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Study of *Arabidopsis thaliana* response to salt stress and endophytic bacteria

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Prepared and presented by:

Hanane BENFARHAT

Evaluation jury:

President: Dr. Benaceur Farouk MCA. Amar Telidji University

Examiner: Dr. Rezzoug Asmaa MCA. Amar Telidji University

Under the supervision of

Supervisor Dr. Fathi Berrabah MCA .Amar Telidji University

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Abstract

Plants can shape an amazing area around their roots, through releasing a plethora of chemical molecules (root exudates) such as sugar, amino acid and secondary metabolites, that are representing a high part of their photosynthesis process. These molecules select mostly beneficial microbes to interact in positive manner leading to making up the second genome of plant a unique entity called holobiont. Even that plants are making healthy relationships, it does not mean that are safe. Soil salinization is one of the biggest challenging facing the development of crops that are considered as glycophytic. Endophytic bacteria provide an eco-friendly and cost-effective natural solution to alleviate salt stress. Five bacterial strains are used to perform this study about *Arabidopsis thaliana* response in salt stress and endophytic bacteria. The seeds of Arabidopsis are sterilized in SDS+70% ethanol, then inoculated with bacterial suspension. After germination, seedlings are incubated in the growth chamber for 10 days. Medicago alfalfa seedlings are used to confirm the efficacy of endophytes. Results show different levels in responses between both inoculated Arabidopsis and Alfalfa. Genomic analyses were performed using selected strains M17 and M67.2 that show a significant protection from salt stress. M17 is identified as *Pseudomonas aeruginosa* strain where M67.2 is *Bacillus amyloliquefaciens*. Comparative genomic for both strains is illustrated. For Pseudomonas strains (M17): three strains associated with plants, phytopathogen and Human pathogen) shows 1427 common genes where four strains are used for M67.2 which are three strains associated with plants and one non associated with plant. Syntenic analyses were performed between these strains using the largest genome (*Pseudomonas fluorescens F113*) as a reference and the closest strain (*Bacillus velezensis FZB42*) for M67.2. The results indicate a high conservation between the bacteria associated with plants in both strains. Genes identification is used to understand the complex network associated with plants. It shows common genes between the two genera. Protein-protein interaction is generated by STRING database for seven genes potentially involved in the protection mechanism; (*cat*, *opuAB*, *betB*, *bcp*, *katA*, *tpx*, *gbsB*). A complex interplay of a multitude of genes mediates salt resistance.

Key words: A. thaliana, M. sativa, salt stress, endophytic bacteria, PGPR, genes**.**

Résumé

Les plantes peuvent façonner une zone incroyable autour de leurs racines en libérant une multitude de molécules chimiques (exsudats racinaires) telles que des sucres, des acides aminés et des métabolites secondaires, qui représentent une grande partie de leur processus de photosynthèse. Ces molécules sélectionnent principalement des microbes bénéfiques pour interagir de manière positive, formant ainsi le deuxième génome de la plante, une entité unique appelée holobiont. Cependant, même si les plantes établissent des relations saines, cela ne signifie pas qu'elles sont à l'abri. La salinisation des sols est l'un des plus grands défis au développement des cultures considérées comme glycophytes. Les bactéries endophytes offrent des solutions naturelles respectueuses de l'environnement et économiques pour atténuer le stress salin. Cinq souches bactériennes ont été utilisées pour étudier la réponse d'*Arabidopsis thaliana* au stress salin et aux bactéries endophytes. Les graines d'Arabidopsis sont stérilisées dans une solution de SDS + éthanol à 70 %, puis inoculées avec une suspension bactérienne. Après la germination, les plantules sont incubées dans une chambre de croissance (phytotron) pendant 10 jours. Des plantules de luzerne (*Medicago sativa*) sont utilisées pour confirmer l'efficacité des endophytes. Les résultats montrent des niveaux de réponse différents entre les Arabidopsis inoculés et la luzerne. Des analyses génomiques ont été réalisées avec les souches sélectionnées M17 et M67.2, qui montrent une protection significative contre le stress salin. M17 a été identifié comme étant une souche de *Pseudomonas aeruginosa* tandis que M67.2 *est Bacillus amyloliquefaciens*. Une analyse génomique comparative a été illustrée pour les deux souches. Pour les souches de Pseudomonas (M17), trois souches associées aux plantes, un phytopathogène et un pathogène humain montrent 1427 gènes communs, tandis que quatre souches sont utilisées pour M67.2, dont trois sont associées aux plantes et une non associée aux plantes. Des analyses syntétiques ont été effectuées entre ces souches en utilisant le génome le plus grand (*Pseudomonas fluorescens F113*) comme référence et la souche la plus proche (*Bacillus velezensis FZB42*) pour M67.2. Les résultats indiquent une conservation élevée entre les bactéries associées aux plantes pour les deux souches. L'identification des gènes est utilisée pour comprendre le réseau complexe associé aux plantes, montrant des gènes communs entre les deux genres. Les interactions protéine-protéine sont générées par la base de données STRING pour sept gènes potentiellement impliqués dans le mécanisme de protection (*cat*, *opuAB*, *betB*, *bcp*, *katA*, *tpx*, *gbsB*). Un réseau complexe de nombreux gènes médie la résistance au sel.

Mots-clés : A. thaliana, M. sativa, stress salin, bactérie endophytes, PGPR (bactéries de la rhizosphère favorisant la croissance de plante).

الملخص

يمكن للنباتات أن تشكل منطقة مذهلة حول جذورها من خلال إطلاق مجموعة كبيرة من الجزيئات الكيميائية تسمى إفرازات مثل السكر والأحماض الأمينية والمركبات الثانوية، التي تمثل جزءًا كبيرًا من نتاج عملية التركيب الضوئي لديها. الجذر تعمل هذه الجزيئات عادة على جذب الكائنات الدقيقة المفيدة بشكل انتقائي للتفاعل بطريقة إيجابية، مما يؤدي إلى تشكيل الجينوم الثاني للنبات ككيان فريد يُسمى الهولوبيونت. وعلى الرغم من أن النباتات تقوم بإقامة علاقات صحية، إلا أن ذلك لا يعني أنها آمنة. يعد تملح التربة واحدا من أكبر التحديات التي تواجه تطوير الزراعات التي تعتبر غليكوفيتية حساسة للملوحة. تقدم البكتيريا الداخلية حلاً طبيعيًا صديقًا للبيئة وفعالًا من حيث التكلفة للتخفيف من ضغط الملوحة. تم استخدام خمس سلالات بكتيرية لإجراء لهذه الدراسة حول استجابة نبات رشاد أذن الفأر للإجهاد الملحي والبكتيريا الداخلية. تعمقت البذور بالإضافة الى إيثانول SDS بنسبة 70٪، ثم زُرعت بمعلق بكتيري. بعد الانبات، تمت مراقبة الشتلات في غرفة النمو لمدة عشرة ايام. استخدم نبات الفِصْفِصَة لتأكيد فعالية البكتيريا الداخلية. أظهرت نتائج تحليلات الجينوم المقارن لكلا النوعين من السلالات مستويات مختلفة لاستجابة النباتين التي أظهرت حماية كبيرة من الاجهاد الملحي. تم تحديد بين M17 كسلالة Pseudomonas aeruginosa ، ثُوضح التحليلات الجينومية المقارنة للسلالتين بوضوح. .Bacillus amyloliquefaciens كـ M67.2 حيث تم تحديد تظهر ثلاث سلالات مرتبطة بالنباتات، واحدة من مسببات المرض للنباتات :Pseudomonas M17 بالنسبة لسلالات وهي ثلاث سلالات مرتبطة M67.2 والاخرى للإنسان حيث تبين وجود 1427 جين مشترك، بينما تُستخدم أربع سلالات لـ Pseudomonas بالنباتات وواحدة غير مرتبطة. أجريت تحليلات التماثل الصبغي بين هذه السلالات باستخدام أكبر جينوم تشير النتائج إلى وجود .M67.2 لـ (Bacillus velezensis FZB42) وأقرب سلالة M17 كمرجع ل fluorescens F113 حفظ عالي بين البكتيريا المرتبطة بالنباتات في كلا السلالتين. ويُستخدم تحديد الجينات لفهم الشبكة المعقدة المرتبطة بالنباتات. وجود سبعة جينات محتملة تشارك في آلية الحماية؛ تُظهر تحليلات التفاعل البروتيني البروتيني بقاعدة بيانات STRING

.gbsB، tpx، katA، bcp، betB، opuAB، cat

تؤدي مجموعة معقدة من الجينات إلى توسيع مقاومة الملح

.

الكلمات المفتاحية: أ. تليانا،م.ألفالفا ، الإجهاد الملحي, البكتيريا الداخلية, بكتيريا نطاق تأثير جذور النبات لتحفيز نمو النبات

Dedication

Before I say anything, I want to thank, who taught man everything. Nothing can describe your greatness, your benevolence and your grace. I want to thank you for all your blessings upon me, and your mercy that has always shown me. I always found you by my side, when the stars don't shine and the moon hides, you were always the light in my darkness. Oh Allah, you are the greatest.

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To my lovely cats (Mimi°, Minouche, Nounou*, Jacki*, Minoucha°, Minou and Absi) I love you all and thank you for waking me up in the morning and making me always happy.*

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List of abbreviations

ABA : Abscisic acid.
AM : Arbuscular mycorrhiza
APX : Ascorbate peroxidase
AtPeps : Endogenous peptide.
BNM : Buffer Nodulation Medium
CAT : Catalase
DAMPs : Damage-associated molecular patterns
DHAR : Dehydroascorbate reductase
ERM : Ericoid mycorrhiza
ETS : Effector-triggered susceptibility
GHR : Guard cell hydrogen peroxide resistant 1
GIPC : Glycosyl inositol phosphorylceramide
IAA : Indoleacetic acid
ISR : Induced systemic resistance
JA : Jasmonic acid
LRR : Related leucine-rich repeat
MS : Murashige and Skoog medium
MscS : Small conductance mechanosensitive ion channel
NADPH : Nicotinamide adenine dinucleotide phosphate
NHX1 : Na$^+$/H$^+$ antiporter
NLRs : Nucleotide-binding leucine-rich repeat receptors
Nod : Nodulation
OD : Optical density
ORM : Orchid mycorrhiza
PAMPs : Pathogen-associated molecular patterns
PGPRs : Plant growth promoting rhizobacteria
PIP2 : Phosphatidylinositol bisphosphate

POD	Peroxidase
PRs	Pathogensis related genes
PRRs	Pattern recognition receptors
PTI	PAMP-triggered immunity
RbohF	Respiratory burst oxidase homologues
RKs	Receptor kinases
RLPs	Receptor-like proteins
ROS	Reactive oxygen species
SA	Salicylic acid
SAR	Systemic acquired resistance
SCaBP8	SOS3-like calcium-binding protein 8
SDS	Sodium dodecyl sulfate
SnRK2s	Sucrose nonfermenting 1-related protein kinases 2
SOD	Superoxide Dismutase
SOS	Salt Overly Sensitive
VOCs	Volatile organic compounds

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Part I. Literature review

Chapter I. Exploring plant-microorganisms interaction’s world

1.1 The rhizosphere: Home shaped by plants

Plants lack the ability to move. This constraint has pushed these sessile beings to develop certain characteristics that permit them to shape their own surroundings, especially in the area surrounding their roots [1]. Such an evolutionary characteristic consists of releasing a plethora of compounds, mainly organic ones, called root exudates, that influence the soil composition and the living communities within it, including fungi, bacteria, archaea, protists, and nematodes [2], [3].This dynamic area, known as the rhizosphere, forms an environment where neighboring microorganisms interact and can be associated with plants to form a unique entity called the holobiont, which can also be considered the second genome of plant [4]. These beings can host important ecological niches for their microbes, not only on roots but also on other parts, such as leaves and stems, collectively termed the phyllosphere, which is the aerial part of plants that hosts and interacts with microbes [5].

1.2 Plants talking and microbes answering

Plants initiate their communications through complex chemical networks that include various molecules that act as messengers between plants and their surrounding rhizospheric communities. Such an exchange can lead to the formation of different interactions with these beings, which, based on the effects that these organisms cause to plants, can be commensal, mutualistic, or parasitic[6]. Generally, plants initiate such interactions through the secretion of root exudates[7] . Rhizodeposits are numerous and can contain large polymers, such as proteins, as well as low-weight compounds like sugars and amino acids, which can attract a myriad community of microbes, like beneficial fungi and bacteria, that can help in nutrient acquisition and protection against pathogens. These beneficial organisms can offer a plethora of different molecules, such as organic acids, amino acids, volatile organic compounds (VOCs), and secondary metabolites (e. g., phenolics)[8].

1.3 Promoting plant health by microbes

Numerous microorganisms can engage in symbiotic interactions with plants, which are based on mutually beneficial exchanges between the plant and its symbiont. Through a variety of mechanisms, these interactions improve plant growth, resistance, and nutrient uptake [4]. The following are the most well-known mutually beneficial interactions that plants can create.

1.3.1 Mycorrhizal symbiosis

Mycorrhizal symbiosis is one of the earliest established relationships with plants, where soil fungi receive fixed carbon from plant's root and then provide good resources of minerals like phosphorus and nitrogen due to their hypha [9]. This interaction initiates by releasing strigolactones (small class of carotenoid-derived compounds) that can take different forms depending on plant and fungi species. Four major forms are known: ectomycorrhiza (located at the roots surface) and endomycorrhiza (colonizing inner tissues, Figure 1) which can take different aspects: arbuscular mycorrhiza (AM), ericoid (ERM), and orchid (ORM) mycorrhiza that can regulate nutrient acquisition, control seedling establishment and assure protection to their hosts from abiotic (osmotic and drought stress) and biotic stresses, increasing defense response [10] [11]

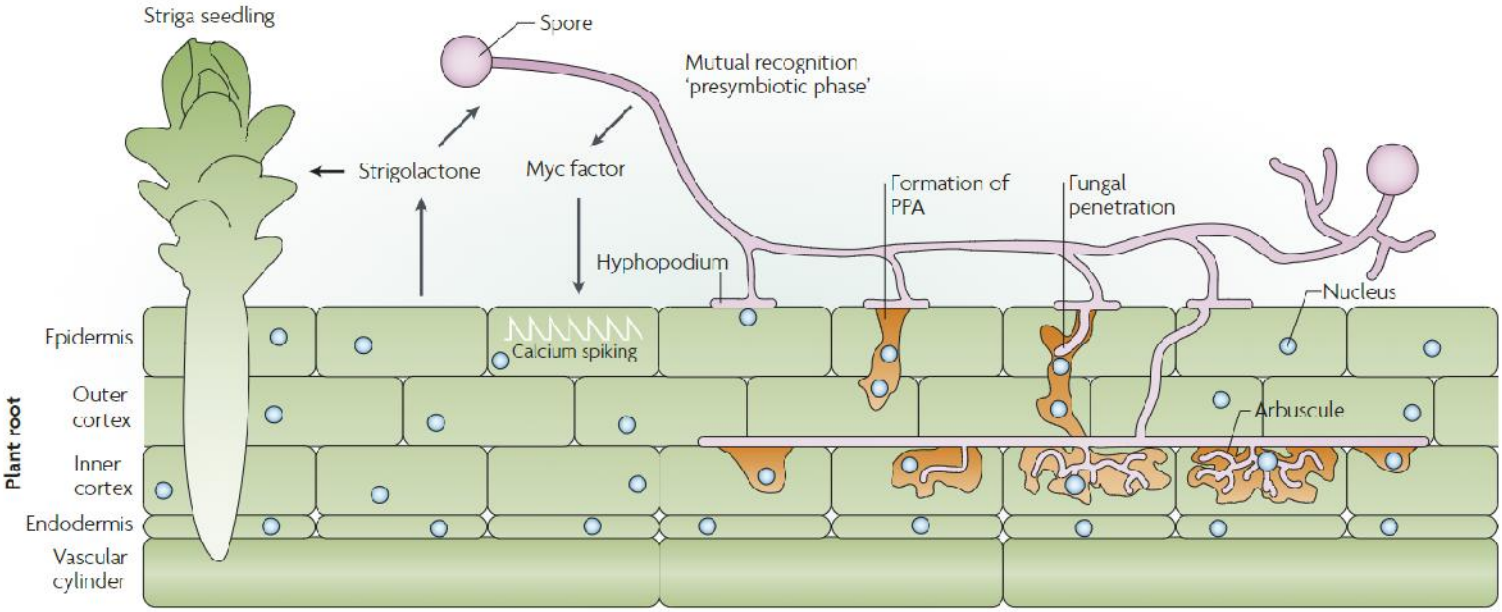


Figure 1. Mycorrhizal colonization [12].

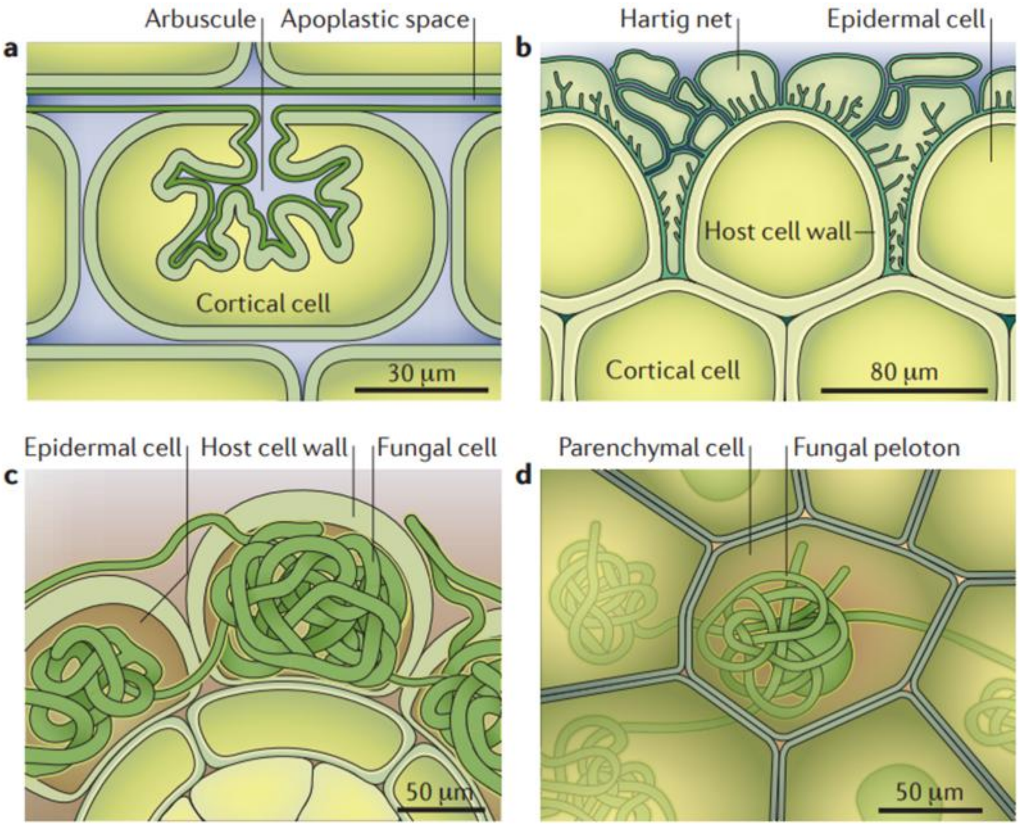


Figure 2. Known forms of mycorrhiza symbiosis [10].

a. arbuscular mycorrhiza; **b.** ectomycorrhiza; **c.** ericoid mycorrhiza; **d.** orchid mycorrhiza.

2.3.2 Nitrogen fixing bacteria and legumes symbioses

Legumes are widely known to make special organ named the nodule, where the important element and limiting factor for growth, nitrogen is fixed by rhizobia a group from proteobacteria or actinobacteria (Frankia) which can be associated with the only nonlegume genus known: Parasponia [13] [14]. The mechanism by which this association is established is been studied for years, it is known that plant release flavonoids[15] that are catched by rhizobia and then activate the expression of nodulation factors (Nod factors) as a response. Once the Nod factors are perceived by the host, a symbiotic signalling pathway is activated leading to calcium oscillations, this facilitates rhizobia entry through root hair curl that are growing around them. Rhizobia invasive the infected thread and enter root tissues and then nodule organ start to be created and ready to converse atmospheric nitrogen to ammoniac [16].

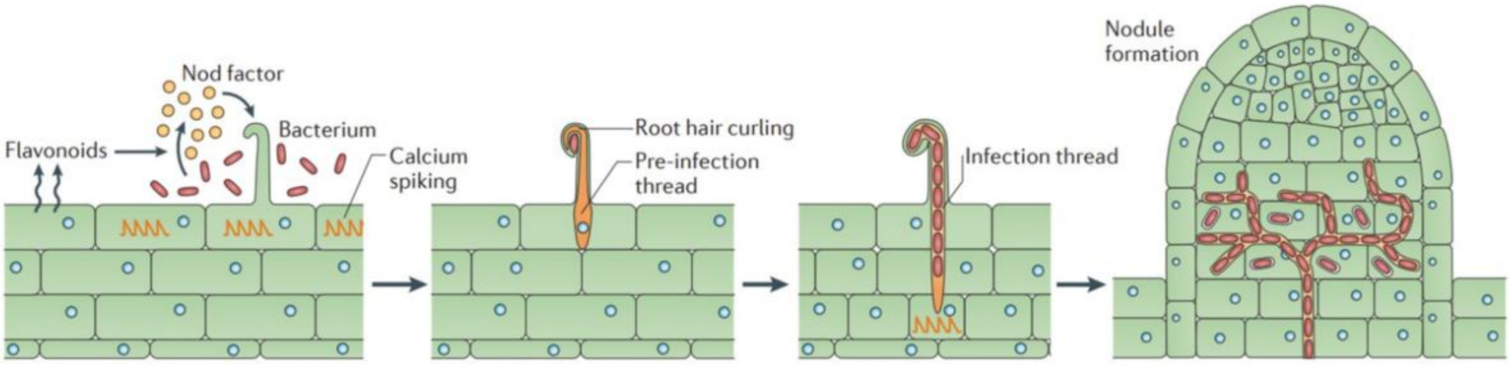


Figure 3. Early symbiosis signaling and nodule formation [16]**.**

1.3.4 Plant growth promoting rhizobacteria

Plant growth promoting rhizobacteria (PGPR) are mostly founded in a beneficial relationship with plants [17]. They are defined by the bacteria present in the rhizosphere and gain the capacity of stimulating plant growth by several mechanisms [18] such as high uptake of minerals, production of siderophore, and growth-promoting metabolites including vitamins and phytohormones like indoleacetic acid (IAA) which is responsible of light and gravity respond in plants [19]. PGPR can be helpful to plants on facing both of abiotic (salt, drought stress) and biotic stress (pathogens) on acting as biocontrol agent [17].

1.3.4 Endophytic colonization

Endophytic bacteria are beneficial colonizers living inside plant tissues [20]. Their microbiome assemblage depends on neighboring selected from the rhizosphere and host species-specific genetic factors. Usually, they are enriched in Proteobacteria and Firmicutes members. The lytic enzymes releasing (e.g. lysozymes) lead successfully to colonize plant tissue [4]. They play a crucial role as a plant component contributing directly in nutrient acquisition, (e.g. phosphate solubilization, iron chelation) [21], regulating phytohormones and indirectly, through targeting phytopathogens with antibiotics [22],[20]. Under abiotic stresses, such as aluminum, drought, temperature and salinity, endophytes can enhance stress tolerance.[23], [24], [25].

Chapter 2: Plants in front of biotic and abiotic stresses

2.1 Biotic stresses

While plants shape their appropriate microbiome, that does not mean they are totally safe but a good destination for various harmful living organisms around them. These biological units are the main cause of biotic stress that induce damage to plants. They can be herbivore[26]nematodes, pests and microorganisms such as fungi, bacteria, viruses and oomycetes [27].

Various symptoms are observed during the pathogenic process, and depending on the mode of nutrition. These pathogens can be classified on the mode of nutrition into three categories: biotrophic which feed on living tissues, conversely to necrotrophic that live on nutrient content of the host cell after destroy it by releasing toxins and enzymes such as *Cochliobolus carbonum* (fungus) and *Erwinia carotovora* (bacterium) [28], [29]. On the other hand, hemibiotrophs use both strategies starting living in live tissues then secreting toxins which kill cells such as the fungus *Mangaporthe oryzae* and the bacterium *Pseudomonas syringae* [30].

2.2 Plants fighting pathogens

Because it is an attractive destination for pathogenic microbes such as bacteria, fungi, and viruses, plants have developed their immune system through sophisticated mechanisms at an increasing level through different layers:

2.2.1 Physical barriers

Plants use physical barriers before inducing specific response to pathogens as a primary stage and remodel its structure in order to impede successful infection besides to less cost energy [31]. Plant cuticle is outermost lipophilic layer with cutin matrix and waxy composition, covering leaves, flowers and fruits[32]. When pathogen penetrates cuticle, cell wall is the secondary challenge. Due to its dynamic and complexity composition made of various pectin polysaccharides, cellulose and hemicelluloses,[33] cell wall provides protection and support for plant [34]. Another element that is considered as physical barriers is the stomata, a natural pore that is closed when pathogen is arrived [31].

2.2.2 Pathogen-associated molecular patterns -triggered immunity

Plants have developed special receptors on the surface of their cells called pattern recognition receptors (PRRs) that are responsible for identifying distinct molecular structures known as "pathogen-associated molecular patterns" that are necessary for the survival of pathogens like fungi like chitin and bacteria like flagellin (PAMPs) [35]. Furthermore, PRRs are able to identify damage-associated molecular patterns, which are generated by infections and cause cell harm (DAMPs). Receptor kinases (RKs) with an internal signaling domain and receptor-like proteins (RLPs) are the two primary types of receptors. PRRs initiate the PAMP-triggered immunity (PTI) program, which is the first line of defense, upon detecting PAMPs or DAMPs. [36], [37].

2.2.3 Effector-triggered immunity

Plants and their pathogens are in continuous co-evolution, often described as an arms race or zig-zag model. Effector proteins are the weapons deployed by pathogens to suppress PTI. The release of these effector proteins leads to effector-triggered susceptibility (ETS). Conversely, plants respond by a more effective immune system, termed effector-triggered immunity (ETI) which perceives these effector proteins and plant elicitor peptides (Pep). Example includes AtPeps (endogenous peptide)[38] in *Arabidopsis* recognized by related leucine-rich repeat (LRR) receptor kinase [37]and in another case using intracellular nucleotide-binding leucine-rich repeat receptors (NLRs) encoded by gene resistance (R)[39]. As PTI, ETI also can enhances the production of ROS that is triggered by other PAMPs (for example, elf18, C10:0, nlp20 and fungal cell wall component chitin [27], [40].

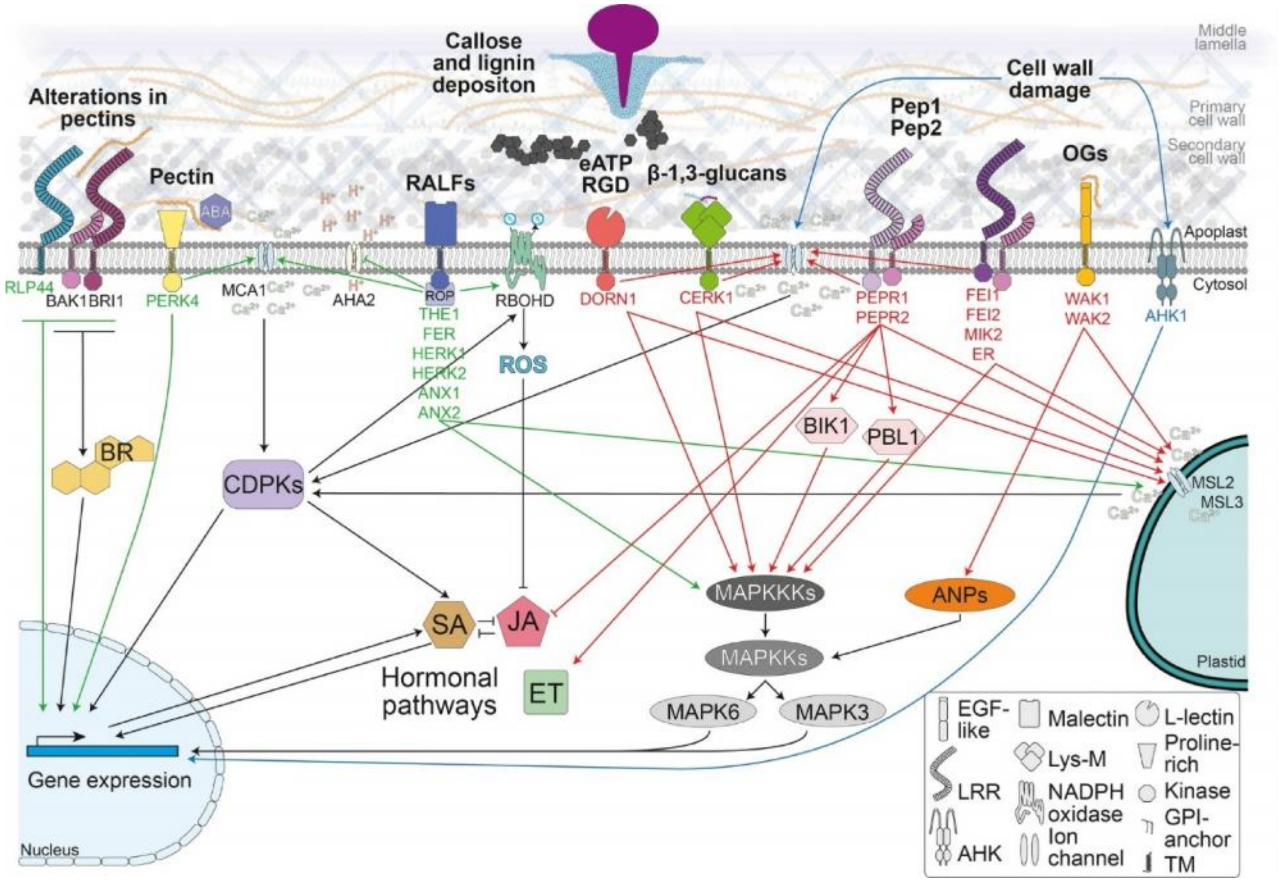


Figure 4. **Plant immune response** [33].

2.2.4 Systemic immunity triggered by plant–microbe interactions (SAR)

It is defense system in distant[41] depending on the localization of pathogenic infection by perceiving effector or MAMPs , it is activated basely by hormone salicylic acid [27] which is a important regulator[42] that is systemically transported from infected tissue to uninfected parts on plant via an apoplastic root in *Arabidopsis thaliana* [42], [43]Activation of pathogenesis-related (PR) genes express antimicrobial proteins, as PR1, PR2, and PR5 is coordinated with high levels of SA [42].

2.2.5 Induced systemic resistance

Where SAR is based on pathogen perception, induced systemic resistance (ISR) is activated by beneficial mutualistic microbes such as *Pseudomonas fluorescens, B. cereus, Bacillus amyloliquefaciens, B.atrophaeus*, etc. that are effective against pathogenic microbes, including necrotrophic pathogens [44], where the key component is jasmonic acid (JA) [13]. In addition to (ET) ethlyn, other hormones enter immune orchestrate signaling networks, such as auxin, abscisic acid, cytokinin, and gibberellin [45]. ISR is a co-evolved useful pathway that is crucial in stimulating plants's immune systems against the most dangerous pathogens [46].The immune system is stimulated by a co-evolved robust pathway called induced systemic resistance (ISR), which plays a pivotal role against a wide range of pathogenic microbes.

2.3 Plants in abiotic stress

2.3.1 Drought stress

One of the most severe stresses that plants can face. Due to decreasing water availability in plant cells, which enhances solute levels in the soil and then inside plant tissues [44],it is considered a limitative factor for growth and productivity, causing osmotic shock [45]. Drought stress can damage several cellular functions like photosynthesis, gas exchange, and nutrient uptake, as well as cellular components including proteins, lipids, and nucleic acid [46]. As a result of this metabolomic dysfunction, changes in the morphology of plant parts such as plant height, leaves, and roots result from a reduction in lateral root branch density[47].

2.3.2 Temperature stress

As any living organism, temperature is a crucial limiting factor affecting physiological processes. Plants are sensitive to whether increasing (heat) or decrease (cold) upon the ideal temperature for its cellular metabolism. [48] .High temperature causes dehydration and consequently damage in plant organelles such as chloroplasts which consequently influence photosynthesis productivity and chlorophyll biosynthesis as well as increasing levels in reactive oxygen species ROS in low temperature[49]. During carbon assimilation, there is an observable suppression in Rubisco activity; in addition to disruption of electron chain transport[50].common plants response to heat stress is synthesis of heat shock proteins (HSPs) that contribute to generate antioxidants, osmolytes and maintain cell integrity[51].

2.3.3 Heavy metal stress

Even that heavy metals are normally founded in soil, and essential nutrients like Fe (iron), Cu (copper) and Mn (manganese) wastes of industrial activities continually contaminate the soil which make it a big face of pollution. that threat plant growth on enhancing toxicity of this minerals and disturbing soil texture [52]. Using bacterial strains resisting to heavy metals to face this stress as a bio-remediators instead of traditional methods is on the top of solutions list [53].

Chapter 3: Plants facing salt stress

3.1 salt penetrating plant tissues

Salt stress is caused by increasing levels of two distinct ions, sodium (Na+) and chloride (Cl-), in plant cells [25]. The roots are the first organ perceivers, but they are not yet identifying special receptors or transporters for NaCl, especially sodium ions, and it is recommended that plants do not directly sense salinity but rather its effects.

Several ways can facilitate the entry of NaCl into plant tissues. The faster one is through the apoplastic pathway by direct flow to xylem, and the second is by symplatsic pathway, characterized by appropriate transports or complements like high-affinity K+ Transporter (HKT) in monocotyledonous, where ions traverse cell to cell [54][55]. Additionally, ionic channels as non-selective cation channels (NSCCs) that allow a direct flow of Na+ to cell across plasma membrane.

Osmotic perturbation is one of the early effects of salt stress, which is essential for NaCl sensing. The hyperosmolality-activated Ca2+-permeable cation channel detects increases in cytosolic calcium $[Ca^{2+}]_{cyt,}$ which acts as an important secondary messenger in osmosensing [56]. In addition, it suggests that the MscS-like (MSL) protein family of mechanosensitive channels could be involved in detecting pressure changes that contribute to membrane depolarization [54].

3.2 Impacts of salt stress on plant health

3.2.1 Osmotic stress

Under saline conditions, osmotic stress is the first short-term salt stress experienced by plants [28]. When soluble salts (Na+ and Cl−) enter plant tissues, it leads to high osmotic pressure [29], making the outside more negative than the inside, which causes lower water potential, leading to deficiency in uptake by roots [26, 30]. Additionally, to elevating Ca+2 concentration in the cytosol [31], osmotic stress can cause many damages to plants, including redox imbalance, stunted root's development [32], plasma membrane shrinkage [29], and altering one of the most important plant's processes, photosynthesis.

Salt stress can affect the most vital process in a plant's life cycle, photosynthesis, and this happens in through two forms. The first is involved in altering stomata's mechanics. Stomata are tiny superficial pores on plants's leaves, responsible for controlling gas exchange, especially the uptake of carbon dioxide (CO2) and water (H2O) transport in transpiration [57]. Salt accumulation leads to disruptive of osmotic pressure inside pair of specialized epidermal cells forming the guard cells, surrounding stomata,[58] and regulating their opening. This disruption leads to close of stomata by contraction and water outflow.

3.2.2 Ionic stress

Roots typically absorb nutrients from the soil, where minerals are around. High concentrations of sodium and chloride especially, compete with some essential nutrient like calcium ($Ca2+$), magnesium ($Mg2+$) and potassium ($K+$) which is important enzyme cofactor and involved in regulating protein synthesis and enzymatic metabolism [59][60],. This ionic competition hinders the uptake of these crucial elements by root, leading to nutrient deficiencies[61]. High levels of chloride impede nitrogen’s absorption while in the presence of sulfate ($SO42-$) ions can reduce the solubility of phosphorus (P). These effects contribute to the short term of salinity stress impacts[62].Furthermore, absorbing saline water causes changes in various plant’s parts such as reduction in number of leaf and size, membrane depolarization and inhibition of root hair development which decrease compactness and plant’s height [62], [63].

3.2.3 Oxidative stress

ROS can play a significant role as signaling molecules [64]in many biological processes such as maintain apex development, where (O_2-·) accumulates in the meristematic zone of the root for cell division and H_2O_2 is accumulated on the elongation zone for cell differentiation in *Arabidopsis thaliana*. [65]. Additionally, to continuous production in chloroplast, mitochondria and peroxisome generally by electron transport systems (ETCs) where O_2 is a powerful electron acceptor[66]. This fundamental molecule has becoming toxic by increasing its levels as a consequence of both osmotic and ionic stresses under salt stress. Due to its highly reaction[67] .ROS induce damage in cellular components Including proteins, lipid[68]. Additionally, to genotoxicity[69] and disturbing physiological processes like photosynthesis[70].

3.3 Plant response to salt stress

After perceiving salt damage, plant respond by many physiological responses including:

3.3.1 Production of osmolytes

In order to decrease osmotic potential and maintain cell turgor, plants produce and accumulate many compatible metabolites such as proline which is free amino acid that strongly hydrate and stabilize protein's structure under stress due to its hydrophobic and hydrophilic ends playing a key role in osmoregulation, additionally to glycine betaine (GB) deriving from oxidation of choline-by-choline monooxygenase (CMO) and betaine aldehyde dehydrogenase (BADH)fig[71] [72] [73].

Glycine betaine is an important osmoprotectant synthesized by the chloroplast, helping plant to assure efficiency of photosynthesis and protection to thylakoid membranes [72].another major osmolytes are sugars like sucrose and fructose and polyols (polyhydric alcohols) as mannitol and sorbitol[74],that aid plant in hydration and membrane fluidity [75].

Figure and table from [72]

3.3.2 Ion homeostasis

Plants tolerant the toxic accumulation of Na+ inside cells by many mechanisms including: select enhancing uptake of K+ over Na+ through specific channels like NSCCs and high affinity potassium transporters (HAKs) in order to maintain K+/ Na+ ratio[76], additionally to over-expression of an Na + and K + permeable HKT transporter [77]. Plants can remove Na+ from leaves then transported into vacuole [76]which potentially modifying lipid content in its membrane called tonoplast[78].

3.3.3 ROS detoxification

Producing antioxidants is commonly natural response in plants to combat damage caused by ROS accumulation [54]. These antioxidants can be non-enzymatic, such as flavonoids and carotenoids that aid in neutralizing ROS [42] and proline that functions as a quencher of singlet oxygen and superoxide that are derived from ROS [57]. Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT) [55], dehydroascorbate reductase (DHAR), and peroxidase (POD)[56].

3.3.4 Activation of SOS signaling pathway

The Salt Overly Sensitive (SOS) pathway takes an important place in regulating salt tolerance[56]. Three main proteins (SOS1, SOS2 and SOS3) additionally to SCaBP8 are forming this conserved evolutionary pathway [79], [80]. High concentration of Na+ outside the plant cell is sensed by membrane lipid named glycosyl inositol phosphorylceramide (GIPC) one of the most important specific sphingolipid classes [81]which is negatively charged and then directly bind with Na+ ions. This interaction leads to activate calcium signaling pathway [79]. SOS3 and SCaBP8 (SOS3-like calcium-binding protein 8) [74]are activated after perceiving calcium ions[56], then they activate and recruiting SOS2 which is serine/threonine protein kinase to membrane in order to phosphorylate SOS1 (PM Na+/H+ antiporter) which rises efflux Na+ outside[80].

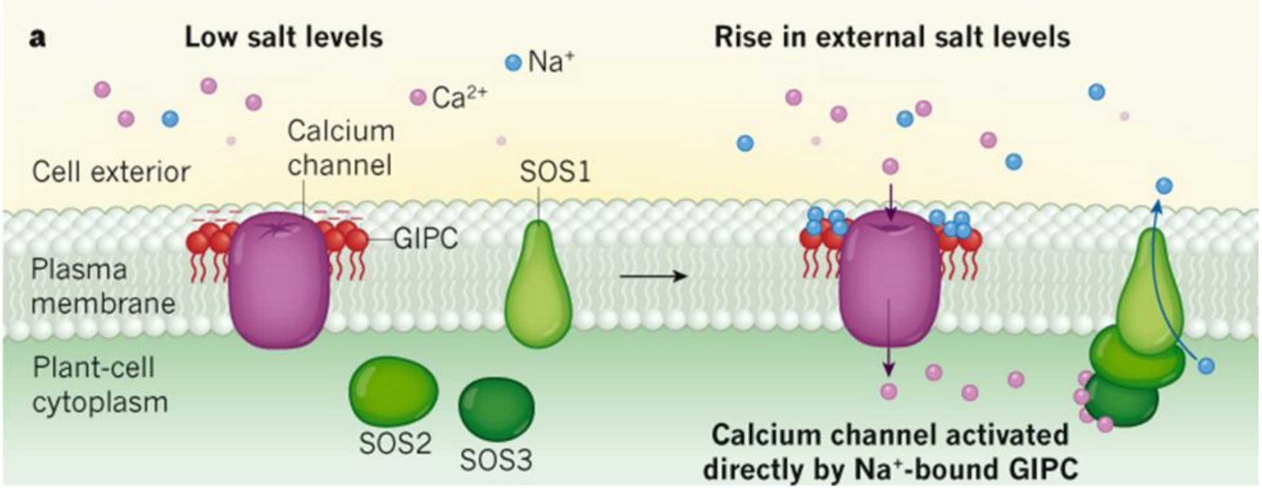


Figure 5. Illustrated exemple of sensing salt by plants [79] **.**

3.3.5 Phytohormones regulation

Salt affects the normal concentrations of many hormones as a result of the plant’s response to stress. One of the critical hormones is abscisic acid (ABA), which plays a master role in the signalling pathway under challenging conditions. High levels of ABA are essential to activating the central stimuli sucrose nonfermenting 1-related protein kinases (SnRK2s) to various responses such as osmoregulation, gene expression, and stomata closure[56]. This latest version is regulated by several processes including: SnRK2s is activated by ABA phosphorylates plasma membrane NADPH oxidase RbohF, which contributes to generating O_2- in the apoplast. O_2- immediately transforms into H_2O_2, an essential signaling molecule in many ABA responses [82]. It is suggested that H_2O_2 can enter guard cells through aquaporin PIP2;1 and stimulate ROS generation. Another crucial process that contributes effectively to this pathway is Ca+2 signals through the activation of its channels in plasma membrane by H_2O_2 which is required by GHR1(guard cell hydrogen peroxide resistant 1) [72].

3.4 Microbes Improve salt resistance to plants

Mutualistic interactions between plants and microbes can effectively enhance plant plasticity performance to resist many biotic and abiotic stresses while at the same time growing healthy and rapidly. Many examples are mentioned. The two fungal endophytes, *Penicillium chrysogenum* and *Penicillium brevicompactum,* showed enhanced photosynthesis production, proline accumulation, and upregulation of the NHX1 gene, which encodes the NHX1 Na+/H+ antiporter in the tonoplast (membrane of vacuole), to assure ion homeostasis for the plants *Lactuca sativa* (lettuce) and *Solanum lycopersicum* (tomato) [83]. On the other hand, several endophytic bacteria can play a role as plant protectants from salinity stress, such as *Bacillus subtilis* (BERA 71), which helps plants increase levels of enzymatic antioxidants such as superoxide dismutase, and non-enzymatic antioxidants like glutathione, to remove toxic ROS and increase the total content of phenols.[84]

Flavobacterium crocinum HYN0056T showed up regulation of gene expression related to enhancing tolerance to both salt and drought stresses and stimulating stomata closure in *Arabidopsis*.[85]

An excellent example of endophytic PGPR is the bacterium *Bacillus megaterium* ZS-3, which regulates many cellular components, like the increasing content of both chlorophyll a and b as well as carotenoid content. Additionally, regulate osmotic substances such as reducing proline levels and enhancing sugar levels, as well as catalase and peroxidase activity. Furthermore, by reducing Na+ toxicity by decreasing Na+ accumulation and increasing the K+/Na+ ratio, the bacterium can produce vesicules in order to isolate Na+ by activating the expression of the *NHX1* and *AVP1* genes. The downregulation of *HKT1* minimizes the uptake of Na+. Salicylic acid and the jasmonic acid/ethylene signaling pathway are also activated [85].

Volatile organic compounds (VOCs) secreted by *Bacillus amyloliquefaciens* FZB42 can effectively increase biomass and activate the anti-oxidation system. Upregulation of jasmonic acid (JA) is observed in *Arabidopsis* mutants as a response to (VOCs)[86].

1- Introduction to the problematic of the study

Soil salinization is a natural phenomenon, where soluble salts accumulated in the soil. Human activities such as irrigation combining with the climate change have the great role to accelerate this phenomenon and make it on the top of challenges in agriculture. Salt stress has become a threat to plant's growth and crop productions that are almost glycophytes (plants that not support growing in salty environment). One of the most effects of this abiotic stress is reducing resilience to plants for adapting in their fluctuant environment and increasing other stresses such as drought stress. This serious challenge needs to be treated using natural solutions, and one of the best eco-friendly solutions is using microorganisms.[73], [87]

Arabidopsis thaliana is a small flowering plant from the mustard family (Brassicaceae). It is widely used as plant biology research model due to its short life cycle and tiny genome size with over than 30 megabases of annotated sequence available in GenBank and ease of genetic manipulation make it a valuable model system to understand cell development and environmental responses [88], [89].

The project aims to study the *Arabidopsis thaliana* response to salt stress and the role of endophytic bacteria in enhancing salt tolerance.

To illustrate this study, five endophytic bacteria strains were utilized and tested on *Arabidopsis thaliana* and *Medicago sativa (alfalfa)*. Genomic comparative and functional analyses are used to identify the common genes and the metabolic pathways by which endophytic bacteria can improve salt resistance to the plant *Arabidopsis thaliana* as well as *Medicago sativa (alfalfa)*.

Part II. Materials and Methods

I. A. thaliana growth and inoculation

Seeds of *Arabidopsis thaliana* col-0 (Colombia) are surface sterilized using 70% (V/V) of ethanol including 0.02% (W/V) of SDS (Sodium dodecyl sulfate). The seed are incubated for 12 minutes with agitation and then rinsed 3 times using ethanol 70% (V/V) in sterile conditions and the last one by using ethanol 100%. The washed seeds are then dried.

The sterilized seeds are then deposited in 25 ml 1/2MS growth medium (Murashige and Skoog medium) gelling with 1.2 % (W/V) of bacto agar. The seeds are recovered with aluminum paper for obscurity and stratified for two days (48h) at 4°C. After two days of stratification, the seeds in Petri dishes were putted in the growth chamber at 27°C for five days.

The plants are inoculated by adding 50 µl of suspension at OD600 = 0.1 previously prepared from solid bacteria to petri dishes containing 25 mL of 1/2 MS cultivated for 48 h at 30 °C in YEB. The following strains were used in this study: M17 (Pseudomonas aeruginosa), M18 (Enterobacter sp.), M50 (*Lysinibacillus sp.*), BT37 (*Streptococcus pseudopneumoniae*), and M67.2 (*Bacillus amyloliquefaciens*).

II. Stress responses analysis

In order to analyze the plant response to the salt stress, after germination, the plants are transferred to a 1/2 MS growth medium containing or not 100 mM NaCl. The seedlings were then incubated for 10 days at 26 °C in the growth chamber.

Pictures were taken every 3 days during the experiment, and the images were analyzed using ImageJ software. Two biological replicates, including at least 16 plants, are analyzed for every condition. For every plant, the length was determined, and a graphic shows the results for each repetition.

III. *Medicago sativa* growth and inoculation

Alfalfa seeds are cleaned with a liquid soap solution (pec) and rinsed four times with distilled water to remove traces of soap. Seeds are then sterilized with a 5° chlorine treatment for 12 minutes and rinsed four times with sterile distilled water. The sterilized seeds are deposited in round petri dishes (150mm) containing a sterile solution of 1.2% agar and distilled water. The seeds are then placed at 4°C in the dark for 48 hours for stratification. Germination is then carried out by placing the seeds for 24 hours in a climatic chamber at 26°C in the dark. The seedlings are then transferred to a modified Buffer Nodulation Medium (BNM) medium containing 1.2% bacto agar and containing or not 150 mM NaCl. The plants are then grown in climate 26°C with 16h of light and 8h of darkness. Freshly prepared bacterial suspensions of solution are added to plants by rinsing the roots and excess inoculum is removed. Inhibition of root growth by NaCl is assessed by measuring growth 5 days after cultivation.

IV. Comparative genomic analyses

To identify putative key genes in the strain M17 (*Pseudomonas aeruginosa*) required for plant infection. Genomic analyses were performed on selected strains of *Pseudomonas* associated with plants: plant growth promoting bacteria (PGPB): *P. koreensis 16610*, *P. atacamensis M7D1*, *P. fluorescence F113*; plant pathogens: *P. syringae DC3000* and human pathogens *P. aeruginosa 138244*.

The genomic analyses were performed in the MicroScope platform (https://mage.genoscope.cns.fr/microscope/compgenomics/pancoreTool.php?).

The protein-protein interaction was illustrated using STRING database. (STRING: functional protein association networks (string-db.org))

Part III. Results

I. M67.2 protect partially *A. thaliana* from the salt stress

In order to evaluate the ability of the studied strains to protect the plants from salt stress, an analysis of A. thaliana growth inhibition by the salt was performed by cultivating the plant in vitro in the presence or absence of NaCl at 100mM and/or the strains M67.2 and M17. After 10 days post inoculation, the plant growth was evaluated by measuring the length of the plants using ImageJ software.

Non-inoculated plants show no variation in plant growth at 1 days; by contrast at 3 and 9 days of culture, the growth of the plant is reduced compared to untreated plants: a reduction of 0.42 to 2.17 cm for the treated vs untreated plants (Figure 6, Ctr). These results indicate that the addition of NaCl at 100mM greatly reduce *A. thaliana* growth.

In the presence of the strain M67.2, the impact of the salt stress is reduced at 3 and 9 days in repetition 2 (Figure 6, M67.2 R2). This reduction is not observed in repetition 1. These results indicate that the strain M67.2 potentially reduce the salt stress effect on *A. thaliana*, however, this hypothesis need further investigation.

In contrast to M67.2, the use of the M17 strain mainly reduces the impact of the salt stress on *A. thaliana*. Except for days 9 in repetition 2, a reduction in the impact of the salt stress is observed for all plants at 3 and 9 days of cultivation.

All these results show that NaCl reduces the growth of *A. thaliana* in our condition. The strains M17 and potentially M67.2 attenuate the impact of the salt stress on *A. thaliana*. Interestingly, at 1 day of plant growth, the addition of the bacteria increases the impact of the salt stress on *A. thaliana*.

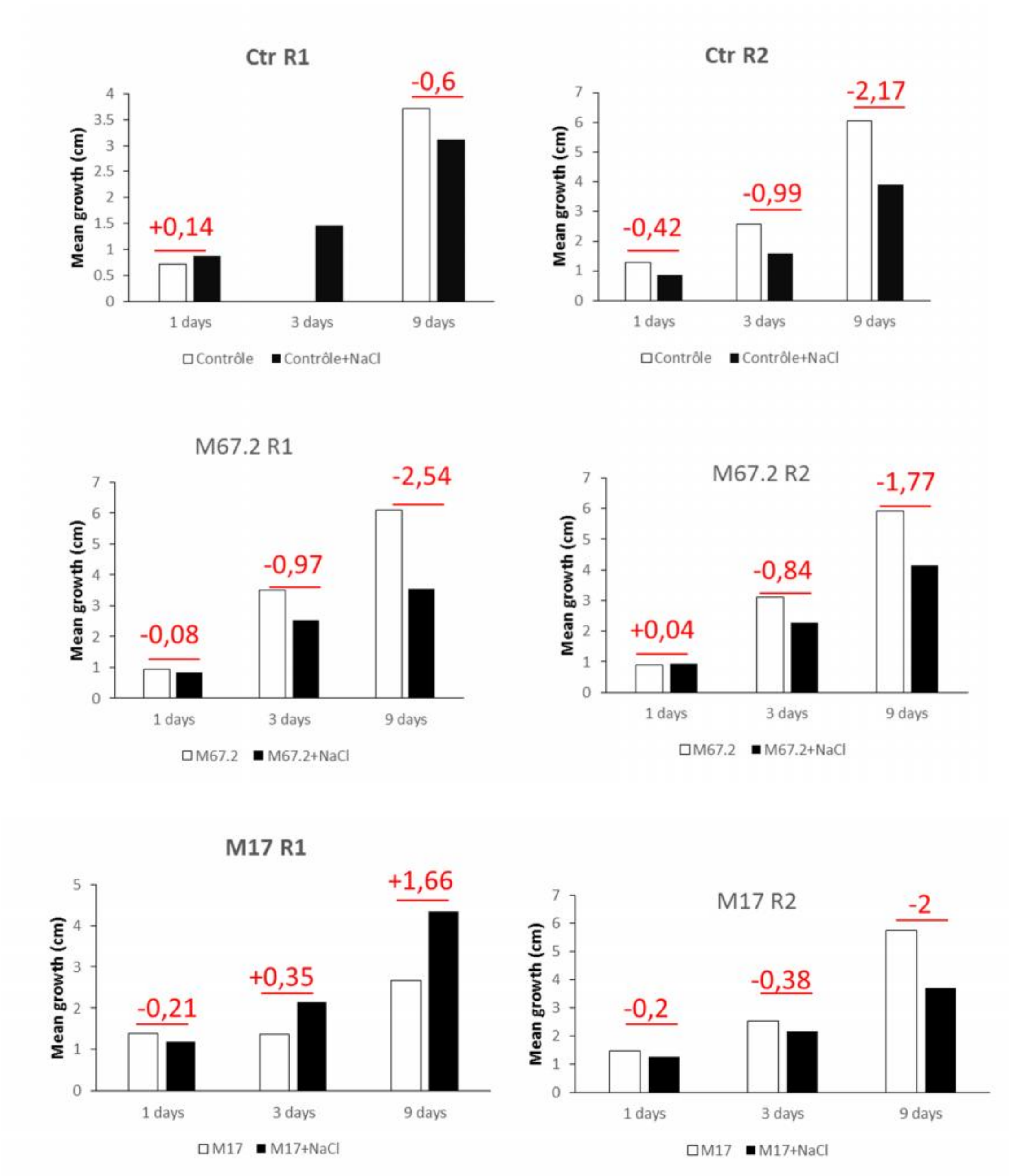


Figure 6. Salt stress response of *A. thaliana* in the presence or absence of M67.2 and M17 strains. In the analyses of *A. thaliana* response to the absence or the presence of NaCl at 100mM, the effect of M67.2 and M17 on *A. thaliana* protection to the salt was evaluated at 1, 3, and 9 days of cultivation. Two biological repetitions including 16 plants were performed for each condition.

II. M17 protect Alfalfa from salt stress

In the aim to evaluate the ability of the stain M17 to protect crops from salt stress, analysis of the salt stress protection of alfalfa (Medicago sativa) by M17 was evaluated at 150 and 200 mM NaCl in plants cultivated *in vitro*.

The addition of NaCl at 150 and 200 mM greatly reduces *M. sativa* growth, interestingly, inoculation of the plant with the strain M17 reduces the effect of the NaCl at 150 and 200mM.

Together, these results indicate that the M17 strain can reduce the impact of the salt stress on Alfalfa crops cultivated *in vitro*.

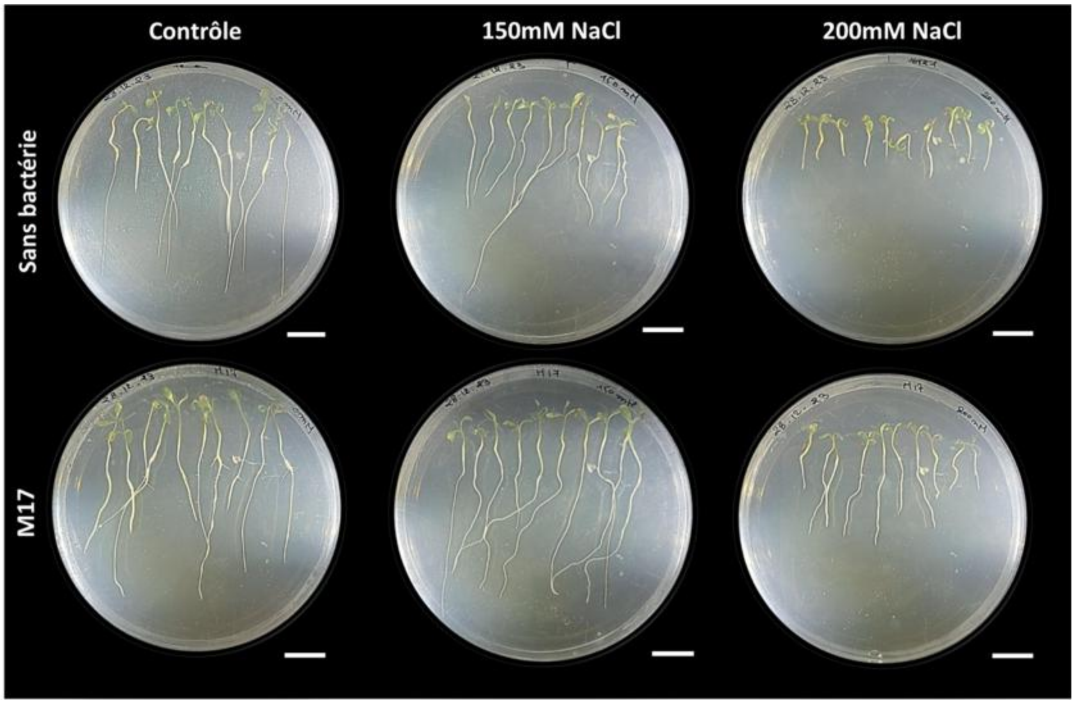


Figure 7. M17 reduces salt stress effect on alfalfa. Alfalfa (M. sativa) plant cultivated *in vitro* in the absence or presence of 150 and 200 mM NaCl, and in the absence (top panel) and presence of M17 (down panel). Scale bars indicate 3 cm.

III. M67.2 displays higher potential for protection Alfalfa than M17.

To go further in the characterization of the impact of the studied strains on Alfalfa response to salt stress, the impact of the addition of 150 and 200 mM of NaCl in the presence or absence of M17 or M67.2 was evaluated.

Interestingly, quantitative analysis reveals that the addition of the strains M17 or M67.2 doesn't impact greatly the salt stress effect at 150 mM, however both strain reduce the impact of the NaCl on Alfalfa growth at 200mM.

These results show that both M17 and M67.2 can reduce the impact of NaCl at 200mM on M. sativa growth.

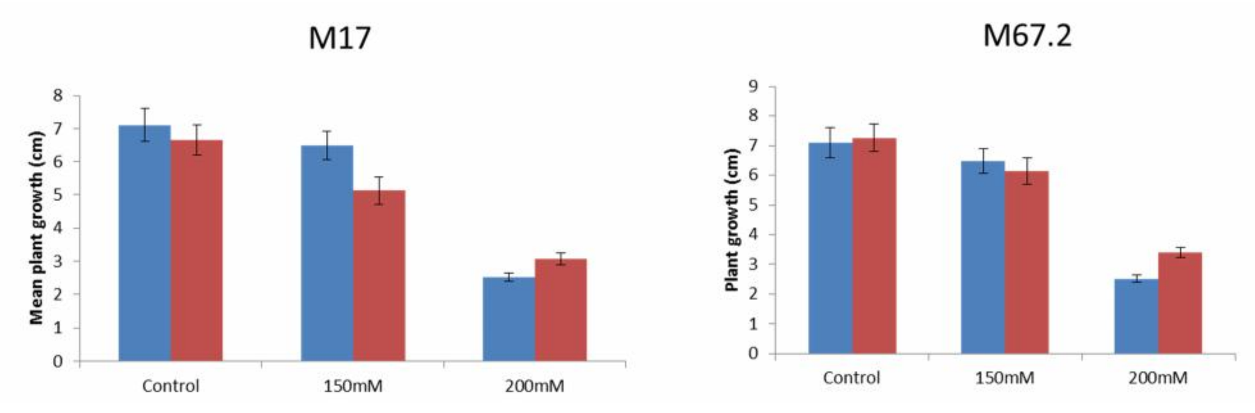


Figure 8. Impact of M17 and M67.2 on Alfalfa in presence or absence of salt stress. Plants cultivated *in vitro* in the absence or presence of 150 and 200 mM NaCl, inoculated or not with M17 or M67.2. Three biological repetitions of 6 plants each were analyzed. The error bars show the standard error (SE).

IV. Genomes of the studied *Pseudomonas* and *Bacillus*.

Molecular identification was previously performed by the lab on the strain M17 and reveals that this strain is *Pseudomonas aeruginosa* and M67.2 is *Bacillus amyloliquefaciens*. The aim of this study was to identify the molecular mechanisms involved in the protection of the plant from salt stress by M17, M67.2 and to identify candidate genes required for the plant bacteria interaction. A comparative analysis of the genomes of five Pseudomonas species was performed, with three Pseudomonas showing plant growth promotion effects: *P. fluorescence F113*, *P. koreensis*

DSM16610, P. atacamensis M7D1, one plant pathogen; *P. syringea DC3000*, and one human pathogen: *P. aeruginosa 138244* and those for Bacillus are three associated with plants : *Bacillus subtilis 168-5* (4,3Mb), *Bacillus velezensis FZB42* (3,9Mb) which is the closest strain to M67.2, *Bacillus siamensis KCTC 13613* (3,9Mb) and *Bacillus amyloliquefaciens DSM 7* (3,9Mb) which is non associated with plants.

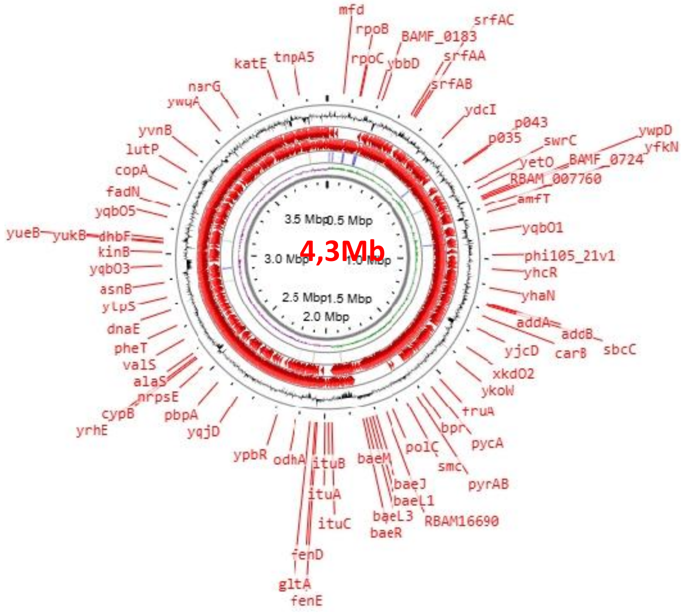
The overview of the genome reveals that the genome of the studied strain shows low sizes variability from 6.03 to 6.8 Mpb (Figure 9), with *P. koreensis DSM16610* and *P. fluorescence F113* showing respectively the lowest and the highest genome size.

P. fluorescence F113 show the highest number of genes with 6340 genes, followed by *P. aeruginosa 138244, P. syringea DC3000, P. koreensis DSM16610* and *P. atacamensis* M7D1showing respectively 6351, 6306, 5616 and 5710 genes.

According to the genome overview; the largest genome of Bacillus (4,3 Mb) is less than the low size of Pseudomonas.

The total number of genes of Bacillus strains are: 4749, 4137, 3876 and 3784 respectively, according to *Bacillus subtilis 168*-5, *Bacillus amyloliquefaciens DSM 7, Bacillus velezensis FZB42* and *Bacillus siamensis KCTC 13613*.

Bacillus amyliliquefaciens DSM 7



Bacillus velezensis FZB42

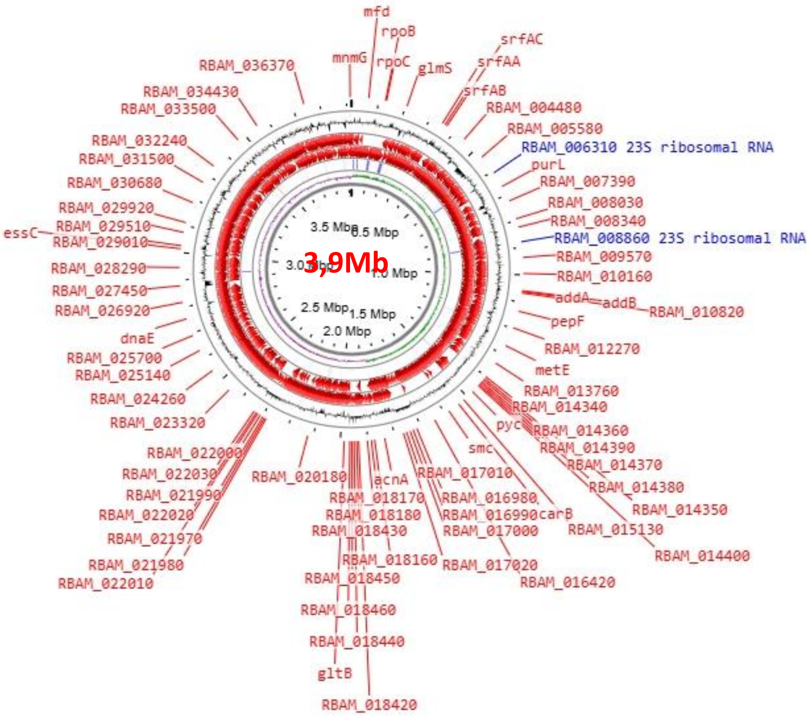


Figure 9. The studied genomes of the closest strain to M67.2 and the largest genome for the comparaison. *Bacillus velezensis FZB42* is the closest strain to M67.2, which is used as a reference

for the study and *Bacillus amyloliquefaciens DSM 7,* is a strain non associated with plants and has the highest genome size, is used for the comparison.

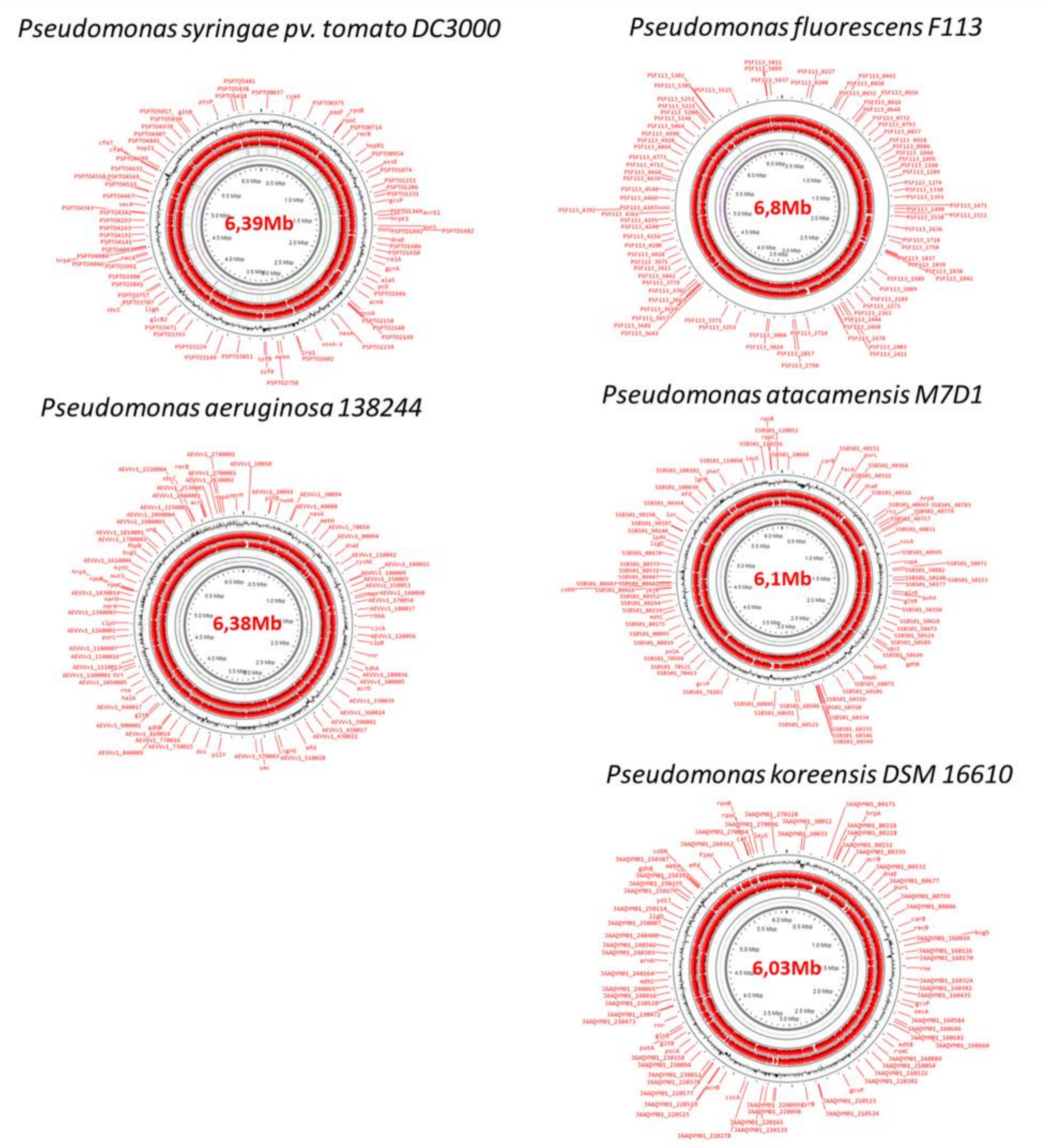


Figure 10. The studied genomes of Pseudomonas strains for the genomic comparison. Circular genome viewers of three Pseudomonas showing plant growth promotion effect: *P. fluorescence*

F113, *P. koreensis DSM16610*, *P. atacamensis M7D1*, one plant pathogen, *P. syringea DC3000*, and one human pathogen: *P. aeruginosa 138244*.

V. Comparative genomic analysis reveals high conservation of genes between studied Pseudomonas strains

In order to determine the Pan/Core genome between *P. fluorescence F113*, *P. koreensis DSM16610*, *P. atacamensis M7D1*, *P. syringea DC3000* and *P. aeruginosa 138244*, a comparative genome analysis was performed on the platform MicroScope (ttps://mage.genoscope.cns.fr/microscope/).

The analysis reveals an important conservation of the gene between the studied strains. *P. koreensis DSM1661*, *P. atacamensis M7D1*, *P. fluorescence F113, P. aeruginosa 138244, and P. syringea DC3000* show respectively, 1208, 1279, 2329, 4263 and 3451 specific genes, by contrast, the genome shared among all strains includes 1427 genes. These results indicate that the core genome remain important compared to the specific genome.

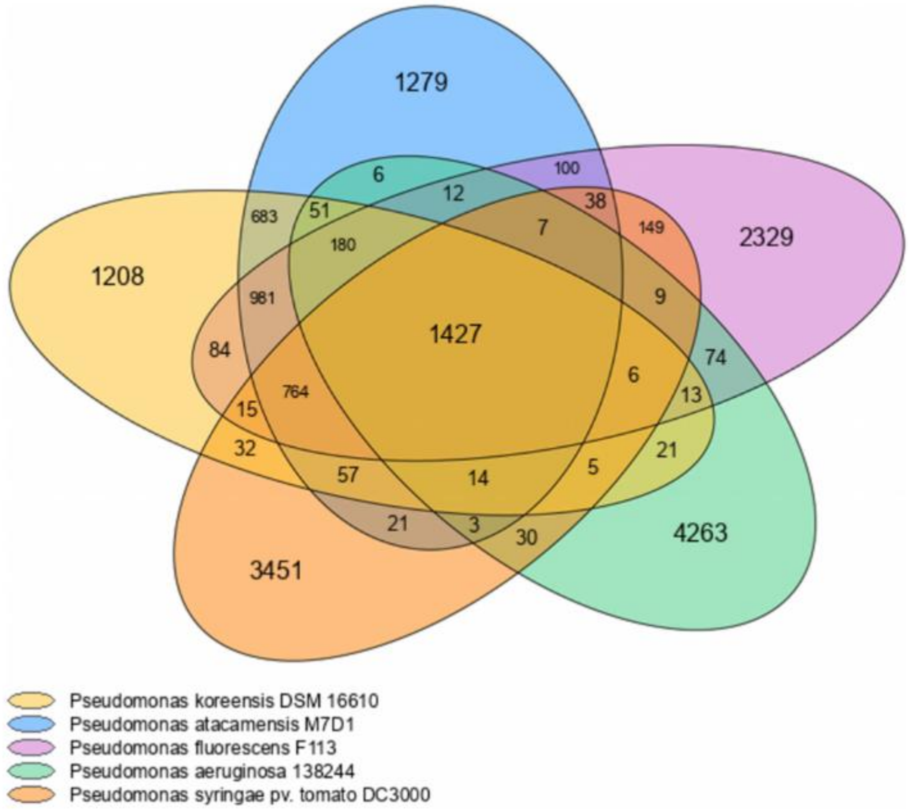


Figure 11. PAN/CORE genome analysis between Pseudomonas strains. The Vann diagram shows the common (CORE) and specific (PAN) genes between *P. koreensis DSM1661*, *P. atacamensis M7D1*, *P. fluorescence F113*, *P. aeruginosa 138244*, *and P. syringea DC3000.*

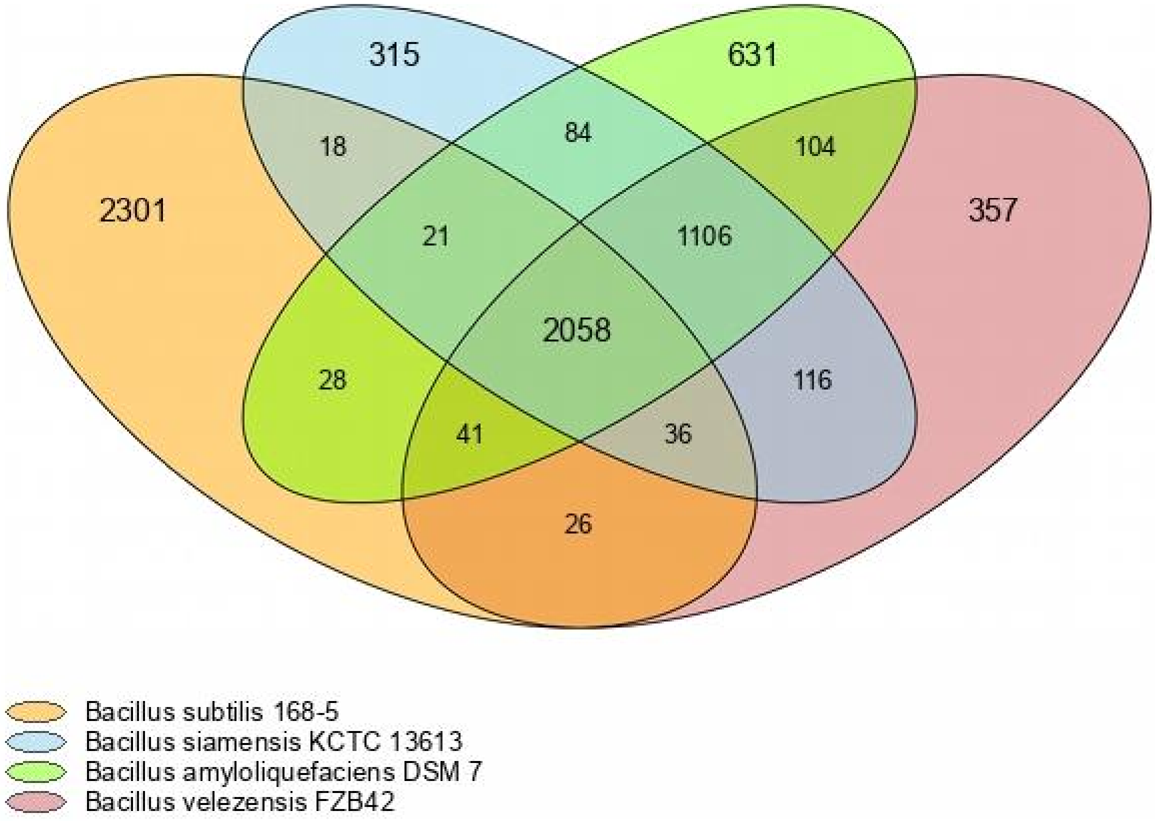


Figure 12. PAN/CORE genome analysis between Bacillus strains. The Vann diagram shows the common (CORE) and specific (PAN) genes between: *Bacillus subtilis 168*-5, *Bacillus velezensis FZB42*, *Bacillus siamensis KCTC 13613* and *Bacillus amyloliquefaciens DSM 7*.

VI. Pseudomonas showing the PGPR effect displays the higher level of genome conservation.

To further characterize the genomic conservation between the studied strains, a synteny analysis was performed using the genome of *P. fluorescence F113*, which shows the largest genome, as a reference. The genome of *P. fluorescence F113* was compared to the genomes of the other strains.

The analysis reveals that *P. fluorescence F113* shows the highest level of conservation of the genome sequence and order with *P. koreensis DSM1661*, *P. atacamensis M7D1* (Figure 10), by contrast, low level of conservation is observed for comparisons with *P. aeruginosa 138244 and P. syringea DC3000.*

The results indicate that the Pseudomonas species displaying the PGPR effect show a high level of genome conservation, when compared to other Pseudomonas species (plant or human pathogens).

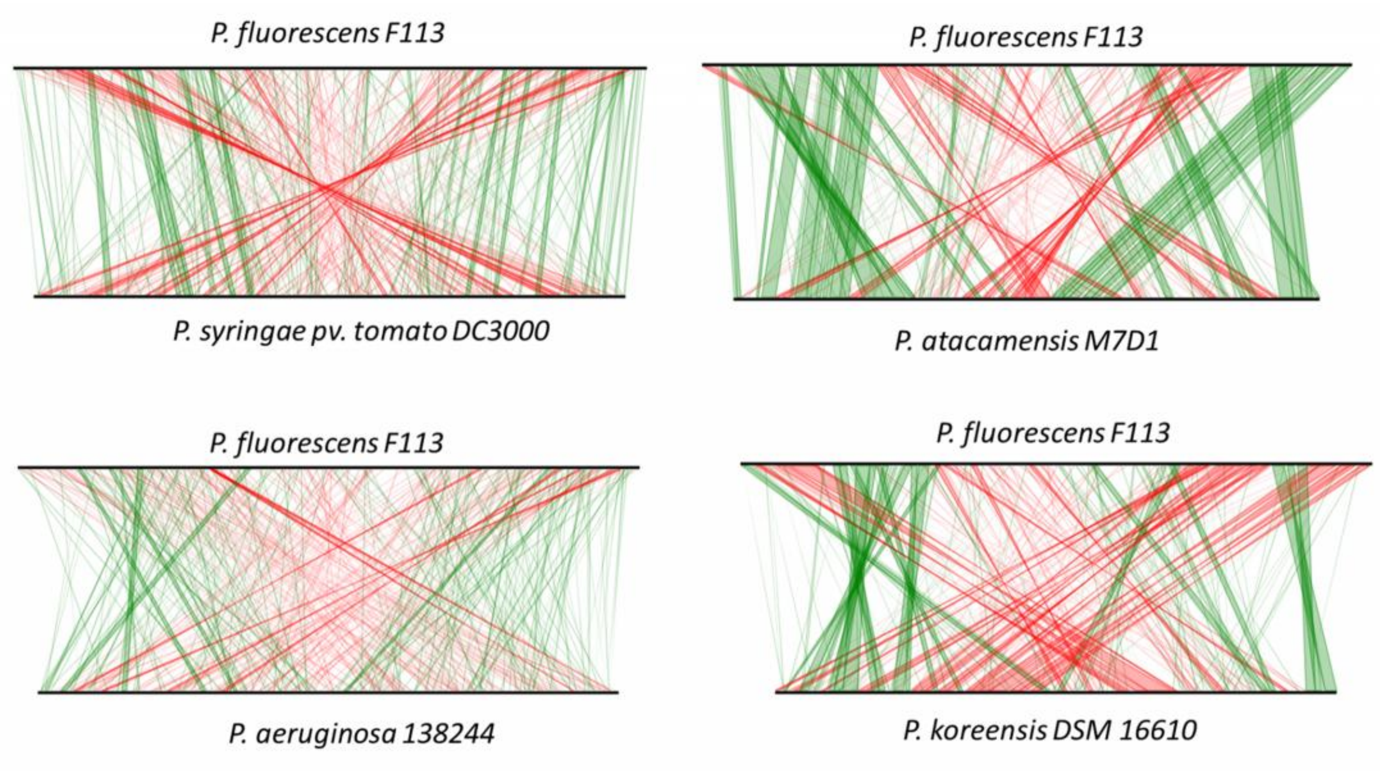


Figure 13. Synteny comparisons between *P. fluorescence F113* and the other Pseudomonas species. Compraison of the genome synteny between *P. fluorescence F113* and *P. koreensis DSM1661, P. atacamensis M7D1, P. aeruginosa 138244, and P. syringea DC3000.* In the red inversion of the gene around the origin of replication, and in green the conservation of the gene order and sequence.

VII. Conserved genes between PGPB are associated with various genes

To identify potential candidate genes involved in the interaction between Pseudomonas strains and the studied plants, the conserved genes between the studied strain and those involved in key aspects of the bacteria's resistance and infection process were identified.

The Table 1 shows the conserved genes associated with bacteria resistance and plant-bacterial infection. We observed a conservation of numerous genes involved in the following processes: resistance to antibiotics, induction of the apoptosis of the host cell, resistance to cooper, production of certain plant hormones like cytokinin, secretion systems and effectors involved in the short-circuit of plant immunity and other enzymes and proteins preventing oxidative damage and detoxification of antimicrobial compounds.

The various genes conserved among the strains indicate the recruitment of complex networks by Pseudomonas strains associated with the plants.

The identified genes can be used for further studies in the M17 strain to validate the genetic network recruited during the interaction with *A. thaliana* or *M. sativa*.

Table 1. Genes potentially involved in the plant-microbe interaction and conserved between studied *Pseudomonas sp.*

Strain	**Genes**
Pseudomonas aeruginosa 138244	Abscisic site processing protein
Pseudomonas atacamensis M7D1	
Pseudomonas koreensis DSM 16610	
Pseudomonas aeruginosa 138244	ABC-type transport system involved in resistance to organic solvents, periplasmic component USSDB6C
Pseudomonas fluorescens F113	
Pseudomonas aeruginosa 138244	ABC-type transport system involved in resistance to organic solvents, permease component USSDB6A
Pseudomonas fluorescens F113	
Pseudomonas fluorescens F113	Acriflavin resistance plasma membrane protein
Pseudomonas fluorescens F113	Acriflavin resistance protein
Pseudomonas fluorescens F113	AmpG protein
Pseudomonas syringae pv. tomato DC3000	
Pseudomonas aeruginosa 138244	Antibiotic biosynthesis monooxygenase
Pseudomonas fluorescens F113	
Pseudomonas atacamensis M7D1	
Pseudomonas fluorescens F113	
Pseudomonas koreensis DSM 16610	
Pseudomonas atacamensis M7D1	
Pseudomonas aeruginosa 138244	
Pseudomonas fluorescens F113	Antibiotic biosynthesis monooxygenase family protein
Pseudomonas koreensis DSM 16610	Antibiotic efflux pump outer membrane protein ArpC
Pseudomonas atacamensis M7D1	
Pseudomonas koreensis DSM 16610	Antitoxin HicB

Pseudomonas aeruginosa 138244	
Pseudomonas atacamensis M7D1	
Pseudomonas koreensis DSM 16610	Antitoxin HigA-1
Pseudomonas atacamensis M7D1	
Pseudomonas atacamensis M7D1	Apoptosis-inducing factor 3
Pseudomonas koreensis DSM 16610	
Pseudomonas atacamensis M7D1	Arsenic resistance transcriptional regulator ArsR2
Pseudomonas koreensis DSM 16610	
Pseudomonas syringae pv. tomato DC3000	Arsenical pump membrane protein
Pseudomonas fluorescens F113	
Pseudomonas aeruginosa 138244	Arsenical-resistance protein Acr3
Pseudomonas atacamensis M7D1	Arsenite/antimonite:H(+) antiporter
Pseudomonas koreensis DSM 16610	
Pseudomonas koreensis DSM 16610	Bleomycin resistance protein
Pseudomonas aeruginosa 138244	
Pseudomonas aeruginosa 138244	Copper resistance protein A
Pseudomonas atacamensis M7D1	
Pseudomonas syringae pv. tomato DC3000	
Pseudomonas atacamensis M7D1	Copper resistance protein B
Pseudomonas fluorescens F113	
Pseudomonas aeruginosa 138244	
Pseudomonas syringae pv. tomato DC3000	
Pseudomonas aeruginosa 138244	Copper resistance protein C
Pseudomonas atacamensis M7D1	
Pseudomonas fluorescens F113	
Pseudomonas koreensis DSM 16610	
Pseudomonas atacamensis M7D1	Copper resistance protein D

Pseudomonas koreensis DSM 16610	
Pseudomonas aeruginosa 138244	
Pseudomonas aeruginosa 138244	Cytokinin riboside 5’-monophosphate phosphoribohydrolase
Pseudomonas atacamensis M7D1	
Pseudomonas koreensis DSM 16610	
Pseudomonas aeruginosa 138244	Drug resistance transporter, EmrB/QacA family
Pseudomonas fluorescens F113	
Pseudomonas syringae pv. tomato DC3000	
Pseudomonas koreensis DSM 16610	
Pseudomonas fluorescens F113	drug/metabolite transporter (DMT) superfamily permease
Pseudomonas fluorescens F113	
Pseudomonas fluorescens F113	drug/metabolite transporter permease
Pseudomonas atacamensis M7D1	
Pseudomonas fluorescens F113	Fosmidomycin resistance protein
Pseudomonas syringae pv. tomato DC3000	
Pseudomonas aeruginosa 138244	Fusaric acid resistance protein
Pseudomonas syringae pv. tomato DC3000	
Pseudomonas fluorescens F113	
Pseudomonas atacamensis M7D1	
Pseudomonas koreensis DSM 16610	
Pseudomonas aeruginosa 138244	Fusaric acid resistance protein FusA
Pseudomonas atacamensis M7D1	
Pseudomonas koreensis DSM 16610	
Pseudomonas fluorescens F113	glyoxalase bleomycin resistance protein dioxygenase
Pseudomonas aeruginosa 138244	
Pseudomonas atacamensis M7D1	glyoxylase I family protein
Pseudomonas koreensis DSM 16610	

Pseudomonas koreensis DSM 16610	Glyoxylase, beta-lactamase superfamily II
Pseudomonas koreensis DSM 16610	
Pseudomonas fluorescens F113	inner membrane component of tripartite multidrug resistance system
Pseudomonas aeruginosa 138244	
Pseudomonas atacamensis M7D1	MarR family transcriptional regulator, organic hydroperoxide resistance regulator
Pseudomonas koreensis DSM 16610	
Pseudomonas aeruginosa 138244	Membrane fusion component of tripartite multidrug resistance system
Pseudomonas fluorescens F113	
Pseudomonas fluorescens F113	Multidrug resistance ABC transporter ATP-binding and permease protein
Pseudomonas aeruginosa 138244	
Pseudomonas syringae pv. tomato DC3000	Multidrug resistance protein
Pseudomonas fluorescens F113	
Pseudomonas aeruginosa 138244	
Pseudomonas syringae pv. tomato DC3000	
Pseudomonas atacamensis M7D1	
Pseudomonas koreensis DSM 16610	
Pseudomonas aeruginosa 138244	Multiple antibiotic resistance protein MarR
Pseudomonas atacamensis M7D1	
Pseudomonas koreensis DSM 16610	
Pseudomonas syringae pv. tomato DC3000	
Pseudomonas aeruginosa 138244	Organic hydroperoxide resistance transcriptional regulator
Pseudomonas atacamensis M7D1	
Pseudomonas fluorescens F113	
Pseudomonas koreensis DSM 16610	
Pseudomonas fluorescens F113	Outer membrane component of tripartite multidrug resistance system
Pseudomonas aeruginosa 138244	

Pseudomonas fluorescens F113	P pilus assembly/Cpx signaling pathway, periplasmic inhibitor/zinc-resistance associated protein
Pseudomonas fluorescens F113	protein VirB4
Pseudomonas fluorescens F113	protein VirD4
Pseudomonas fluorescens F113	protein VirJ
Pseudomonas fluorescens F113	putative arsenic resistance flavin-binding monooxygenase
Pseudomonas aeruginosa 138244	putative bacteriocin resistance protein
Pseudomonas aeruginosa 138244	
Pseudomonas aeruginosa 138244	putative multidrug resistance protein NorM
Pseudomonas atacamensis M7D1	
Pseudomonas koreensis DSM 16610	
Pseudomonas atacamensis M7D1	putative multidrug resistance protein YoeA
Pseudomonas koreensis DSM 16610	
Pseudomonas koreensis DSM 16610	putative quorum-sensing-regulated virulence factor
Pseudomonas koreensis DSM 16610	putative Virulence factors positive transcription regulator BvgA
Pseudomonas aeruginosa 138244	
Pseudomonas atacamensis M7D1	
Pseudomonas koreensis DSM 16610	
Pseudomonas atacamensis M7D1	Quinolone resistance transporter
Pseudomonas koreensis DSM 16610	
Pseudomonas atacamensis M7D1	reactive chlorine species resistance protein C
Pseudomonas atacamensis M7D1	Regulatory protein VirG
Pseudomonas koreensis DSM 16610	Regulatory protein, luxR family
Pseudomonas aeruginosa 138244	Regulatory protein, LysR:LysR, substrate-binding
Pseudomonas fluorescens F113	

Pseudomonas aeruginosa 138244	RND multidrug efflux membrane fusion protein MexE
Pseudomonas fluorescens F113	RND multidrug efflux transporter, Acriflavin resistance protein
Pseudomonas aeruginosa 138244	RND transporter
Pseudomonas aeruginosa 138244	Salicylate biosynthesis isochorismate synthase
Pseudomonas fluorescens F113	Signal transduction response regulator / Disease resistance domain-containing protein
Pseudomonas atacamensis M7D1	small multidrug resistance family-3 protein
Pseudomonas koreensis DSM 16610	
Pseudomonas syringae pv. tomato DC3000	Tabtoxin resistance protein
Pseudomonas fluorescens F113	
Pseudomonas syringae pv. tomato DC3000	TetR-like virulence regulator
Pseudomonas syringae pv. tomato DC3000	type II/III secretion system protein
Pseudomonas aeruginosa 138244	Type II/IV secretion system ATP hydrolase TadA/VirB11/CpaF, TadA subfamily
Pseudomonas syringae pv. tomato DC3000	type II/IV secretion system protein
Pseudomonas aeruginosa 138244	Type IIA topoisomerase (DNA gyrase/topo II, topoisomerase IV), A subunit
Pseudomonas fluorescens F113	
Pseudomonas fluorescens F113	type III effector 1
Pseudomonas atacamensis M7D1	
Pseudomonas fluorescens F113	Type III effector HopPmaJ
Pseudomonas aeruginosa 138244	
Pseudomonas atacamensis M7D1	Type III pantothenate kinase
Pseudomonas koreensis DSM 16610	
Pseudomonas syringae pv. tomato DC3000	Type III secretion ATP synthase HrcN

Pseudomonas atacamensis M7D1	type IV fimbrial biogenesis protein FimT
Pseudomonas koreensis DSM 16610	
Pseudomonas fluorescens F113	type IV pilin
Pseudomonas fluorescens F113	type iv pilus assembly
Pseudomonas atacamensis M7D1	Type IV pilus assembly protein PilC
Pseudomonas koreensis DSM 16610	
Pseudomonas aeruginosa 138244	
Pseudomonas atacamensis M7D1	type IV pilus assembly protein PilE
Pseudomonas koreensis DSM 16610	
Pseudomonas aeruginosa 138244	Type IV pilus assembly protein PilF
Pseudomonas atacamensis M7D1	
Pseudomonas koreensis DSM 16610	
Pseudomonas atacamensis M7D1	type IV pilus assembly protein PilV
Pseudomonas koreensis DSM 16610	
Pseudomonas atacamensis M7D1	type IV pilus assembly protein PilW
Pseudomonas koreensis DSM 16610	
Pseudomonas atacamensis M7D1	type IV pilus assembly protein PilX
Pseudomonas koreensis DSM 16610	
Pseudomonas atacamensis M7D1	type IV pilus assembly protein PilY1
Pseudomonas koreensis DSM 16610	
Pseudomonas aeruginosa 138244	Type IV pilus ATPase PilU
Pseudomonas atacamensis M7D1	
Pseudomonas koreensis DSM 16610	
Pseudomonas aeruginosa 138244	Type IV pilus biogenesis factor PilY1
Pseudomonas atacamensis M7D1	Type IV pilus inner membrane component PilN
Pseudomonas koreensis DSM 16610	
Pseudomonas aeruginosa 138244	

Pseudomonas aeruginosa 138244	Type IV pilus inner membrane component PilO
Pseudomonas atacamensis M7D1	
Pseudomonas koreensis DSM 16610	
Pseudomonas atacamensis M7D1	Type IV pilus inner membrane component PilP
Pseudomonas koreensis DSM 16610	
Pseudomonas aeruginosa 138244	
Pseudomonas aeruginosa 138244	Type IV pilus non-core minor pilin PilE
Pseudomonas atacamensis M7D1	Type IV secretion protein Rhs
Pseudomonas koreensis DSM 16610	Type IV secretory pathway, VirJ component
Pseudomonas aeruginosa 138244	
Pseudomonas atacamensis M7D1	type VI secretion system protein ImpI
Pseudomonas koreensis DSM 16610	
Pseudomonas atacamensis M7D1	type VI secretion system protein ImpK
Pseudomonas koreensis DSM 16610	
Pseudomonas atacamensis M7D1	type VI secretion system protein ImpL
Pseudomonas koreensis DSM 16610	
Pseudomonas atacamensis M7D1	type VI secretion system protein VasD
Pseudomonas koreensis DSM 16610	
Pseudomonas atacamensis M7D1	type VI secretion system protein VasJ
Pseudomonas koreensis DSM 16610	
Pseudomonas koreensis DSM 16610	type VI secretion system secreted protein VgrG
Pseudomonas atacamensis M7D1	
Pseudomonas koreensis DSM 16610	Type VI secretion system spike protein VgrG4b
Pseudomonas atacamensis M7D1	
Pseudomonas koreensis DSM 16610	
Pseudomonas aeruginosa 138244	Type VI secretion system spike protein VgrG5
Pseudomonas aeruginosa 138244	Type VI secretion-associated protein

Pseudomonas fluorescens F113	virulence gene repressor
Pseudomonas atacamensis M7D1	Virulence plasmid 28 protein
Pseudomonas atacamensis M7D1	Virulence sensor protein BvgS
Pseudomonas koreensis DSM 16610	
Pseudomonas aeruginosa 138244	
Pseudomonas fluorescens F113	virulence-associated e family protein
Pseudomonas syringae pv. tomato DC3000	
Pseudomonas aeruginosa 138244	choline dehydrogenase
Pseudomonas atacamensis M7D1	
Pseudomonas fluorescens F113	
Pseudomonas koreensis DSM 16610	
Pseudomonas syringae pv. tomato DC3000	
Pseudomonas aeruginosa 138244	osmotically inducible peroxiredoxin OsmC
Pseudomonas atacamensis M7D1	
Pseudomonas fluorescens F113	
Pseudomonas koreensis DSM 16610	
Pseudomonas syringae pv. tomato DC3000	
Pseudomonas aeruginosa 138244	Glutathione reductase
Pseudomonas atacamensis M7D1	
Pseudomonas fluorescens F113	
Pseudomonas koreensis DSM 16610 *Pseudomonas syringae pv. tomato DC3000*	
Pseudomonas aeruginosa 138244 *Pseudomonas atacamensis M7D1* *Pseudomonas fluorescens F113* *Pseudomonas koreensis DSM 16610* *Pseudomonas syringae pv. tomato DC3000*	Organic hydroperoxide resistance protein

Table 2. Common genes between Pseudomonas and Bacillus strains that are potentially involved in the protection of plants from salt stress and other stresses.

Strains	Common genes
Pseudomonas aeruginosa 138244 *Bacillus amyloliquefaciens DSM 7* *Bacillus siamensis KCTC 13613* *Bacillus subtilis 168-5* *Bacillus velezensis FZB42*	Stress protein, member of the CspA family (cspC) Cold-shock protein (cspC)
Pseudomonas aeruginosa 138244 *Pseudomonas atacamensis M7D1* *Pseudomonas fluorescens F113* *Pseudomonas koreensis DSM 16610* *Pseudomonas syringae pv. tomato DC3000* *Bacillus velezensis FZB42*	Betaine aldehyde dehydrogenase (betB)
Bacillus siamensis KCTC 13613 *Bacillus subtilis 168-5* *Pseudomonas aeruginosa 138244* *Pseudomonas atacamensis M7D1* *Pseudomonas fluorescens F113* *Pseudomonas koreensis DSM 16610*	Proline permease (putP) Sodium/proline symporter PutP Proline: Na (+) symporter
Pseudomonas atacamensis M7D1 *Pseudomonas fluorescens F113* *Pseudomonas koreensis DSM 16610* *Pseudomonas syringae pv. tomato DC3000*	Glutathione peroxidase

Bacillus velezensis FZB42	Glutathione peroxidase
Pseudomonas aeruginosa 138244 *Pseudomonas atacamensis M7D1* *Pseudomonas fluorescens F113* *Pseudomonas koreensis DSM 16610* *Bacillus siamensis KCTC 13613* *Bacillus subtilis 168-5* *Bacillus velezensis FZB42*	Proline:Na(+) symporter Protein PutP Proline permease Sodium/proline symporter PutP
Bacillus amyloliquefaciens DSM 7 *Bacillus siamensis KCTC 13613* *Bacillus subtilis 168-5* *Pseudomonas fluorescens F113*	Glycine betaine/carnitine/choline transport ATP-binding protein opuBA Choline ABC transporter (ATP-binding protein) Protein OpuBA
Pseudomonas syringae pv. tomato DC3000 *Bacillus amyloliquefaciens DSM 7* *Bacillus siamensis KCTC 13613* *Bacillus subtilis 168-5*	Glycine betaine/choline ABC transporter ATP-binding protein (opuCA)
Bacillus siamensis KCTC 13613 *Bacillus subtilis 168-5* *Pseudomonas aeruginosa 138244*	Peroxiredoxin with versatile activity Peroxiredoxin Bcp

Pseudomonas koreensis DSM 16610 *Pseudomonas atacamensis M7D1* *Pseudomonas fluorescens F113* *Bacillus subtilis 168-5* *Bacillus velezensis FZB42* *Bacillus amyloliquefaciens DSM 7*	Thiol peroxidase (*bcp*) Protein Bcp Thiol peroxidase (*tpx*) (lipid hydroperoxide reductase) Thiol peroxidase
Pseudomonas aeruginosa 138244 *Pseudomonas atacamensis M7D1* *Pseudomonas koreensis DSM 16610* *Bacillus amyloliquefaciens DSM 7* *Bacillus siamensis KCTC 13613* *Bacillus subtilis 168-5*	Glycine betaine transport system permease protein OpuAB Glycine betaine ABC transporter permease (opuAB) L-proline betaine and betonicine ABC transporter (permease) (opuAB)
Pseudomonas fluorescens F113 *Bacillus velezensis FZB42*	Na(+)/H(+) antiporter Na(+)/H(+) antiporter subunit B
Pseudomonas fluorescens F113 *Bacillus amyloliquefaciens DSM 7*	Oxidoreductase
Pseudomonas aeruginosa 138244 *Pseudomonas atacamensis M7D1* *Pseudomonas koreensis DSM 16610*	Glycine decarboxylase

Pseudomonas syringae pv. tomato DC3000 *Bacillus siamensis KCTC 13613* *Bacillus subtilis 168-5*	Glycine dehydrogenase [decarboxylating] Fragment of glycine decarboxylase (part 1)
Bacillus amyloliquefaciens DSM 7 *Bacillus siamensis KCTC 13613* *Bacillus subtilis 168-5* *Pseudomonas aeruginosa 138244* *Pseudomonas atacamensis M7D1* *Pseudomonas koreensis DSM 16610* *Pseudomonas syringae pv. tomato DC3000* *Pseudomonas fluorescens F113*	Choline dehydrogenase (gbsB) Choline dehydrogenase (betA) Protein BetA
Pseudomonas aeruginosa 138244 *Pseudomonas atacamensis M7D1* *Pseudomonas fluorescens F113* *Pseudomonas koreensis DSM 16610* *Pseudomonas syringae pv. tomato DC3000* *Bacillus siamensis KCTC 13613* *Bacillus velezensis FZB42* *Bacillus amyloliquefaciens DSM 7* *Bacillus siamensis KCTC 13613* *Bacillus subtilis 168-5*	Catalase (katB) Catalase Vegetative catalase 1 (katA) Catalase 2 (katE)

Bacillus amyloliquefaciens DSM 7 *Bacillus siamensis KCTC 13613* *Bacillus subtilis 168-5*	Catalase 2 (katE)

IX. Protein-protein interaction network

We aim to mention how some potential genes probably contribute in assuring salt resistance to plants. The STRING database is used to illustrate this network, where the chosen strains are *Pseudomonas fluorescens* and *Pseudomonas koreensis* for M17, and *Bacillus subtilis 168*, *Bacillus amyloliquefaciens DSM 7* and *Bacillus velezensis* for M67.2. The letters (**a**, **b**, **c**, **d**, **e**) in figure present prediction of protein-protein interaction for the genes *cat*, *opuAB*, *betB*, *betB_2*, and *bcp* respectively where the letters (**f**, **g**, **h**, **i**, **j**, **k**, **l**) present these genes respectively *betB*, *opuAB*, *katA*, *tpx*, *tpx*, *gbsB.*

a. *cat* gene is encoded to catalase protein which has interactions with *sodB_1* encoded to superoxide dismutase [Mn/Fe] and *sodB_2* encoded to superoxide dismutase (Fe) additionally to *tsaA_2* encoded to putative peroxiredoxin.

b. *opuAB* gene encoded to glycine betaine transport system permease protein has interaction with *opuAA* which is glycine betaine transport ATP-binding protein, *opuAC_1* (glycine betaine-binding protein OpuAC precursor) additionally to *proV* encoded to glycine betaine/proline transport system ATP-binding protein and *osmF* which encodes to Putative osmoprotectant uptake system substrate-binding protein OsmF precursor.

c. *betB* in *Pseudomonas koreensis* is encoded to betaine-aldehyde dehydrogenase which is responsible of catalyzing the reversible oxidation of betaine aldehyde in the biosynthesis of the osmoprotectant glycine betaine. It has interactions with *betA*, *betI* that encode to choline dehydrogenase and DNA-binding transcriptional regulator BetI respectively. *gltB* is encoded to glutamate synthase large subunit.

d. *betB_2* in *Pseudomonas fluorescens* is encoded to NAD/NADP-dependent betaine aldehyde dehydrogenase catalyzing the reversible oxidation of betaine aldehyde which is implicated in osmoprotectant glycine betaine biosynthesis. It has network with *fmt* (Methionyl-tRNA formyltransferase), *putA* (bifunctional protein PutA) which is responsible on proline oxidation on glutamate to use it as carbon nitrogen source and finally, *betT_1* encoded to high-affinity choline transport protein.

e. *bcp* in *Pseudomonas fluorescens* presents peroxiredoxin has interactions with *trxA_1* which is thioredoxin, additionally to *resA*_1 and *ykuV* presenting thiol-disulfide oxidoreductase ResA and thiol-disulfide oxidoreductase YkuV respectively. Finally, interaction with *tsaA_3* (putative peroxiredoxin) and *rubA* (rubredoxin-1).

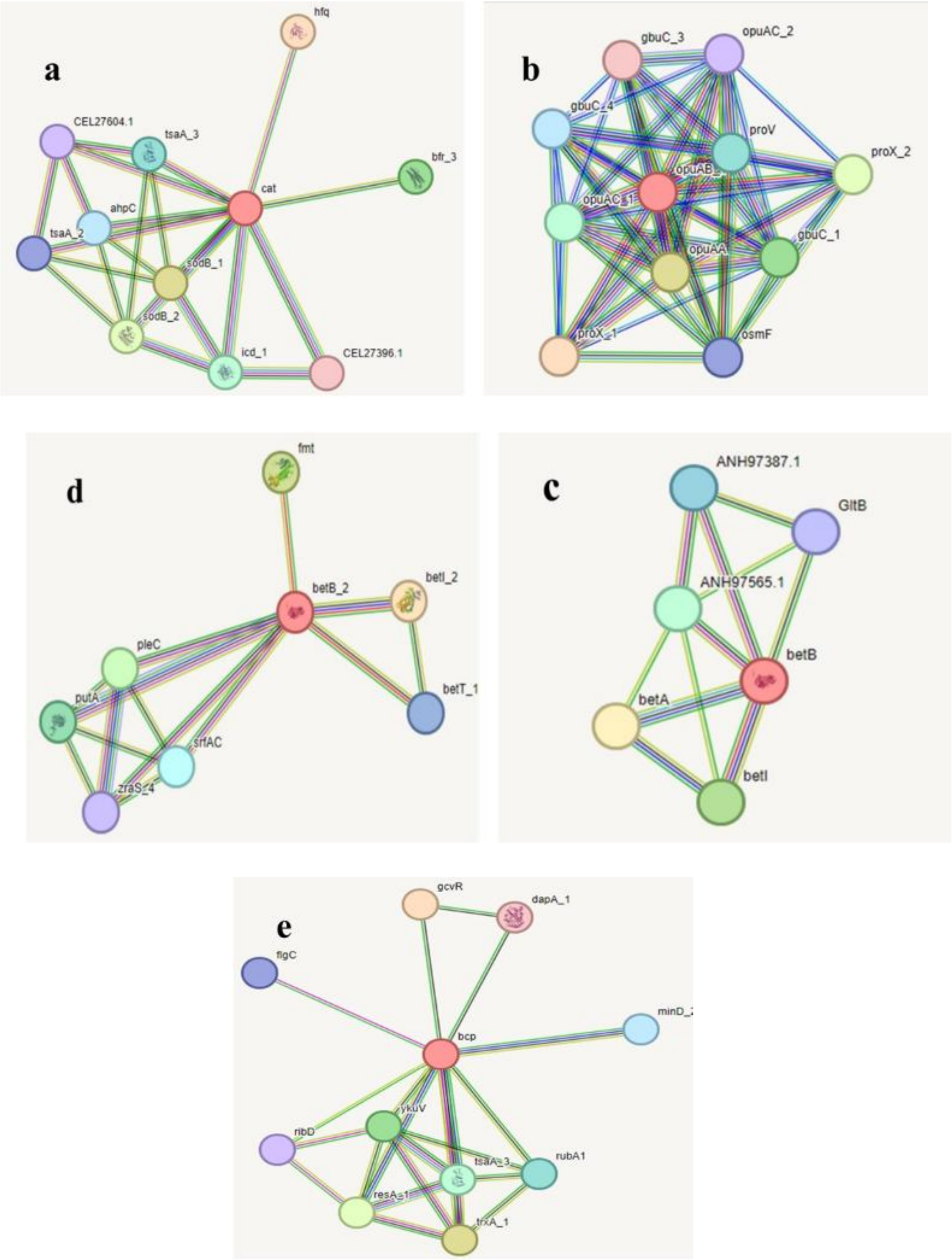


Figure 14. Network of candidate genes in enhancing salt resistance in plant inoculated with M17. a. presents the network of *cat* gene encoded to catalase protein in *Pseudomonas fluorescens*, **b.** presents *opuAB* gene network encoded to glycine betaine transport system permease protein OpuAB in *Pseudomonas fluorescens*, **c.** present *betB* network in *Pseudomonas koreensis* encoded to betaine aldehyde dehydrogenase, **d.** refers to *betB_2* gene network in *Pseudomonas fluorescens* presenting NAD/NADP-dependent betaine aldehyde dehydrogenase.

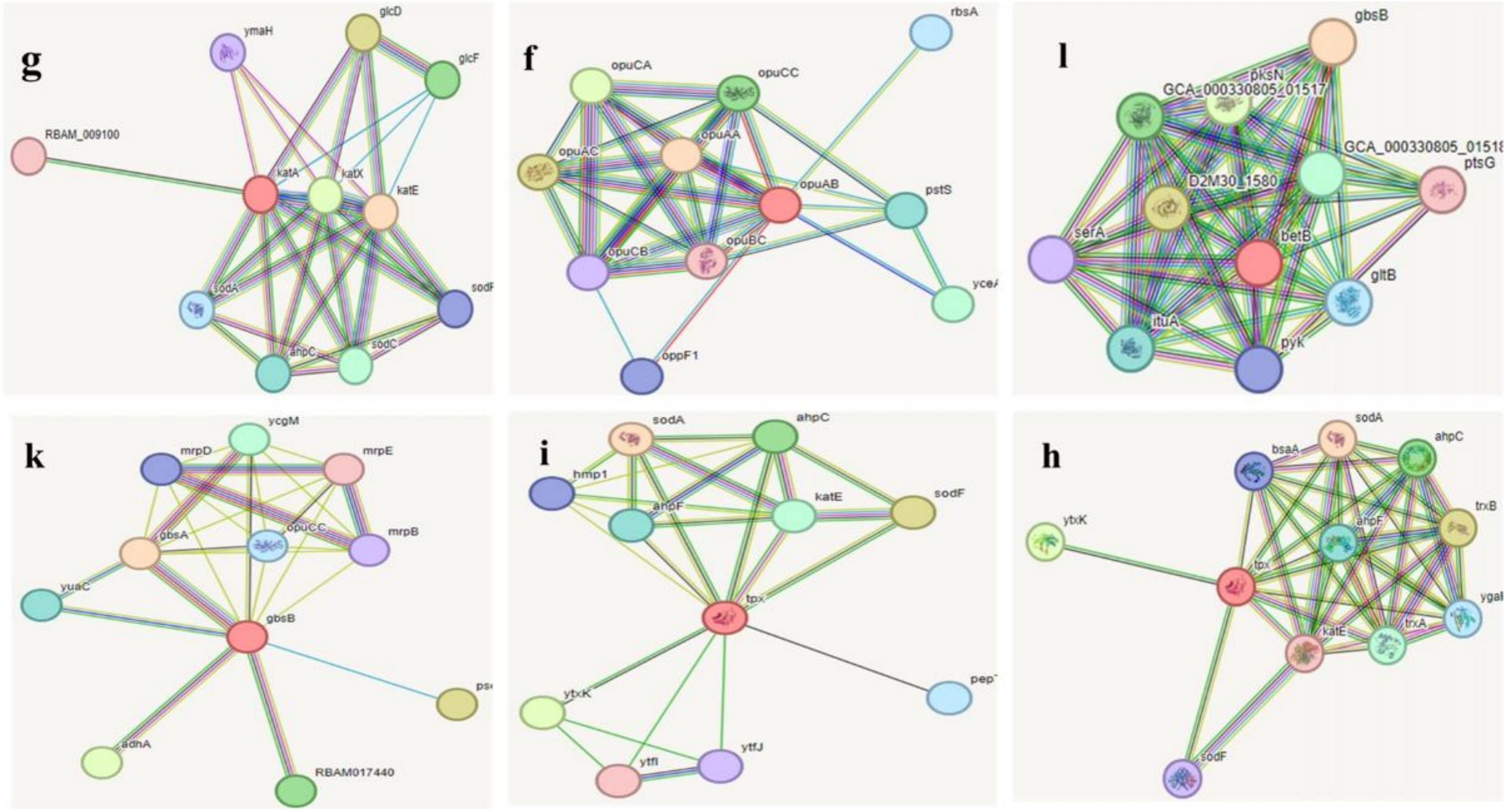


Figure 15. Protein-protein interactions of genes of Bacillus strains that are common with those of Pseudomonas strains. l. presents *betB* network (in red) in *Bacillus amyloliquefaciens*, **f.** presents *opuAB* network (in red), **g.** is for *katA* interaction in *Bacillus amyloliquefaciens DSM 7*, **h.** reveals to *tpx* interaction in *Bacillus subtilis 168***, i.** presents *tpx* in *Bacillus velezensis*, **k.** reveals to *gbsB* presenting by *Bacillus amyloliquefaciens DSM 7*.

Part IV. Discussion

It is amazing that living beings have the ability to communicate and transform this communication to complex symbiosis even they are from different kingdoms. Plants can have a myriad of interactions at the same time in the same space. One of their symbionts is the endophytic bacteria. They are considered as microorganisms live within plant's tissues without causing any form of danger to plant's life. Soil salinization is a serious challenge threating crops production especially with the whole number of our populations. Scientists are taking care of using eco-friendly solutions where growth promoting plant microbes present an excellent choice with cost effectiveness. Myriads of studies on identifying new strains with effective mechanisms are illustrated [90]. We aim to study the response of plants (Arabidopsis and Alfalfa) to salt stress in the presence of endophytic bacteria and characterizing their mechanisms to alleviate stress through gene identification.

We decided to investigate this study about the importance of endophytic bacteria in alleviating salt stress in both plants models, *Arabidopsis thaliana* and *Medicago sativa L.* (*alfalfa*). Firstly, we used five bacteria already known by their ability to colonize plant's roots. Variable potential in alleviating salt stress is observed in different NaCl concentrations comparing with control. Two endophytic bacteria (M17 and M67.2) showed high potential in protecting plant from salt stress. They belong to Pseudomonas (Gram -) and Bacillus (Gram +) genera respectively. Similar results are observed in other studies showing significant promoting growth under 100mM NaCl during early Arabidopsis seedling development due to the positive effects of inoculated bacteria [90]. M67.2 showed similar promoting plant in both stress and non-stress condition, but significant promotes when NaCl concentration up to 200mM in Alfalfa which can be explained by the effect is depending on stress conditions and plant host. While M17 showed positive promoting in both plants host in different NaCl concentration. Many studies reveal that under salt conditions, PGPRs control stress through several ways. In order to understand how our bacteria strains promote Arabidopsis and Alfalfa under salt stress and the other stresses associated with (osmotic, ionic and oxidative stress). We decided to perform genomic analyses starting by comparative genomic to each genus. We used for M17, three bacteria associated with plant, one is not and one Human pathogen to select more specific genes related to the phenomenon observed. We found 1427 common genes (core-genome). The same study is performed to M67.2, we choose three bacteria associated with plant, one of them is the closet strain to M67.2 (*Bacillus velezensis FZB42*) and one non-associated. The core-genome in Venn diagram shows 2058 common genes. However, we aim to identify conserved genes that are potentially associated with core functionalities during the evolutionary relationships between these strains. So, we perform synteny analyses (figure). Instead of a high degree of synteny is revealed which is depended on species (figure). Correlating synteny results (figure) with phenotypic data (figure) can provide insights into the genetic basis in alleviating stress. According to the Table 2 we suggest that Pseudomonas strains and Bacillus ones, are sharing functional similitaries, so we selected seven genes (*cat*, *betB*, *opuAB*, *katA*, *tpx*, *bcp*, *gbsB*) to study their mechanisms implicated in the protection.

Oxidative stress is a harmful face of salt stress, where its biomarkers such as ROS, causing great damage to plant components such as DNA mutations and proteins denaturation. Antioxidants accumulation in different plant species inoculated with bacteria indicates that is an important common response [91], [92]. Many genes encoded to antioxidants are found in Pseudomonas and Bacillus strains (Table2) .*cat* is one of these genes encoded to catalase, an enzymatic antioxidant, its content was increased by 19,8% at 200mM [90]. Notably, the protein-protein interaction showed its network with other antioxidants for instance, superoxide dismutase [Mn/Fe], superoxide dismutase (Fe) (SOD) and peroxiredoxin (Prxs) (figure). Additionally, to other genes linked to antioxidant biosynthesis such as *bcp*, *katE*, *katB*, *katA*, and *tpx* which encoded respectively to thiol peroxidase, catalase 2, catalase, vegetative catalase 1 and thiol peroxidase [93], [94],[85],[95], [96],[97].Thus, the presence of these genes can explain in a part reducing negative effect of this kind of stress.

Drought stress also share this face of stress. A recent study on the effects of *Bacillus amyloliquefaciens QST713* on antioxidants and photosynthesis production of Alfalfa under drought stress showed decrease in ROS content with increasing in the activities of peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT), and superoxide dismutase (SOD) [98]. Similar result is obtained in tomato inoculated with *Pseudomonas aeruginosa HG28-5* [99]. However, proline is a famous amino-acid playing a role as compatible osmo-protectant (osmolyte) where its content only increases after inducing salt stress and can rise the plant growth by 89% using Pseudomonas strain at 200mM [90],[100]. In addition, *Bacillus amyloliquefaciens SQR9* can succefully elevate glutathione content in Arabidopsis through increasing expression of glutamine synthase and glutathione reductase genes. Moreover, we found *gor*, *gor-1*, *gor-2* genes encoded to glutathione reductase as well as *ghsB* encoded to glutathione synthase [101]. This provides a plausible explanation for positive Pseudomonas effect besides Bacillus. Furthermore, glycine betaine (GB) is also an effective osmolytes reducing water loss[92]. Our Bacillus strains present *opuAC* and *opuD* genes encoded to glycine betaine suggesting that is potentially involved in reducing salt negative effect which confirmed by other studies[102] . The difficult uptake of nutrients by root plant is a frequently observed effect of increased salinity, in ionic and osmotic stress forms. Many essential ions for growth such as phosphate, potassium and iron are not absorbed because of the accumulation of Na+ and Cl- ions leading to disrupting Na^+/K^+ homeostasis [103] ,[99] [60] ,[62]

This problem can be controlled by PGPRs, which raise K+ and decrease Na+ to maintain homeostatic equilibrium [103], [104] . PGPRs (endophytic bacteria) remarkably, induce changes in plant to become more adaptable to stress conditions. SOS pathway, is a mechanism used by plant to maintain ion homeostasis, notably the plant inoculated with PGPRs demonstrated high expression of *SOS* genes that have interwork with ions transporter like Na^+/H^+ antiporters which are essential to control ionic stress[105][94]. They are founded in our Pseudomonas and Bacillus strains mentioned in the Table2, so we suggest that is in other ways explain their mechanisms of protection. Correspondingly, endophytic bacteria facilitate access to nutrient particularly, phosphate, potassium, and nitrogen through solubilization as well as production of siderophores for iron chelation [106],[107][108]. Moreover, we found genes involved in siderophores biosynthesis in Pseudomonas which are pyoverdines and pyochelin corresponded to *pvdH*, *pvdM*, *pvdS* and *pqqB*, *pqq*, *pqq* respectively.

Interestingly to notice that we found in Pseudomonas *pqq* genes encoded to pyrroloquinoline quinone and I suggest that might be play a vital role in alleviating salt stress due to its properties as an antioxidant and vitamin-like-accessory factor involved in biological processes as growth and reproduction [109], [110].

The potential growth of both Arabidopsis and Alfalfa can be directly related by regulating phytohormones by endophytes, for exemple in Pseudomonas we found genes related to cytokinin productions (Table1). Concomitantly, ABA is a mandatory hormone regulating plant metabolism. Under stress its content increases as a response and it's revealed as well as other phytohormones such as auxin, ethylene and jasmonic acid. In line with the expectation, multiple studies investigated the upregulation of these phytohormones in plant inoculated with endophytic bacteria such as *Pseudomonas aeruginosa HG28-5*, *Bacillus G7*, *Bacillus amyloliquefaciens FZB42*, *Bacillus amyloliquefaciens SN13, Pseudomonas putida MTCC 5279* [99], [86], [111], [112], [113], [114].

Strikingly, volatile organic compounds can orchestrate plant to elevate salt tolerance. We found that our chosen bacterium to represent M67.2 has genes responsible for VOCs biosynthesis which a study provides evidence for it. Arabidopsis salt tolerance is improved through VOCs secreted by *Bacillus amyloliquefaciens FZB42*. Activating anti-oxidation system, limiting sodium intake, triggered the transcriptional activation of *NHX1, HKT1, high-affinity* K^+ *transporter 1*and Na^+/H^+ *exchanger 1* in Arabidopsis. Subsequently, modulating the jasmonic acid signaling cascade is crucial to conferring salt tolerance [85], [86].

Conclusion

In conclusion, this study sought to explore the potential of endophytic bacteria as a natural approach to elevate plant salt resilience. Passing by inoculation and stress induced processes, we explore the feasibility of these endophytic bacteria as a sustainable eco-friendly solution to mitigate of salt stress damage. Our findings revealed that two strains (M17 and M67.2) from five endophytic bacteria that are used, have a significant positive effect on reducing salt negative influence in both of Arabidopsis and Alfalfa. To elucidate the mechanism underlying this stress tolerance, we employed genomic analyses. Our initial step involved in confirming identities to of M17 and M67.2 which are already identified. According to their classification, they belonged to the Bacillus and Pseudomonas genera, respectively. Furthermore, comparative genomic is performed to get the core-genome of each genus. Several genes are having functional similarities between Pseudomonas and Bacillus. Seven genes encoded to proteins potentially involved in the mechanism were selected to perform protein-protein interaction encompass antioxidants, phytohormones, enzymes and other stress-related functionalities as confirmed by numerous studies. While a complete understanding of the intricate mechanisms by which endophyte-mediated salt tolerance remains elusive research. This study, pave the way for future investigation. Overall, our findings underscore the promising role of endophytic bacteria in enhancing salt resistance in plant. By harnessing this natural symbiosis, we can develop sustainable eco-friendly solutions in agriculture to combat soil salinization negative effects.

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