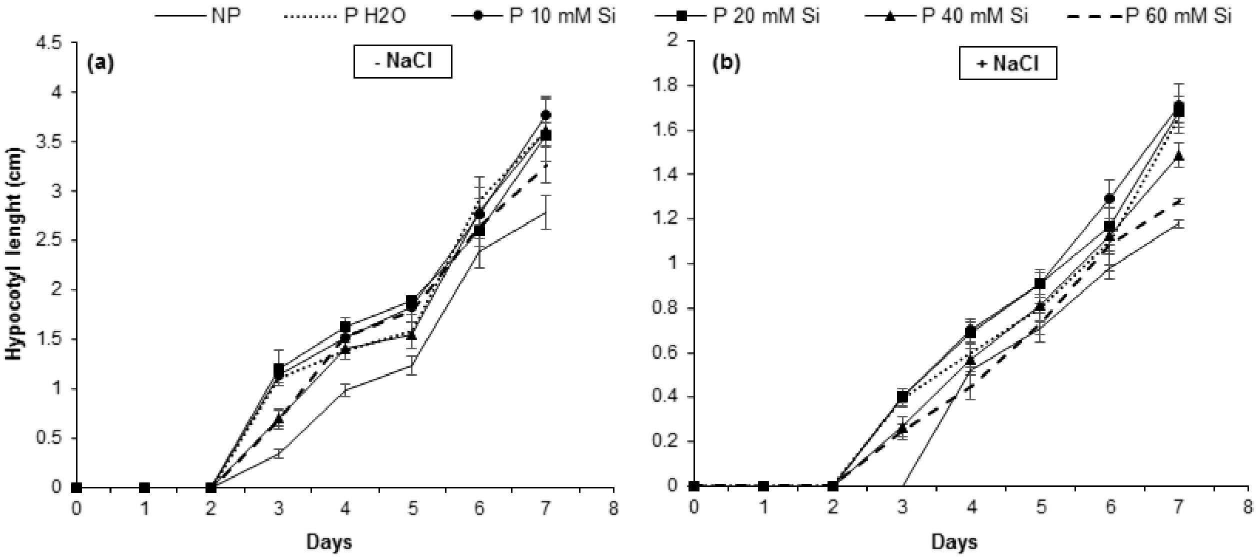

Figure 33: Effect of Si seed priming on hypocotyl length of fenugreek seeds during 7 days of germination under unstressed (-150 mM NaCl; a) and stressed (+150 mM NaCl; b) conditions. NP; unprimed seeds, P; primed seeds.

III.3 Embryo viability

Figure 34 presented the effect of Si priming and salinity stress on embryo viability, where the obtained results indicated that the embryo of fenugreek seeds showed different staining intensity dependent on the pretreatment and the germination conditions (stressed or unstressed). Indeed, after 24 and 48h of germination, only the embryo of primed seeds with 60 mm Si which was stained yellow when compared with other embryos under unstressed conditions. However, after exposition to 150 mM NaCl stress, embryos from unprimed and primed seeds with 40 and 60 mM Si were presented with yellow staining after 24h, 48h and 72h of germination, suggesting that embryo from these seeds become unviable after 24h of germination under salt stressed conditions. In addition, the embryo from stressed unprimed seeds rest unviable until the end of experiment, reflecting by yellow color after 96h of germination. In contrast, the embryos from primed seeds with H_2O, 10 and 20 mM of exogenous Si were bright red during the whole experiment under both stressed and unstressed conditions.

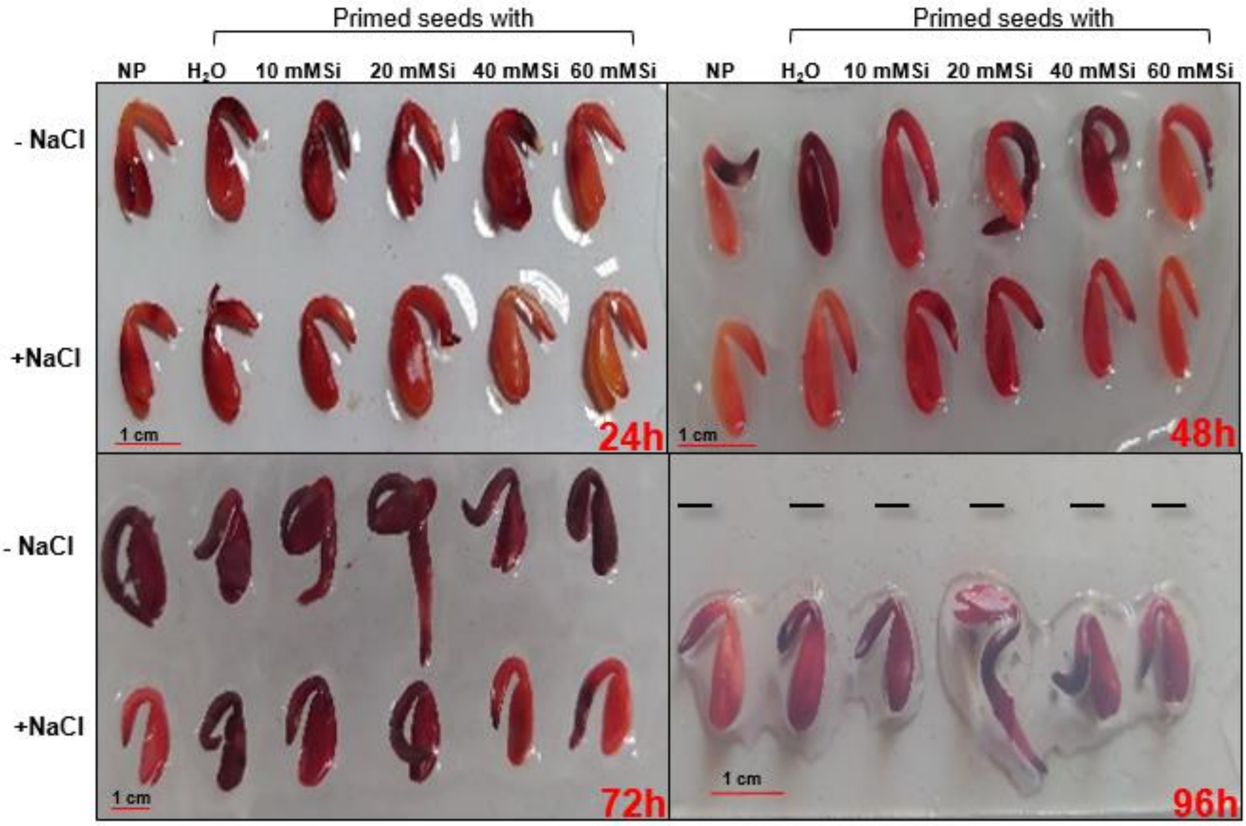

Figure 34: Effect of Si seed priming on embryo viability at 24h, 48h, 72h and 96h of germination under unstressed (-150 mM NaCl) and stressed (+150 mM NaCl) conditions. After seeds immersion in 2, 3, 5 triphenyltetrazolium chloride (TTC) solution, the embryo viability was evaluated by staining pattern and red color intensity of embryo cell. Living embryo tissues are red stained, while dead embryo tissues are unstained with red. At 96 h of germination time, hyphens mean that all tested seeds have been germinated. NP; unprimed seeds.

III.4 Lipid peroxidation and hydrogen peroxide content in fenugreek seedlings

The accumulation of oxidative stress markers, such as H_2O_2 and MDA, in 7-day-old fenugreek seedlings under stressed and unstressed conditions was presented in figure 35 (a and b). The results showed that, under unstressed conditions, the priming with H_2O or different concentrations of exogenous Si significantly ($p \leq 0.05$) reduced H_2O_2 and MDA contents in fenugreek seedling relative to control. The same findings were observed about MDA content under stressed conditions, where the priming with H_2O or different concentrations (10, 20, 40 and 60 mM Si) of Si was significantly($p \leq 0.05$) decreased the production of MDA by 22%, 24%, 31%, 15% and 12%, respectively, in fenugreek seedling relative to non-primed stressed seedlings. In contrast, only pretreatment with 20 mM Si that was significantly ($p \leq 0.01$) reduced H_2O_2 content by 37% when compared to the seedling of unprimed stressed seeds.

While, the priming of fenugreek seeds with H_2O or different Si concentrations of Si (10-60 mM) had no significant ($p \geq 0.05$) effect on H_2O_2 accumulation relative to unprimed seeds under stressed conditions.

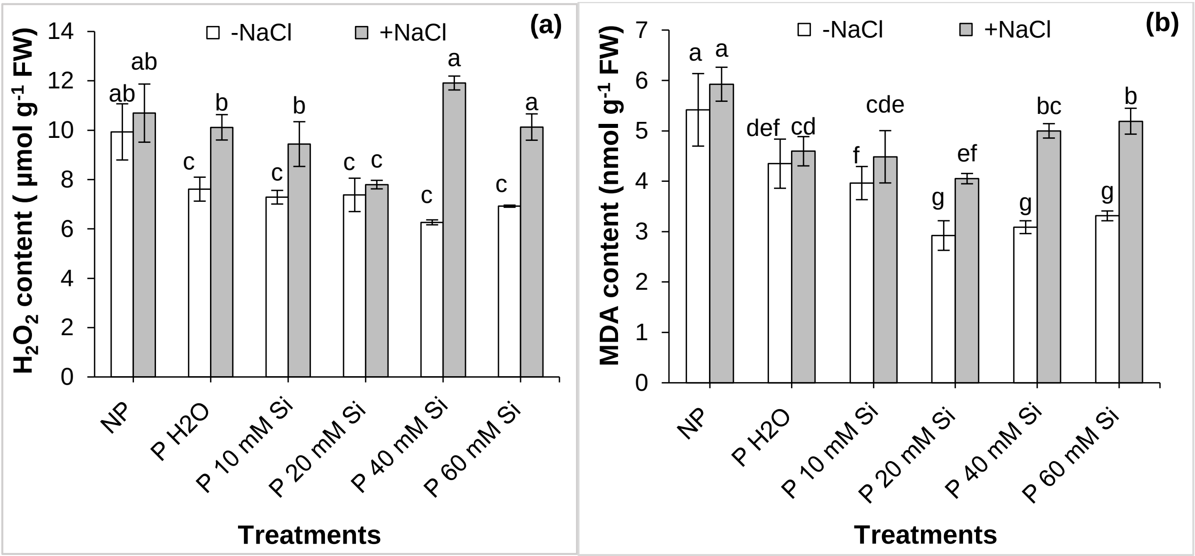

Figure 35: Effect of Si seed priming on H_2O_2 (a) and MDA (b) content in 7-day-old fenugreek seedlings under unstressed (-150 mM NaCl; a) and stressed (+150 mM NaCl; b) conditions. Data are mean value ± SE. Bars with different letters are significantly different at $p \leq 0.05$ level. H_2O_2; hydrogen peroxide, MDA; malonyldialdehyde, NP; unprimed seeds, P; primed seeds.

Based on the above results of germination parameters, embryo viability and oxidative stress induction in fenugreek seedling, we suggested that priming with 20 mM of exogenous Si was the optimal concentration to alleviates the harmful effect of salt stress on germination seeds. This latter was the reason to selected 20 mM Si in the present study, to investigate the effect of Si seed priming on growth and yield of salt stressed fenugreek plants under field conditions.

III.5 Plant, pod and seed biomass

As presented in table 14, the exposed unprimed fenugreek to salinity stress (100 mM NaCl) had significant ($p \leq 0.01$) lower dry weight of plant, pod and seed. In fact, the dry weight of plant, pod, seed per pod and seed per plant were significantly decreased by 50%, 23%, 49% and 55%, respectively, relative to control (NP treatment). However, priming with 20 mM Si resulted in significant ($p \leq 0.05$) higher dry biomass of plant (0.26 g), pod (0.11 g), seed per pod (52.66 mg pod^{-1}) and seed per plant (0.21 g $plant^{-1}$) compared to that of unprimed fenugreek under salinity stress. In addition, the biomass of 100 seeds, as an important parameter of plant production, was significantly ($p \leq 0.05$) enhanced in primed fenugreek relative to unprimed under stressed conditions. Likewise, under unstressed conditions, priming with 20 mM Si also enhanced all of dry biomass of plant (83%), pod (15%), seed per pod (48%) and seed per plant (61%), while it significantly ($p \leq 0.05$) reduced the dry weight of 100 seed by 13% when compared to unprimed control.

Table 14: Effect of Si seed priming and salt stress on dry weight of plant, pod and seed of fenugreek plants under field conditions. Data are mean value ± SE. The data followed by the different letters are significantly different at $p \leq 0.05$ level.

Treatments	Dry weight				
	Plant (g plant^{-1})	Pod (g pod^{-1})	Seed per pod (mg pod^{-1})	Seed per plant (g plant^{-1})	100 seeds (g)
NP	0.24 ± 0.02^{b}	0.13 ± 0.04^{a}	53.06 ± 1.95^{b}	0.18 ± 0.01^{ab}	0.68 ± 0.02^{a}
P	0.44 ± 0.01^{a}	0.15 ± 0.06^{a}	78.93 ± 5.67^{a}	0.29 ± 0.03^{a}	0.59 ± 0.10^{b}
NP + NaCl	0.12 ± 0.01^{c}	0.10 ± 0.05^{b}	27.06 ± 3.59^{c}	0.08 ± 0.01^{b}	0.48 ± 0.01^{d}
P + NaCl	0.26 ± 0.02^{b}	0.11 ± 0.03^{ab}	52.66 ± 8.95^{b}	0.21 ± 0.03^{a}	0.50 ± 0.03^{c}

NP; unprimed seeds, P; primed seeds, NaCl; 100 mM NaCl.

III.6 Yield related parameters

In addition to plant, pod and seed dry weight, the effect of Si priming and salinity stress on fenugreek yield and production was also evaluated by other yield related parameters like, number of pod per plant (NPP), number of seed per pod (NSP), number of seed per plant (NSp), pod length (PL) and harvest index (HI). The obtained results (Table 15) indicated that, under field conditions, the exposition of unprimed fenugreek to 100 mM NaCl importantly ($p \leq 0.05$) reduced all assessed yield related parameters (NPP, NSP, NSp, PL and HI) by 47%, 27%, 66%, 29% and 7%, respectively, relative to the control (NP treatment). In contrast, the NPP was interestingly improved by 77% in Si primed fenugreek compared to unprimed stressed plants. More than that, in primed stressed fenugreek, the obtained results of NSP, NSp, PL and HI were statistically similar to those observed in unstressed treatments (P and NP). Under unstressed conditions, the priming with Si had no significant ($p \geq 0.05$) effect on the above investigated yield parameters, exempt HI, which was significantly ($p \leq 0.01$) decreased by 16% in primed fenugreek relative to unprimed control.

Table 15: Effect of Si seed priming and salt stress on yield related parameters of fenugreek plants under field conditions. Data are mean value ± SE. The data followed by the different letters are significantly different at $p \leq 0.05$ level.

Treatments	Number of pod per plant	Number of seed per pod	Number of seed per plant	Pod length (cm)	Harvest index
NP	3.4 ± 0.21^{ab}	8 ± 0.53^{a}	26 ± 2.30^{a}	7.5 ± 0.17^{a}	0.81 ± 0.19^{a}
P	4.2 ± 0.14^{a}	10 ± 0.71^{a}	27 ± 2.30^{a}	7.9 ± 0.14^{a}	0.68 ± 0.10^{b}
NP + NaCl	1.8 ± 0.14^{c}	5.8 ± 1.14^{b}	8.66 ± 1.28^{b}	5.3 ± 0.16^{b}	0.75 ± 0.08^{b}
P + NaCl	3.2 ± 0.14^{b}	8.4 ± 0.85^{a}	23 ± 1.53^{a}	7.2 ± 0.25^{a}	0.90 ± 0.25^{a}

NP; unprimed seeds, P; primed seeds, NaCl; 100 mM NaCl.

III.7 Nutritional quality of harvested seeds

Under field conditions, to evaluated the nutritional quality of harvested seeds from applicated treatments, the content of proteins and soluble sugars was assessed and the obtained results were presented in figure 36 (a and b). The presented data showed that the content of proteins was significantly ($p \leq 0.05$) decreased by 18% in the seeds of unprimed stressed fenugreek, while salt stress had no significant effect ($p \geq 0.05$) on the amount of soluble sugars ($p \geq 0.05$) when compared with unstressed control. The inverse effect was observed in the seeds of primed stressed fenugreek, where the content of proteins was significantly improved by 5%, while those of soluble sugars was importantly decreased by 23% when compared to unprimed stressed fenugreek. In contrast, Si priming has no significant ($p \geq 0.05$) effect on seed content of proteins and soluble sugars relative to unprimed unstressed control under normal field conditions.

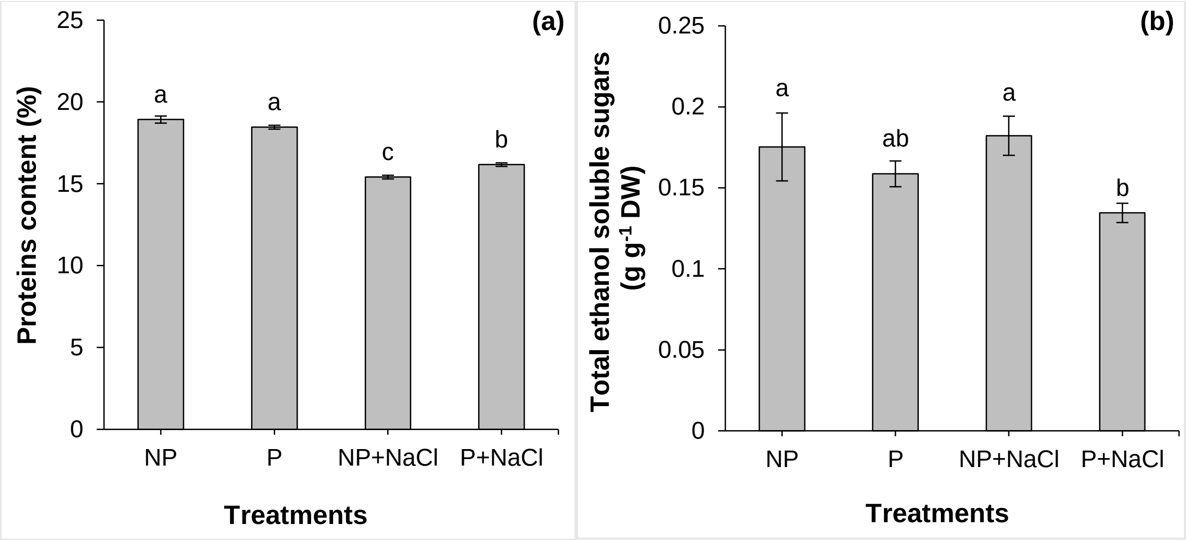

Figure 36: Effect of Si seed priming and salt stress on proteins (a) and total ethanol soluble sugars (b) content in harvested fenugreek seeds under field conditions. Data are mean value ± SE. Bars with different letters are significantly different at $p \leq 0.05$ level. NP; unprimed seeds, P; primed seeds.

III.8 Antioxidant activity of harvested seeds

In the present study, the content of total polyphenol and flavonoid was determined in harvested fenugreek seeds to evaluate the effect of Si priming and salt stress on antioxidant activity of seed extract under field conditions (Figure 37). The obtained results suggested that exposition to 100 mM NaCl stress significantly ($p \leq 0.01$) decreased total polyphenols and flavonoid contents by 22% and 11%, respectively, relative to the control. However, the priming with Si significantly alleviated this negative effect and importantly ($p \leq 0.01$) improved the antioxidant activity of seed extract, in terms of polyphenol and flavonoid, by 18% and 20%, respectively, when compared to the seed extract of unprimed stressed fenugreek. In addition, under unstressed conditions, Si priming had no significant ($p \geq 0.05$) effect on seed content of total polyphenols, but it significantly ($p \leq 0.01$) reduced flavonoid content by 23% in harvested seed relative to control.

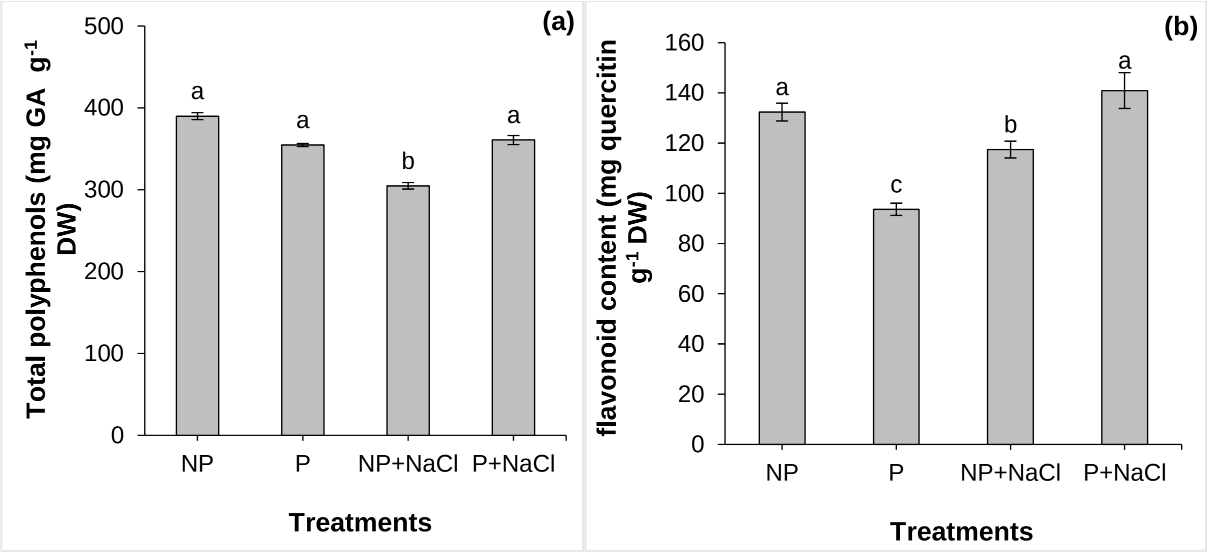

Figure 37: Effect of Si seed priming and salt stress on total polyphenols (a) and flavonoid (b) content in harvested fenugreek seeds under field conditions. Data are mean value ± SE. Bars with different letters are significantly different at $p \leq 0.05$ level. NP; unprimed seeds, P; primed seeds.

IV. Discussion

In many studies, the germination stage was evaluated by several parameters including final germination percentage (FG %), germination rate index, mean germination time, germination speed (GS), time to reach 50% of germination (T50), velocity index and germination value (GV) (Czabator 1962; Calone et al. 2020). Similarly, in the present study, to investigate the effect of salt stress and priming with Si on germination of fenugreek seeds, FG%, GS, T50 and GV were assessed. The obtained results indicated that salinity stress significantly reduced all of studied germination parameters, while these latter were importantly improved in Si primed seed under salt stress. Although seed priming with Si alleviated the depressive effect of salt stress on seed germination, but this positive effect was reversed where fenugreek seeds were primed with Si concentration beyond 20 mM. Therefore, Si-priming could alleviate the inhibitory effect of 150 mM NaCl stress on seed germination in a dose-dependent manner, with the optimal beneficial concentration is 20 mM. In addition, during seven days of germination, the maximal radicle and hypocotyl lengths were observed in primed fenugreek seeds with 20 mM Si compared to unprimed stressed seeds. The same findings were reported by Alves et al. (2020), where the obtained results indicated that priming with Si improved germination rate index, reduced mean germination time and enhanced survival of lettuce seedlings in salt-stressful conditions. The same results were showed by Azeem et al. (2015) in wheat under saline environments. More than that, under field conditions in semi-arid environment, Ayed et al. (2021) found that Si seed priming significantly increased the germination percentage, the germination index, the length of shoot and roots, the seedling fresh weight and the seedling vigor index of wheat. Also, it was reported that under other abiotic stresses like drought (Ali et al. 2021) and heavy metal accumulation (Pereira et al. 2021), seed pretreatment with Si increased seed germination, as well as seedling growth.

Decreased seed germination under salt stress could be a result of affected embryo viability by destruction of membrane structure of embryonic cells. In fact, the embryo viability was highly alterded in stressed unprimed seeds, explaining by the yellow color in embryonic cells during all germination experiment. However, in 20 mM Si primed seed the embryo viability was kept throughout the seven days of germination under salt stressed conditions. On the other hand, to explain the positive effect of priming with 20 mM Si on germination parameters and seedlings development, H_2O_2 and MDA contents were determined as oxidative stress indicators. The results showed that a significant decrease of oxidative stress markers was obtained in the seedlings of 20 mM primed seeds when compared to other treatments under salt stressed conditions. In the same way, Alves et al. (2020) reported that Si-priming alleviated the depressive effect of salt stress on seed germination of lettuce by reducing MDA and H_2O_2 content and enhancing enzymatic antioxidant activity, in terms of superoxide dismutase, catalase and glutathione reductase. Similarly, in a study conducted by El-Serafy et al. (2021) the results showed that priming with Si or SiNPs (silicon nanoparticles) significantly increased lathyrus seedlings salt tolerance by activating the enzymatic and non-enzymatic antioxidant machinery, which detoxifies reactive oxygen species (ROS). To confirm, Ellouzi et al. (2022) also reported that the beneficial effect of Si seed priming was more correlated with ROS-scavenge and early activation of antioxidant defence, recorded in barley seedlings when exposed to salt stress.

According to the hypothesis who reported that, successful plant growth and development are conditioned and predicted by successful germination stage. In the current study, following the above-cited results of germination parameters and seedling growth, we selected primed seeds with 20 mM Si to evaluate the role of Si-priming on plant biomass and yield of stressed fenugreek under field conditions. The results indicated that, salinity significantly decreased all studied yield parameters. This results was confirmed by those of Amiri et al. (2020), who showed that salinity stress reduced pod length, pod weight, number of seeds per pod, number of pods per plant, number of filled seeds per plant, filled to unfilled seeds ratio, shoot dry weight, straw weight, thousand-seed weight, grain yield, harvest index and water use efficiency and increased percentage of unfilled seeds of fenugreek. However, in the current study, when compared to salt stress, Si priming remarkably increased the dry biomass of plant, pod and seed, correlating with a significant increment in all mentioned yield related parameters, in terms of harvest index, number of pod per plant, number of seed per pod and weight of hundred-seeds in salt stressed fenugreek under field conditions. In concordance, alleviation of depressive effect of salt stress on plant biomass by Si seed priming was showed in several plant species like Alfalfa (Liu et al. 2018) and maize (Abdel Latef and Tran 2016). Where, the authors correlated the positive effect of Si seed priming with its role in amelioration of photosynthetic pigments and activation of antioxidant systems, inhibiting, as a result, oxidative stress induction. Regarding yield parameters, according to the previous literature, there is a lack of information about the effect of Si seed priming on yield, particularly of salt stress fenugreek under field conditions. However, under drought stress it has been reported that seed priming with Si increased spike length, hundred-grain weight and grain yields of drought-stressed wheat plants

(Ahmed et al. 2015). Also, under cadmium stress, priming with Si had a significant ameliorative effect on wheat growth and yield (Hussain et al. 2019).

Fenugreek is known as an important and oldest aromatic and medicinal plant, because it is used in several areas, like pharmacy, cosmetics and traditional medicine (Basch et al. 2003; Nagulapalli et al. 2017). These properties are given to it by its seeds, which are rich on antioxidant and bioactive molecules (Yadav and Chowdhury 2017; Alrumaihi et al. 2021). However, despite the importance of fenugreek seeds, the combined effect of salt stress and Si priming on seed quality and antioxidant activity has not yet been studied under field conditions, although it is more studied on plant growth. For this reason, in the present study, in addition to yield-related parameters, we also performed an analysis to assess the quality of harvested seeds obtained from each treatment. In fact, previous studies found that fenugreek seeds are riche in flavonoids, saponines, proteins and polyphenol (Benayad et al. 2014; Khorshidian et al. 2016). However the present studies showed that, under field conditions, exposition of fenugreek plant to salt stress negatively affected the nutritional quality, in terms of proteins content and soluble sugars, and significantly reduced the antioxidant activity of seed extract, reflected by reduced polyphenols and flavonoids content. In contrast, priming with Si alleviated these harmful effects of salt stress, where it significantly improved the content of proteins, carbohydrate, flavonoids and polyphenols. Therefore, based on the above-cited results, priming with Si could be a successful technic to enhance growth and yield of stressed fenugreek under field conditions and more interestingly it able to maintain de nutritional quality and antioxidant activity of harvested seed under salt stress.

V. Conclusion

According to the obtained results, Si seed priming moderated the adverse effects of salt stress on germination parameters, embryo viability and seedling growth in fenugreek, where the Si effect was in a dose-dependent manner, and 20 mM is the beneficial concentration. Additionally, under field conditions, Si seed priming was also effective to overcome salt stress damages on fenugreek biomass and yield. Moreover, characterization of harvested seeds indicated that exposition to salt stress significantly decreased the content of nutritional and antioxidant molecules, while Si seed priming alleviated these depressive effects and significantly improved the grain quality in salt stressed fenugreek under field conditions. Taken together, Si seed priming not only enhanced seed germination and early seedling growth, but also mitigated the harmful effect of salt stress on yield and quality of harvested seed from fenugreek under environmental conditions.

General discussion

The application of exogenous Si in response to abiotic stresses, including drought and salinity, is reported that among the promi strategies to help many plant species to survive or adapt under several environmental constraints (Rizwan et al. 2015; Hu et al. 2020; El Moukhtari et al. 2021b, 2022b). However, in the case of AMPs, there is a lack of information about the combined effect of salt stress and Si treatment on the tolerance of these important plants under environmental constraints. For this reason, the present thesis aimed to investigate the beneficial effect of Si treatment on the tolerance of fenugreek under salt stressed conditions, detailing the role of Si at different growth processes, such as germination, growth and yield.

The obtained results of seed germination exhibited that salinity stress significantly decreased germination percentage, germination energy, germination speed, velocity index and the growth of fenugreek seedling, together with a significant increase in the mean germination time of fenugreek seeds. This abiotic stress has also altered the viability of embryo and their ability to mobilize seed reserve, in terms of soluble sugars and proteins. Similarly, it was reported that salt stress also reduced seed germination of various AMPs such as *Thymus daenensis, Thymus kotschyanus* and *Origanum compactum* (Khoshsokhan et al. 2012; Laghmouchi et al. 2017). According to Moukhtari et al. (2022b) the negative effects of NaCl on seed germination could be attributed to osmotic and/or ionic toxicity. In this way, imbibition of fenugreek seeds with 200 mM NaCl led to a high accumulation of Na^+ and a decrease in K^+. In contrast, the treatment of seed with 3 mM Si importantly improved the tolerance of fenugreek at germination stage to salt stress. This latter was demonstrated by a higher germination percentage and vigor index and a decrease in mean germination time. Similarly, Si application significantly improved embryo viability, seed reserve mobilization and fenugreek seedling growth under salt stress. The tolerance of fenugreek seedling to salinity stress was significantly associated with a high activity of SOD and PPO antioxidant enzymes and a strong accumulation of antioxidant compounds like total polyphenols and flavonoids. This activation of antioxidant systems explained the obtained significant amelioration of membrane stability reflected by reduction in MDA and H_2O_2 contents and lower values of electrolyte leakage in fenugreek seedlings under stressed conditions. Therefore, exogenous Si application significantly improved the seed germination of fenugreek under salt stressed environments, where it could make the germination of NaCl-treated seeds comparable to untreated seeds. These obtained findings was confirmed by those previously reported by Wang et al. (2010) in *Momordica charantia,* Shi et al. (2014) in *Solanum lycopersicum* L. and Zhang et al. (2015) in *Glycyrrhiza uralensis*. In this context, it was documented that the positive effects of Si treatment could be associated with induction of hydrolytic enzymes and accumulation of organic solutes (Biju et al. 2017), regulation of hormonal metabolism and enhancement of antioxidant pathways (Wang et al. 2010; Moukhtari et al. 2022b).

Additionally to seed germination, the current work evaluated also the effect of Si supplementation on growth, photosynthesis, biological nitrogen fixation and yield of fenugreek plants under salt stressed conditions. Indeed, exposition to salinity stress (150mM NaCl) has drastic effects on many growth attributes like plant biomass, leaf area and length of fenugreek plants. These toxic effects of salinity on plant growth could due to excessive accumulation of salt ions and osmotic stress, together with oxidative stress through the production and accumulation of ROS (Van Zelm et al. 2020). In addition, accumulation of salt ions, like Na^+, could also disturb photosynthesis machinery (Farissi et al. 2018). In this context, ours result showed that the high content of Na^+, recorded in salt stressed fenugreek plants, was significantly correlated with a high reduction in chlorophyll content, photosystem II efficiency, stomatal conductance and photosystem I oxidation. According to Alamri et al. (2020), the accumulation of Na^+ ions in leaves increased the activity of chlorophyllase, chlorophyll-degrading peroxidase and pheophytinase, and in return inducing a reduction in chlorophyll content. On the other hand, it also has been reported that during response to salt stress, plants close their stomata to limit the water loss by transpiration and therefore lead to a reduction in CO_2 assimilation and, as a result, disrupt the photosynthetic activity (Antolín et al. 2010). Furthermore, the results of the present study showed that salt stress increased the accumulation of ROS, inducing an oxidative stress, which was demonstrated by high electrolyte leakage and lipid peroxidation (MDA). Osmotic stress, oxidative stress and ionic toxicity that have been reported in stressed fenugreek plants, reduced symbiosis establishment reflected by obtained decreases in nodule number and nitrogen content. Likewise, the similar results have been reported in *Medicago sativa* (Elgharably and Benes 2021; El Moukhtari et al. 2021a) and *Melilotus indicus* (Sunita et al. 2019). In this way, Faghire et al. (2011) reported that salinity could reduce the respiration of bacteroides and the carbonate metabolism of nodule, which negatively affects their functioning and consequently reduced biological nitrogen fixation.

In contrast, our findings indicated that supplementation of exogenous Si in the growth medium significantly mitigated the inhibitory effects of salt stress on growth of fenugreek plants. In fact, the treatment of salt stressed fenugreek plants with 3 mM of exogenous Si importantly improved dry and fresh biomass of shoot and root, plant height and leaf area. Likewise, in other plant species, like wheat, cucumber and basil, adding exogenous Si to the growth medium significantly enhanced plant biomass, plant length and leaf area under salt stress (Hajiboland et al. 2017; Robatjazi et al. 2020; Zhu et al. 2020). Improvement of plant growth might be the result of an enhancement in photosynthesis machinery (Sudhir and Murthy 2004b). In this context, in the present work, the photosynthesis process was detailed and the obtained results showed that Si treatment diminished the deleterious effects of NaCl on photosynthetic parameters such as chlorophyll content, stomatal conductance and photosystem II efficiency (*Fv/Fm*). Chlorophyll fluorescence, maximal quantum yield, energy conversion, energy regulation, electron transporter rate and photosystem I oxidation were also improved in Si treated fenugreek plant under salt stressed conditions. This latter was reported also by Ksiaa et al. (2021) in sea barley, where the treatment with exogenous Si significantly improved PSII efficiency under iron deficiency conditions. Under cadmium toxicity, Vaculík et al. (2015) showed that Si treatment could alleviate cadmium toxicity by enhancing the photosynthetic rate and electron transporter rate in PSII, which may be due to silicon-mediated enhancement of thylakoid formation in the chloroplasts of bundle sheath's cells. According to Alamri et al. (2020), Si may increase chlorophyll content under salt stress by increasing the activity of some chlorophyll synthesis enzymes, including δ-aminolevulinic acid dehydratase and porphobilinogen deaminase, and inhibiting those responsible of chlorophyll degradation, such as chlorophyllase, chlorophyll-degrading peroxidase and pheophytinase. Zhu et al. (2020) reported that exogenous Si supplementation significantly increased photosynthetic performance, which explained the enhancement in plant growth under stressed conditions. On the other hand, in legume species, biological nitrogen fixation is a vital process for plant development. This was confirmed in our study by the highly significant correlation between nodule number, nitrogen content and growth attributes. Essentially, the incorporation of Si to the growth medium significantly improved nodule number, which in return enhanced nitrogen content in both shoot and root of salt-stressed fenugreek plants. In the same way, the results reported by El Moukhtari et al. (2021a) on alfalfa and Steiner et al. (2018) on soybean also indicated that Si application significantly increased symbiosis establishement reflected by high nodule number and nitrogen content in stressed plants. Si supplementation could enhance nodule formation and act on nodule functionality by increasing symbiosome number, nitrogenase activity and leghemoglobin content under salinity (El Moukhtari et al. 2021b). Taken together, Si application raised the tolerance of fenugreek plants against salt stress by improving photosynthesis and biological nitrogen fixation, which are among the vital biological processes.

To confirm the above-cited beneficial effects of Si treatment under field conditions, the fenugreek seed were pretreated with different Si concentrations and then grown under salt stress (150 mM NaCl). The obtained results about germination test presented that Si seed priming alleviated the deleterious effects of salt stress on seed germination of fenugreek. Indeed, under salt stressed conditions, germination percentage and germination speed were improved, while the mean germination time (T_{50}) was decreased in Si primed seeds. Si seed priming improved embryo viability and reduced accumulation of oxidative stress, which could explain the enhanced obtained germination parameters and seedling growth of Si primed seed under salt stressed conditions. Nevertheless, this benefic effect of Si seed priming was with a dose-depending manner, with the benefice concentration was 20 mM. The similar results were reported by El-Serafy et al. (2021), showing that priming with Si or SiNPs (silicon nanoparticles) significantly increased lathyrus seedlings salt tolerance by detoxifying ROS. Alves et al. (2020) and Ellouzi et al. (2022) also reported the same benefic effect of Si seed priming on the salt tolerance of lettuce and barley seedlings, respectively. More than that, the evaluation of Si seed priming under field conditions indicated that Si seed priming ameliorated all of plant biomass, plant length, number of pod per plant, number of seed per pod, weight of 100 seeds and harvest index in salt stressed fenugreek. Moreover, salt stress drastically affected harvested seeds composition, while priming with Si significantly alleviated these harmful effects, where it significantly improved the content of proteins, flavonoids and polyphenols. Therefore, Si seed priming not only mitigated the adverse effect of salt stress on plant growth but it enhanced also the yield and harvested seed quality in salt stressed fenugreek plants under field conditions. Thus, regardless the used technic, direct treatment or seed priming, application of Si could be a promoting method to enhance the tolerance of fenugreek, as both important AMPs and legume, against salt stressed environments. Additionally, because of climate change pronounce the appearance of several abiotic stressors, the use of exogenous compound, as biostimulants, could be an effective and ecofriendly ways to enhance plant growth and yield under stressed environments

Conclusions and perspectives

Taken together, exposition of fenugreek to salt stress significantly induced many drastic effects during different growth stages. This abiotic constraint considerably reduced seed germination, embryo viability, carbohydrate and protein reserves mobilization and seedlings growth performance. Salinity stress also induced an oxidative stress reflected by high content of MDA, H_2O_2 and EL in fenugreek seedlings. Conversely, treatment through 3 mM of exogenous Si importantly improved fenugreek seed germination by enhancing embryo viability and seed reserve mobilization in 200 mM NaCl salt stressed seeds. Si application also improved membrane stability, reduced MDA levels and induced both enzymatic and non enzymatic antioxidant systems in fenugreek seedling under salt stress. Moreover, under salt stressed conditions, a significant reduction in salt ions content (Na^+) and a high accumulation of osmolytes compounds, like proline and glycine betaine, were showed in Si treated salt stressed fenugreek seedling. Therefore, Si is directly involved in central defence mechanisms, such as seed reserve mobilization, osmoregulation, salt ions reduction, oxidative stress detoxification and antioxidant systems activation, to enhance salt tolerance of fenugreek seed germination.

In addition to seed germination, salt stress besides reduced the growth and yield of fenugreek plants grown in a growth chamber at 25 ± 1 °C, 60% - 80% relative humidity and 16h photoperiod. In the same experiment, we noted that salinity (150 mM NaCl) stress also caused a significant induction of oxidative stress, reflecting by high accumulation of MDA and ROS in salt stressed fenugreek plants. However, when salt stressed fenugreek plants were treated with 3 mM of exogenous Si, a significant amelioration of plant biomass, yield parameters, photosynthesis, relative water content and nutrient uptake was showed. Exogenous Si also decreased Na^+ accumulation and maintained cell membrane integrity, due to a decrease in lipid peroxidation and ROS accumulation. In addition, their supplementation to the growth medium induced defence-related mechanisms through induction of antioxidant enzymes and biosynthesis of antioxidant molecules, like polyphenols and flavonoids. The production of organic compound, acting in osmoregulation process, was also noted in Si treated fenugreek plants under salt stressed conditions. Likewise, the results of chapter four showed that Si treatment has the same effects in salt stressed fenugreek even the experiment was carried out in a greenhouse under sunlight conditions (23 - 25 °C). This act as a confirmation of the beneficial role of Si, where the results indicated that exposition of fenugreek plant to salt stress caused a significant induction of oxidative stress, reflecting by the obtained raise in the content of oxidative stress indicators. The induction of oxidative stress could be the reason for obtained decreases in photosynthesis and biological nitrogen fixation, as well as plant biomass. In contrast, application of exogenous Si importantly alleviated the harmful effect of salt stress on fenugreek growth, by enhancing photosynthesis machinery and symbiosis relationship, which are among the vital biological processes. Production of oxidative stress markers was also reduced after Si treatment in salt stressed fenugreek, this reduction was significantly correlated with a significant increase in endogenous Si content and enzymatic antioxidant systems in both shoot and root of fenugreek plants. According to the above-cited effects, exogenous Si was directly involved in the central and vital biological processes to enhance the tolerance of fenugreek, as an important AMPs and legume species, against salt stressed environments.

The effect of seed priming with Si under salt stress was also investigated in the present work. The obtained findings showed that Si seed priming importantly improved fenugreek seed germination, by enhancing germination parameters, embryo viability and seedling growth of salt stressed seeds. While, this beneficial effect was in a dose-dependent manner and the benefic Si concentration to primed fenugreek seeds was 20 mM $CaSiO_3$. To confirm this impact, primed seeds with 20 mM $CaSiO_3$ were used to assess the effect of Si priming in salt stress tolerance of fenugreek plants under field conditions. As a result, Si seed priming significantly moderated the adverse effects of salt stress on fenugreek biomass and yield related parameters under filed conditions. More interestingly, evaluation of harvested seeds exhibited that Si seed priming significantly enhanced the nutritional and antioxidant quality of harvested seeds, in terms of protein, carbohydrate, polyphenol and flavonoid content. Hence, the positive effects of Si seed priming have not been observed in germinated seeds only, but also on the growth and yield of salt stressed fenugreek under filed conditions.

To conclude, according to our results, exogenous Si treatment mitigated the destructive effects of salinity in fenugreek at different growth processes (germination, growth and yield) regardless the application method, priming or direct application. Therefore, Si treatment could be an effective and ecofriendly approach to maintain plant growth and production under salt stressed environments.

Following our findings, we cited some perspectives to better understand the mechanisms by which Si improved the tolerance of fenugreek to salt stress. For example, at the germination stage, we propose to study the effect of Si on the GA/ABA ratio and on the activation of amylase and glucosidase enzymes in germinated fenugreek seed under salt stress. Additionally, in plant stage, we recommended to investigate the effect of Si on expression of salt tolerant genes, notably the expressing genes for SOS and HKT that are among the crucial transporters, involving in salt ions exclusion. On the other hand, because of the importance of legume plants in food and agricultural worldwide, a study to detail the effect of Si on nitrogen nutrition and nitrogenase activity is recommended, to investigate the effect of this exogenous molecule in the biological nitrogen fixation process as a vital process of this plant species. The fenugreek seed extract, such as essential oils, has several roles in prevention and treatment of many diseases, thus we propose to assess the effect of Si application on yield and composition of essential oils from salt stressed fenugreek. Furthermore, supplementation of exogenous compounds to salt stress conditions has a present interest in the development of sustainable agriculture, thus our results suggested that the development of Si-enriched fertilizers is highly recommended to improve crop productivity under stressed environments. On the other hand, the study of the effects of the Si application on fertility and soil composition is also one of the interesting perspectives of our work.

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