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Enhancing of Salt Stress Tolerance in Mung (*Vignaradiata*) Seedlings Inoculated with the Halotolerant *Bacillus* spp.

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ABSTRACT: Biotic and abiotic factors have been responsible for the productivity of agriculture all around the world. Leguminous plants have been beneficial to the mankind for various reasons such as biological nitrogen fixation and improvement of soil fertility. Salinity is a serious threat responsible for the productivity, vitality, growth and survival of plants. Increased soil concentration extensively hinders the plant health. Salinity negatively affects plants not only by salt stress, but water stress, ion toxicity, oxidative stress, nutritional disorders, etc. All of these in turn harm the processes of plant growth, development and survival. To overcome this issue, plant seeds can be inoculated with halotolerant plant growth promoting rhizobacteria that would enhance the tolerance of plants to salt stress as well as improves the plant health.

KEYWORDS: Halotolerant, Salinity, Salt tolerance, Plant growth promoting rhizobacteria(PGPR), *Vignaradiata*.

I. INTRODUCTION

Depending on the geography of region where these plants have been grown several obstructions cause degrading effects on the plant by various means. Plants can be stressed by a wide variety of factors resulting in primarily two types of stress, biotic stress and abiotic stress. Biotic stress as the name suggests is caused by living organisms such as viruses, bacteria, fungi, nematodes, arachnids, insects and weeds.(Gull et al., 2019) Whereas, abiotic stress is caused by environmental factors such as heavy metals, temperature, water, ultraviolet radiation, high salinity, etc.(He et al., 2018) Each one of these factors shows different type of deficient effects in plants. Heavy metals such as Fe, Cu, Co, Zn, Mn, Mo are required in minute quantities by plants. Though, higher quantities may affect the plants deficiently. Excessive heavy metals cause toxic effects on plants, both directly and indirectly. Direct toxicity includes the inhibition of cytoplasmic enzymes and damage to the cell structures. Indirect toxicity includes the anti-microbial activity of heavy metals. As heavy metals accumulate excessively the favorable soil microorganisms reduce and thus indirectly damage the plant.(Chibuike & Obiora, 2014)

Depending upon the geographical locations, temperature may vary and negatively affect the plants in correlation with the temperatures which primarily include three temperature stresses. High temperature stress can cause growth retardation, necrosis, decrease in photosynthesis, and recurring death of plant. Low temperature stresses include two types of stress chilling stress and freezing stress. Chilling stress includes stress caused in temperature ranging from 0-15°C and it causes pollen sterility. Freezing stress is caused when temperature ranges below 0°C which causes ice formation in the apoplasts, injures the cell. The ice formation leads to dehydration and osmotic stress because of the limited water transport.(Kai & Iba, 2014)

Water stress can be caused in two conditions. One is when the water does not reach the roots, also known as drought. When water is not available the enlargement of cells decreases resulting in growth inhibition and reproductive failure. Drought can affect the stomatal closure, which limits the gaseous exchange, reduces transpiration and thus negatively affects the carbon assimilation. The water insufficiency disrupts the uptake and transport of nutrients. Another condition of water stress is when there is excess water. When water is available in excess, it causes oxygen depletion which in turn reduces root respiration and often results in root death. Excess water affects the stomatal opening and the transpiration occurs and it may lead to wilting.(S. Lisar et al., 2012)

The depletion of stratospheric ozone layer is becoming more concerning as the time passes as it is responsible for filtering out the UV-C (200-280nm) wavelength, which can be very harmful to organisms but the absorption coefficient

of ozone decreases rapidly at 290nm and reaches zero at 330nm. UV-B radiation is harmful to living organisms. UV radiation damages the DNA, proteins, lipids, plant growth regulators, pigments and membranes as well as disrupting the photosynthesis of sensitive plants.(Hollósy, 2002)

II. SALINITY STRESS

Salinity stress is responsible for various negative effects in plant growth such as decrease in leaf water potential, because of the presence of sodium ions closure of stomata in turn inhibiting the leaf expansion and disrupting the photosynthesis. The plant growth is disrupted because of the presence of sodium and chloride ions. The salinity disrupts the nutrient uptake as well as water. These toxic ions accumulate in older leaves, reduce the yield and they cause the death of the plant.(Carillo et al., 2011)

III. SALT TOLERANCE

In plants, the genetic control mechanisms of salt tolerance have yet to be fully understood because it is very complex.(Carillo et al., 2011) Salt tolerance can be assessed as the percent of biomass produced in saline versus control conditions over a definite period of time. It can also be assessed in terms of survival.(Munns, 2002) The tolerance for salinity of a plant species would be overridden by sudden exposure to salinity, even if the species were a halophyte.(Albert, 1975) So different adaptation like gradual acclimation of salinity can be efficient in order to prevent sudden shock. The sensitivity to salinity may change during ontogeny. Salinity tolerance is dependent upon the increase or decrease at the plant species and/or environmental factors. For different species, salt sensitivity may be increasing in different stages of life cycle for example, germination and reproduction.(Howat, 2000; Marschner, 1986)(Carillo et al., 2011) Plants in general have adapted to the salinity stress by three types of plant responses or tolerance: i.osmotic tolerance, ii) Na^+ exclusion and iii) tissue tolerance(Munns & Tester, 2008)

i.Osmotic tolerance: The salt-stressed plants show limited growth because of the osmotic effect of salinity. The ability of the plant to exclude the salt cannot prevent the reduction of growth rates and the stomatal conductance.(James et al., 2008) Osmotic tolerance is the ability of the plant to tolerate the drought aspect of salt stress as well as the maintaining of leaf expansion and stomatal conductance.(Rajendran et al., 2009) A study suggests that there is a positive relationship between the relative growth rate of the plant and stomatal conductance in salt-treated plants. Higher stomatal conductance in turn results in higher CO_2 assimilation rate.(James et al., 2008) But if salt accumulation exceeds the toxic concentration, the old leaves would die whereas the young leaves would be independent of the export of photosynthesis, which undergoes growth reduction and new leaves production. This is why osmotic tolerance includes ability of continuing production and growth of new and greater leaves as well as higher stomatal conductance.(Munns & Tester, 2008)

ii. Na^+ exclusion: In order to reduce the ionic stress on the plant, the amount of Na^+ needs to be minimized that would be accumulated into the cytosol of cells, especially in the transpiring leaves.(Munns & Tester, 2008; Rajendran et al., 2009) Thus, Na^+ exclusion done by roots can ensure that the Na^+ does not accumulate up to toxic concentration within plant leaf blades.(Munns & Tester, 2008)

iii.Tissue tolerance: This leads to an increase of survival of old leaves. Here, Na^+ and Cl^- are compartmentalized at the cellular and intracellular level to suppress toxic concentrations within cytoplasm including mesophyll cells in leaf(Munns & Tester, 2008) and synthesis and accumulation of compatible solutes within the cytoplasm. Compatible solutes protect enzymes from denaturation, stabilize membrane or macromolecules or mediate osmotic adjustment.(Ashraf & Foolad, 2007)

Mungbean (*Vignaradiata*) is a fast-growing leguminous plant cultivated for its edible seeds and sprouts. The mature seeds of Mungbean are an invaluable source of digestible protein for humans. Mungbean has the ability to fix atmospheric nitrogen in symbiotic association with Rhizobium bacteria, which enables them to meet their own nitrogen requirement and benefit succeeding crops.(Mehandi et al., 2019) They play a very important role in soil fertility.

IV. SALINITY TOLERANCE IN MUNGBEAN(VIGNA RADIATA)

In a study by(Patel et al., 2015b), out of 50 bacteria only 12 isolates were grown on Nutrient agar (12 on King's B base medium, 12 on Ashby's mannitol agar and 14 isolates had grown on Yeast mannitol agar with Congo red). All cultures



were able to grow in 5% NaCl. From the total isolates, some were able to grow on media containing 10%, 12% and 18% NaCl concentration. 40 out of 50 isolates were able to produce IAA (indole acetic acid). 18 out of 50 showed zone of clearance in pikovskaya medium. Some cultures showed pH decrease/ drop in growth medium. The same isolates showed quantitative phosphate solubilisation. 13 out of 50 were able to fix 100% nitrogen from atmosphere. Siderophore production in 11 isolates was observed. 13 out of 50 were able to produce chitinase, 40 out of 50 produced cellulase. 21 isolated produced zone of clearance in skimmed milk agar. Two isolated showed plant growth promoting trait production such as IAA production, phosphate solubilisation, nitrogen fixation, siderophore production and tolerance to NaCl up to 18% in growth medium. The same isolates showed antifungal activities due to the presence of cell wall degrading enzymes. In presence of saline soil, plant growth reduced significantly, but in the presence of salt with PGPR (plant growth promoting rhizobacteria), the plant growth improved considerably. In presence of salt, total chlorophyll content reduced, but treatment with PGPR increased chlorophyll content. Total protein content did not change significantly due to PGPR treatment to plant under saline stress. (Patel et al., 2015a)

V. *BACILLUS* SP. IN SALINITY TOLERANCE

Bacillus species are rod-shaped, endospore formers, aerobe or facultative anaerobe, gram-positive bacteria. Their endospores are resistant to heat, cold, radiation, desiccation and disinfectants.

In a study by (Singh & Jha, 2016), *Bacillus licheniformis* HSW-16 strain was used as a test subject for induced tolerance to salt stress in wheat plant (*Triticum aestivum*). For salt tolerance screening the bacterium was tested by growth in tryptic soy broth with different NaCl concentrations ranging from 2-11% w/v. after the experiments, the presence of bacteria improved the plant growth. The shoot length, root length, fresh weight, dry weight, chlorophyll a content and chlorophyll b content showed a significant amount of increase. Thus, it improved health of plant under stress-induced conditions. HSW-16 strain showed decrease in Na⁺ content but K⁺ and Ca⁺² content increased. (Singh & Jha, 2016)

In another research, bacterium *Bacillus aryabhata* strain H19-1 and *Bacillus mesona* strain H20-5 were inoculated in the soil containing tomato plants to observe the effect of salinity stress. Both strains showed increase in fresh weight and dry weight. Chlorophyll content showed significant increase in plants having both strains, but only H19-1 elevated carotenoid. Salinity stress resulted in decreased electrolyte leakage in plant leaves. Stress induced the increase in K⁺ concentration and Na⁺ concentration, but showed decrease in Ca⁺² concentration. *Bacillus mesona* strain H20-5 increased anti-oxidant enzyme activity. (Yoo et al., 2019)

VI. CONCLUSION

Salinity is a serious hazard to agriculture. The effects of salinity on plants and their mechanisms, employed to survive have been studied for over 100 years now. Salinity negatively affects the plant productivity. Enhanced salt tolerance is helpful as to protect the plant as well as provide other multiple benefits. Enhanced salt production leads to increase in crop productivity that will eventually result in eradication of the malnutrition problems.

CONFLICT OF INTEREST

The authors have no conflict of interest in preparing this article.

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