Survival of Root Nodule Bacteria Under Salinization Conditions

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Abstract: Agricultural production is now diminishing because of the detrimental climate change impacts, global temperature and rising environmental tension. Therefore, it seems important to use suitable solutions and ecologically compatible and environmentally beneficial technology to achieve sustainable agricultural production and increase farm goods for feeding all people in the world to minimize the adverse effects of such stresses on plants. Soil salinity is a worldwide concern in terms of crop production. According to the studies, osmotic stress, poor physical conditions of soil, nutritional disorders and toxicity and reduced crop yields on crops that grow in salt-affected soils. Limiting crop losses caused by salinity stress is a significant environment in which food demand increases. In order to increase food production in soles affected by salt, new agricultural technologies are required. Plant-based beneficial halotolerant Rhizosphere bacteria have been known to enhance plant tolerance to salinity through mechanics such as root systems growth, soil structure improve, water and nutrient absorption increase, sodium absorption decreases, stress ethylene decreases negative effects and genes expression associated with salinity stress resistance. A more economic-efficient environment-friendly alternative, which could be possible in less time, could require microbial inoculation in order to reduce tension. These inoculants contribute to the production of salinity-stressed conditions of sustainable agriculture.

Keywords: Halotolerant PGPR · Salinity stresses, Sinorhizobium meliloti, Microbe interactions, Saline soilbased agriculture.

Introduction:

Food protection is one of the fundamental needs of a population and cannot be overlooked by any organization. In 2030 the overall demands for agricultural goods are 60% higher than they are at present, says the FAO (Food and Agriculture Organization). In order to supply increasing food demand for more than half a century, the world relies on improved crop returns[1]. As the population growth is increasing, which according to UN projections, will reach 8.9 billion by 2050[2]. The fertility rate in agricultural soils is diminishing, the proportion of suitable farmland to populations is steadily decreasing. Therefore, one of the key requirements is to increase crop yield per unit area of soil cultivation, particularly under salt conditions. The soil of 4 dS/m (approximately 40 mM NaCl at 25oC) with the electric conductivity (EC) and 15 percent sodium exchangeable of saturation extracts (ECe) shall be saline soil. Most cultivated plant yields in this soil are decreased, although many cultivated plants showed a lower yield. Various meanings for salinity have been made. The concentration of salt in water and soil solution, which results in the accumulation of salt in the root region, is too high, according to Shannon & Grieve (1998). The plant is struggling in the absorption of adequate soil water. Another definition of salinity refers to an excessive anion and cation accumulation, which causes plant growth [3].

Soil salinization is a great challenge to agriculture. At present, about 40% of lands, including irrigated lands, contain elevated concentrations of mineral salts. Use of leguminous crops, forming symbioses with root-nodule bacteria (rhizobia), is a promising method of soil improvement. They are stress-resistant and capable of improving soils by fixation of atmospheric nitrogen. Cultivated alfalfa (Medicago sativa L.) is a candidate for bioremediation. It is highly resistant to drought and frost and moderately tolerant to salinity.

Root nodule bacteria S. meliloti form a nitrogen-fixing symbiosis with the genus Medici plants, which includes 83 species and 18 interspecies forms of alfalfa plants differing significantly in their response to drought, cold, and soil acidity [4]. For example, M. varia grows on slightly saline soils of the arid climate (Kazakhstan). In contrast, black medic (M. lupulina) or sickle alfalfa (M. falcata) grow on acidic soils of humid temperate continental (Northwestern region of the Russian Federation) or marine temperate, arctic types of climate (Murmansk, Arkhangelsk region), respectively [5]. At the same time, new species and biovars of root nodule bacteria have been recently revealed (owing to the interest in rare forms of alfalfa),

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the species S. Meliloti bv. meliloti remains the most widespread and well-studied. The practical significance of alfalfa-based legume–rhizobium symbioses has been shown for the temperate and subtropical climatic zones of the Mediterranean coastline, North Africa, Morocco, Mexico, as well as for Russia [6]. Simultaneously, while natural symbioses adapted to temperate continental and temperate, arctic northern waterlogged acid soils may also be of no small interest due to the current global climatic changes. They remain to be studied.

The genotypic characteristics of bacteria (micro) and plants (macro symbionts) forming nitrogen-fixing symbioses are very important [7]. Matching of selected strains of root nodule bacteria substantially increase the viability and seed production of alfalfa on degraded soils [5]. The influence of the genotypes of inoculant strains and host plants on symbiosis formation is different under controlled conditions and on exposure to stress. For example, under salinity stress, the inoculant strain's role increases, and the effect of the interaction between both symbionts increase almost sevenfold. Root nodule bacteria are, therefore a promising subject for agricultural biotechnology, while the investigation of genetic regulation of tolerance to stress factors is a prerequisite for the directed construction of symbiotic systems with a predetermined adaptive potential.

Due to their excessive salt concentrations and high pH values, saline and alkali soils form an unsuitable habitat for the growth of most of the leguminous plants and their root-nodule bacteria. It seems unusual to expect the natural occurrence of rhizobia in these soils of as high pH levels as 10.5 (in 1:2 soil-water suspensions). Little is known about the survival and symbiotic characteristics of rhizobia in saline and alkali soils though considerable information is available on the growth and symbiotic activities of these bacteria under in vitro conditions of salinity and alkalinity.

Whether exotic strains of rhizobia introduced as inoculants into saline-alkali soils would survive, proliferate, and exhibit effective symbiosis in these soils' unfavourable habitat is an aspect that needs consideration. Observations have been made that local strains of rhizobia adapted to grow under certain soil conditions may make more effective inoculants for such soils than the strains of exotic natures. This aspect needs even greater attention in respect of saline and alkali soils than in normal grounds where the soil environment's reaction may not differ much from soil to soil.

Therefore, the present studies were carried out to ascertain the presence and survival of rhizobia of some commonly cultivated legumes in saline-sodic soils and to compare the symbiotic characteristics of exotic and native rhizobia strains of lentil and berseem in the salt-affected soils of different degrees of alkalinity.

Diversity and salt tolerance of rhizobia:

It was often of concern to investigate indigenous populations of root nodule bacteria, primarily to study the rhizobial disease's natural resistance to various abiotic stress factors[8]. In the centres of origin of host plants, where germ plasma polyps are huge, significant genotypic diversity of rhizobia has been shown[9]. Therefore, it is promising to study the native rhinoplastic populations from these centres to look for stresstolerant isolates that are symbiotically successful. The likelihood of isolating symbiotically successful strands of S. meliloti from root nodules of wild host plants is 5% greater even in the affected salinity region (surface adjacent to the Aral Sea) [10]. The positive association between salt-resistance and symbiotic effectiveness was demonstrated for strains isolated from root nodules of wild plants. As compared to the isolates of salt sensitivity, strains that have a high degree of Salt Tolerance mainly form a symbiosis with a higher efficiency [10][11]. The inherent immunity to relatively high levels of sodium ion (Na+) characterizes alfalfa rhizobia. Thus, the strain 102F24 of S. meliloti is, thus, 0.3 M NaCl resistant, far greater than the host plant's resistance (M. varia)[12]. Similar resistance was observed for Agrobacterium tumefaciens GMI 9023, while the species strains R. leguminosarum (all biovars), R. etli, A. rhizogenes and Bradyrhizobium japonicum are resistant to 0.2 M, whereas the varieties R. tumefaciens GMI 9023, R. tropici (Strain IIB), S. fredii and Mezo-rhizobium huakuii, are tolerant to 0.1 M [13]. High degree of S. meliloti salt resistance comes from the coevolution of rhizobia and its host plants, as osmolitis is 600 mOsm/L (or 0.3 m NaCl), equal to hyperotic conditions in alfalfa root nodules and bacteroids. [14]. In the absence of a host plant, rizobia, usually the soil biota leader, is at 3.85 mSm/cm (0.36 M NaCl) in the absence of a heavily salinized plant[15]. The predominant majority of native S. meliloti strains (71.4 percent) are able to expand at 0.6 M NaCl under laboratory conditions. Based on the study of 650 isolates from five geographically distinct areas, the trapping procedure was used to separate the later root nodules (hereinafter, Nisolates) and soils (hereafter, following Sisolates). However, salt tolerances below the specified threshold were slightly higher

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among the souls of saline soils adjacent to the Aral Sea region (0.6 M). The rhizobial adjustment to normal hyperosmotic conditions has been suggested as reducing their levels of salt tolerance to 0.3 M, which is the minimum amount needed for the development of the symbiotic system (see below), while being adequate for root nodule bacteria to survive in the saprophytic form[17]. This suggests further study of salt-tolerance pathways, a trait that could have a significant evolutionary effect on bacterial survival.

The genes affecting salt tolerance of S. Meliloti and other proteobacteria:

Rhizobia accumulates K+, glutamate and/or trehalose ions in response to salt tension, close to Escherichia coli and other bacterial species. But separate rhizobia species differ in the control of trehalose biosynthesis, which is essential for tolerance with desiccation. For example, the function of the gene in trehalose biosynthesis in R. leguminosarum bv. trifolii does not depend on ionic power in S. meliloti, on the contrary, the SMa0233 gene is triggered in the case of increased osmolarity [18] [19].

The discovery by Tn-mutagenesis or by sit-directed injection of transposons of genes involved in stress tolerance processes has been carried out actively enough. The molecular marker of the type GFP, lacZ reporter gene or GUS-based structures has been used frequently to research mutants[20]. In a variety of instances, the salt-sensitive phenotype has been formed by the Tn5mutants of the vines fredii, R. leguminosarumby. viciae or M. ciceri and, at the same time, the nitrogen-fixing of the symbiosis of the relevant host plant species has lost their potential [21]. Our colleagues N. Pobigaylo and A. Becker[22] have contributed to alfalfa root nodule genes' functional role. These authors developed the method of gathering transposants based on the insertion of miniTn5 in mass with short genome-specific oligonucleotide sequences (the STM mutagenesis method), which subsequently permitted the identification and detection of the target gene at the site of insertion of the minitransposon. A 10000 STM Rm2011:mTn5 mutant library was built, with five thousand genes evaluated for participation in cultural and symbiotic properties regulation [23]. Analysis by 50 transposants from this set at salinity stress ex planta reveals that mini TN5 inserts have either triggered an improvement in the salt tolerance levels of rhizobia or a substantial raise in the dry mass of the inoculated M. varia plants grown under saline conditions at comparable frequencies (0.2 average); however, the efficacy level under nonsaline conditions has not increased considerably (V.S. Muntyan, personal data). Our results contribute to the conclusion that various gene groups in S. meliloti regulate saltresistance and impact the adaptability of host plants to salinity under conditions of symbiosis.

Bacterial-Mediated Alleviation of Salinity Stress in Crop Plants:

The assortment of plant tolerance to salinity by plant-associated beneficial microorganisms such as rhizoplane, rhizosphere and endophytic bacteria and mycorrhizal fungus may be of considerable significance. The rhizosphere of various plants is colonized by PGPR (plant growth-promoting rhizobacteria) and has beneficial effects. Plants inoculated with these bacteria induce biochemical and morphological changes which increase abiotic stress tolerance, including the IST (induced systemic tolerance) salinity stress[24].

The current research focuses primarily on micro-organisms that can relieve abiotic stress like soil salinity relative to previous research centred on micro-organisms that enhance the soil's consistency and fertility. Salinity has a known effect on soil microorganisms' behaviour, including soil bacteria, due to its high osmotic intensity and toxic effects. However, bacteria in sites subjected to repeated stresses in salinity or associated with halophytic plants are possibly more resilient or resistant and can thrive and proliferate in harsh soil and rhizosphere[25]. Salinity-tolerant bacteria have been shown to be stronger promoters of plant growth under conditions of salinity. In the region around the root (rhizosphere), among bacteria whose habitats include the substrate, the rhizobacteria (root-associated bacteria) are more resistant to salinity stress as the water depletion of the plant root results in increased osmolity and an increase in intensity of ion. These PGPRs can thrive in the plant rhizosphere due to their durability and competitiveness under salty and arid soil conditions, tolerant to salinity of up to 3 percent NaCl[26]. In these regions, bacteria areolated have growth-promising properties such as developing indole 3-acetic acid (IAA), solubilizing phosphate, the deaminase (ACC) function of ammonia, and fixing nitrogen. In stress conditions osmolytes can accumulate in halotolerants and halophilic microorganisms. Extensive study has been performed in order to demonstrate the positive effect on plant growth of halotolerant PGPR[27]. PGPR salt-tolerant greatly improved numerous

plants' growth and development, such as maize, cucumber, sweet potato, ocimal basil, tomatoes, peppers, canola, beans, salt-treated soil, glycin Maximum L., potato, glycines max L., oryza sativa L.

Mechanisms of Action by Which PGPR Alleviate Salt Stress:

Bracibacterium, Brachy Bacterium, Brevibacterium, Haererohalobacterium, Staphylococcus, Halotolerant of the PGPR including Azaspirillum, Rhizobium, Bacillus, Pseudomonas, Pantoea, Paenibacillus, Enterobacter, Burkholderia, Achromobacterium, Microbacterium, Methylobacterium, Flavobacterium, Arthrobacterium, Serratia, Mycobacterium, Oceanobacillus sp., Exiguobacterium s. The following processes are:

- (i) The development of auxin, cytokinin, and gibberellins for phytohormones. Phytohormones are known to be involved in plant growth and stress response, including salinity stress, and to strengthen cell protection mechanisms to defend the plants against stress. The synthesis in the root and leaves of salinity-stressed plants of hormones, auxins, gibberellins and zeatin decreases and so germination and plant growth and development are also decreased under high salinity conditions. In addition to a decline in hormone production, salinity also decreases hormone supplies (e.g. cytokinin) from root to shoot. Salinity has been shown to have little impact on the generation of PGPR salt-tolerant hormones (i.e. auxin). For example, in a previous analysis, PGPR salt tolerance like A. brasilense were found to develop IAA at a 200-mm NaCl concentration. In another analysis, the development of IAA at 1.5 percent was observed for S. Plymuthica RR2-5-10, S. rhizophila e-ps10, P. chlororaphis TSAU13 and P. fluorescens SPB2145. Bacterial hormones induce enhanced root growth, radical length (root system architecture modulation), root surface area and the number of root tips while enhancing nutrient uptake in salinity stress conditions to promote plant growth. In other words, increased rooting (a root system with a wide area and an increased amount of root hairs) forms a larger root surface area which helps the plant to generate more soil nutrients. For example, in contrast to noninoculated wheat plants, the PGPR (i.e., P. aurantiaca TSAU22, P. extremorientalis TSAU6, and P. extremorientals TSAU20) dramatically improved wheat root growth to 40 per cent and firegrowth to 52 per cent at 100 mM NaCl. Plants develop abscisic acid (ABA) in reaction to salt stress, which allows stomach closure to decrease sweat loss and media the root branch to increase water in salinity stressed plants and induces the growth of leaf. Some PGPR (i.e., lipoferumazopirillum) have been identified in the stressed plant to increase the ABA material. PGPR strains may alter the hormonal equilibrium of plant hormones by generating phytohormones, thus improving plant growth under salinity stress conditions.
- (ii) Ion homeostasis, increased plant nutrient supply by processing of siderophores (increased iron nutrients), increased nitrogen (N) transition and acquisition, solubilization of inorganic phosphates and K-bearing mineral solubilization. High Na+ concentrations are known to inhibit the intake of nutrients K, P and N which are essential to growth and development. In other words, salinities produce nutrient imbalances in the retained intracellular ionic system due to disruption. Salinity-stressed plants are secured by Na+ entry, xylem recirculation to roots, and expulsion of roots during salt stress[29], which may avoid Na+ entry. Previous studies indicate that multiple PGPRs could minimize excess accumulations of Na+ in plants stressed by salinity and sustain ion homeostatic stress conditions (higher N, P and K intake contributing to the increase of the K+ / Na+ ratio in plants).
- (iii) Reduced development of ethylene caused by stress. Ethylene can be inhibitory (a plant root elongation inhibitor), or relaxing depending on its concentration in the plant growth cycle (i.e., seed germination, root hair development, stem elongation, fruit ripening, etc.). Excessive ethylene hormone development leads to lowered root and shoots growth under salinity stress conditions. ACC bacteria causing deaminosis are converted into α-ketobutyrate and ammonia (ACC) for ethylene processing (supply of nitrogen and energy). Besides, eliminating ACC decreases ethylene's deleterious effect, increases tension, and promotes salinity-stressed plants' production. In general, the number of lateral roots, lateral root and root dryweight of salinity-stressed plants are increased by decreasing stress ethylene containing ACC deaminase PGPR. Direct ties between bacterial development of ACC deaminase and root growth have been documented. Bacterial ACC-deaminase, in particular, uses more deep soil water under conditions of salinity stress and thus improves the productivity of water usage (WUE) in salinity-stressed plants. In several PGPRs like PGPR salt resistant, the enzyme ACC deaminase is active. Many

studies have shown that PGPR can improve plant growth and salinity resistance through this enzyme's production[30].

- (iv) Compatible solutes synthesis, also called compatible osmolytes, including prolines, carbohydrates, betaine glycine, polyols and choline. Compatible osmolytes constitute a group of chemically varied, polar, uncharged and soluble, organic compounds which, at high levels, do not interfere with cell metabolism. In building up compatible solutes, the plants that stress cells can be covered against salt stress under limits that are appropriate to natural cellular physiology and osmotic change. Past studies suggest that the concentration of compatible solutes, including proline, glycine betaine, free Amino acid, and soluble sugars in salinity-stressed plants, could relieve salinity stress in different plants by PGPR as Azospirillum, P. pseudo alquigenics, Burkholderia, Arthrobacter and Bacillus.
- Antioxidant enzymes synthesis. As the by-products of cell metabolism, the production of (v) reactive oxygen species (ROS) is normally poor under normal growth conditions in different plant organels. Low ROS levels are known to act as a signalling molecule, activating stress responses and defences. However, a response from plants to stress at the salinity is the increase of ROS production (i.e.hydroxyradic, radical super-oxide, singlet oxygen, hydrogen peroxide and superoxide) resulting in oxidative damage to various cellular elements, such as the DNA, lipid (lipid peroxidation). This means that salinity stress causes a secondary stress called oxidative stress, which means the production of ROS higher than threshold levels that lead to a cell homeostasis imbalance. Plants that are equipped with enzyme defensive antioxidants (CAT, CAT), GPX, SOD, (Superoxide Dismutase), APX and GR (GlutathonReduitase), ASC and nonzymatic elements such as glutathione, cysteine (APX) can extract or neutralize ROS to cope wi-fi and ascorbate Plants that are equipped with enzyme-based enzyme components (CAT, CAT, CAT and GPX). Different research also shown that there is a clear link between oxidative stress resistance and enhanced plant concentration of antioxidant enzymes. Some PGPRs may also degrade ROS by producing enzymes of antioxidants. Often related to oxidative stress resistance are strong antioxidant enzymes function. There is important evidence that PGPR can relieve salinity-induced oxidative stress (ROS) by controlling antioxidant enzymes in various plants[31]. It is important to remember that the processes behind the increases in antioxidant enzyme levels are still unknown in plants that cause stress when they are impacted by bacteria.
- (vi) Antibiotics, competition for space and nutrients and induction for systemical tolerance in plants to control complex plant diseases (a number of root and foliar pathogens).

Conclusions:

Soil salinization is currently one of the main issues impacting farm production worldwide. In addition to primary salinization, secondary salinization from anthropogenic activity would have a much more important impact on crop production and food safety. Not many reconstruction solutions for saline soils have been successful to date. The use of PGPR and its metabolites as a salt-tolerant may play a major part in growing and remediating saline soils' productivity in the event. The plants (grown under the stress of salt) can also be covered against plant pathogens with the use of the salt-tolerant PGPR. The analysis demonstrated the feasibility and the viability of the application of salt-tolerant PGPR in salty soil. Bioformulations may be produced with these strains or metabolites to boost and green saline soils efficiently.

In order to give plants resistance to salinity stresses a sustainable and environment-friendly approach, the microbial communities in the rhizosphere of halophytic plants, also known as a rhizosphere microbiome, and the microbial communities in salt-affected land will thus ensure a higher yield of plant products on salt-affected soils and open a new advanced plants PGPR will also improve the absorption of nutrients from the soil affected by salinity, thus eliminating the need for the use of chemicals in soil saline. Such inoculants contribute to sustainable agriculture production under salinity-stressed conditions (helpfully minimize the use of agrochemicals and restore soil health). Despite several studies carried out in this field, a significant impediment is still to understand the cross-discussions between halo-tolerant rhizobacteria and plants mediating this response. More study in field conditions is also required in order to apply halotolerant rhizobacteria as a biological fertilizer.

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