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Salinity stress, its physiological response and mitigating effects of microbial bio inoculants and organic compounds

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Abstract

Around 20% of irrigated land globally affected due to salinity stress. This condition is more severe in some states where the effect is more than 35%. Salinity stress caused mainly due to two reasons one of them is the poor quality of irrigated water and other is the excess concentration of salts present in the soil. There are two different mechanisms in plants in response to salt stress, which further provides signals to various parts of plants and enacts according to that response. Based on the ability to tolerate the stress plants classified as glycophytes, which can tolerate a low level of saline stress while others are halophytes, which can tolerate more level of saline stress. Halophytes like mustard can cope up with the situation of salinity stress because they have a special mechanism for it. There many ill effects of salinity stress has adverse effects in all around the world along with India, especially in arid regions. There are various mitigation methods to cope with this salt stress and thus preventing its negative effects on production and reduce our economic loss. The application of microbial bio inoculants that are trichoderma, mycorrhiza, rhizobium, and organic compounds that are cycocel and benzyl adenine reviewed in this paper. It also involves various mitigating effects of them, which is, observed by application of them in Mustard plants.

Keywords: Agriculture, biotic, crop, density, forage, grass, salinity

Introduction

The Brassica juncea L. that is usually named, as Indian mustard is a plant of Brassicaceae family and used in various purposes. The chief component of the mustard plant is its oil, which is famous for its comestible nature and medicinal usefulness in not only India but also whole over the world. It has some other purposes also like biofuels, feed for cattle and fertilizer for the land. It is widely cultivated in arid and many semi-arid zones of the world. India positioned second in all over the earth in terms of cultivation of Brassica as well as contributes to 7 per cent of edible oil's supply in all countries but still, its cultivation is not fulfilling the needs of the population of the country and also not sufficient for export. The reason behind very less production is highly susceptible to nature to various stresses. In among all of them; saline stress is a major concern. Soil is considered to be saline if its electrical conductivity is more than 4 dS/m which is equivalent to 4 mmhos/m. The salinity stress is of two major types one of the natural salinity is due to the number of salts present naturally in soil and other is induced salinity, which is due to the accumulation of salt due to various human activities. The plant characterized into two ways based on the tolerance of their salt stress. One of them is a halophyte, which could easily tolerate this salt stress, and others are Glycophytes, which are sensitive to saline conditions and unable to grow under salinity stress. Halophytes like mustard can cope up with the situation of salinity stress because they have a special mechanism for it. The first step it includes is Exclusion in which the entry of toxic ions at root level 2nd is compartmentation at plant level in which toxic ions transferred to older leaf sheath and older plant tissues. The third includes the excretion of toxic salt through salt hairs, salt glands and or bladders. The fourth step includes cell level compartmentation in which sequestration of toxic ions to vacuoles and cell wall. Salinity stress causes types of stress one of them is Ionic and other is Osmotic. In ionic stress plant, there is an accumulation of Na ions and K deficiency, which leads to Na toxicity which results in plant senescence. In response to its plants to avoid this stress plant, maintain ion homeostasis and Na extrusion. While on other stress i.e. osmotic stress it leads to dehydration and low leaf development. The salt stress has also been found responsible for an increased respiration rate, ion toxicity, changes in C and N metabolism,

mineral distribution, membrane instability (Marschner, 1986) and permeability (Gupta et al., 2002) decreased biosynthesis of chlorophyll (Khan, 2003) and inefficiency of photosynthesis, all of which ultimately lead to lowered economic productivity. Ahmad et al, 2010 examines "A prolonged salinity stress is responsible for secondary stress, i.e.; oxidative stress that generates reactive oxygen species (ROS) deleterious to biomolecules like, proteins, nucleic acids (DNA/RNA), membrane lipids etc." Sánchez-Rodríguez et al., 2010 states "Plant cells generate ROS even under normal conditions but they are balanced by the scavenging system of the cell. When the generation of ROS exceeds its quenching capacity, oxidative stress appears. Polyunsaturated fatty acids (PUFA) are more vulnerable to ROS attack and this leads to lipid peroxidation". However, plants have protective mechanisms like enzymatic and non-enzymatic antioxidants against these ROSs.; Ahmad and Sharma, 2008, 2010) observes "Enzymatic antioxidants are superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR) and non-enzymatic are ascorbic acid (ASA), glutathione (GSH). The major factors involved in declination in plant's growth includes saline stress as it leads to excessive concentrations of Na⁺ and Cl⁻ ions imbalance in osmosis and proper nutrition. It has adverse impacts on C as well as the N metabolism biosynthetic process of chlorophyll and photosynthetic activity. The retardation in photosynthetic activity is mainly due to the sensitive nature of Photosystem II light inhibition. (Chl) is an effective measure to check the harm caused to Photosystem II for detection in salt tolerated varieties. In the adoption of a saline environment by plants, they have developed several methods to combat salinity. These include an increase in the production of Osmolytes metabolites like proline, which helps in cell osmotic adjustment and stabilizing membrane, and detoxifying of harmful cations and anions. Also, help in the protection of PS II from high salt concentrations. Moreover, proper managing of minerals increases the osmotic synthesis for improving response mechanism of plants.

Types of salt-affected soils

These salt-affected soils include Sodic and Saline soils. They both possess high levels of pH usually more than 7.8. Both cause a problem in the proper growth of plants and hampers their some essential physiological processes, which lead to a reduction in the yield of crops. There is also quite a difference in these types of soil, which are following. They both have high pH while sodic have more 8.5 pH while saline has less than 8.5 pH. Electrical Conductivity, which signifies the more presence of soluble salt ions as more amount of soluble ions will be there, more will be the electric current. Therefore, in saline soils, it has more than 4mmhos/cm while in sodic soils; it is less than 4mmhos/cm. Exchangeable Na% which involves% saturation of sodium ions in the adsorption complex of soil. It calculated by the ratio of exchangeable of Na+ ions to the cation exchange capacity. Its value is more than 15 in sodic soils while less than 15 in saline soils. Sodic soils have a high content of sodium while in saline soils; there is more amount of salt content however, these soils may have both sodium and the salt amount at the same time. In sodic there is high pH due to the presence of CO_3^{2-} and HCO_3^{-} ions while in saline soils, there is more amount of water dissolvable salts, which comprises of Na, Ca and Mg chlorides.

Symptoms of saline soil: There are signs of deficiency of Fe, P and Zn nutrients, which is limited in saline conditions, which involve purple or dark green colouring of lower leaves and stems by a deficiency of P and yellow colour stripes on upper leaves by Zn and Fe deficiency. The other commons. There is also the distribution of powdery material on the soil 'surface as the salts diffused on the soil surface.

Symptoms of Sodic soils: In sodic soils, there is similar symptoms of famine but also it causes the highly damaging effect of germinating of seeds, as there is a reduction in the viable capacity of seedlings. In severe cases, there is a distribution of organic content around the soil.

Physiological response to salinity stress: There two different ways in which plant response to deal with salt stress observed.

Osmotic Effect: It is a rapid and non-specific response. In this, there is a decrement in the osmotic potentiality of atmosphere, water and soil and have a straight affect on the status of root cell water. This is resulted due to the distinction of the potentiality of cytoplasm and solution of soil water surrounded it. This stress leads to the decrement of available water and decrement in up taking of water, which results dehydrating of cells. This type of effect is similar to all dehydrating stress, which involves drought, saline stress, and very low temperature, physical wounding and quite -non-specific in nature. There is an increment in osmotic potentiality and percolation of salt ions into cells of plants, which cause a stimulation for specific signalling events in plants.

Signalling events in osmotic stress

There is a decrement in turgidity of plant cells due to cellular dehydration and act as an alert for osmotic stress, which recognized by plasmalemma. There is an alteration in expression of a gene induced to the nucleus by signalling of calcium and series of phosphorylation, which transduced through osmotic signalling. The effect on ABA function is another essential constituent of this osmotic stress signalling which leads to enhancement of its synthesis. Due to the enhancement of ABA synthesis, there are types of responses observed. A first response which rapid in nature, there is a reduction in pH which induces turgor loss in guard cells of stomata which to the closing of stomata. The other response, which in nature, involves initiation ABA-responsive transcription factors, which adhere to ABA-responsive promoter elements, which help in the accumulation of few genes in more amount to help in tolerating the stress. There is an osmotic modification in retaliation to osmotic impact. There is a decrement in osmotic potentiality, which is due to increased aggravation osmotically active compounds leading to reduction of variation of atmospheric and cellular osmotic potentiality. One of the easiest response to lower osmotic stress includes the aggravation of salt ions in cells. Meanwhile, this leads to the destruction of cellular enzymes. Therefore, plants less follow this mechanism. To overcome this osmotic effect, several LEA (late embryogenesis abundant) proteins which include betaines like glycine betaines, polyamines like (spermine, spermidine) and sugars. These help in the decrement of osmotic potentiality and protection of dehydration affected compounds of cells.

Tolerance towards osmotic stress: Due to the effect of osmotic stress, there is a reduction in the capability of dilation of cells especially at root tips and younger leaves. This leads to the closing of the stomata. There is an increase in more growth in leaf area and conduction in stomata in a response to the osmotic stress. The change in the increase of leaf area is desirable only an area where there is good irrigation supply otherwise in other areas where irrigation supply is limited, this is unenviable change as it leads to the utilization of water present in soils even when the grains have not reached the maturity stages.

Avoidance: In this procedure of avoidance plants, keep salt ions away from the parts where they can because more harm so that the concentration does not reach up to toxic level. For this, they adopt two methods.

Salt exclusion: Through this ability in which there is the exclusion of salts is by filtering the salts at the root level, which prevents the salt to enter the root membranes while water allowed moving through these membranes. For example, mangrove plants in which there is a mechanism of salt exclusion through filtration providing tolerance against these toxic ions.

Salt extrusion: In this mechanism, salt removed through various glands or bladders or cuticle present on leaves. These could be done by following methods

Expulsion of Na⁺ from leaf blades: Important mechanism to combat salt stress. In this, there is an expulsion of Na⁺ through roots by ensuring that there is no aggregation of toxic compounds up to unbearable level within leaves. Failure of the expulsion of the toxic compounds leads to an increase in the toxic effect of sodium ions, which further causes the death of cells.

Expulsion of salt ions through salt bladders: For sequestration of Na+ ions there is the presence of salt bladders in some plants. For example *Mesembryanthemum crystallinum* L., A triplex.

Salt glands: These are the dumping sites for excess salts present in plant parts, which are being absorbed through water from the soil. It is a very beneficial organ for adapting plant life in salt excessive conditions.

Salt Dilution: In process plants maintain succulence in them by diluting the salt ions present in the tissue of them. There are various methods for increasing succulence in plants, which includes an extension in their storage capacity by developing various fleshy, thick and calculating modifications, which are predominantly due to the increment in the size of vacuoles of mesophyll cells by filling with water.

Compartmentation of ions-

Organ level: Accumulation of more salts predominantly in roots in comparison to shoots specifically leaves.

At the cellular level: Accumulation of ions in vacuoles as compared to the cytoplasm, which helps in protection of enzymes from high saline conditions.

Tissue tolerance: As there aggravation of Na⁺ or in some

species Cl⁻, there is a requirement of compartmentalizing of these ions to provide the tolerance against these ions mainly in mesophyll cells of leaves. The more accumulation of these ions more than bearable amount leads to toxicity in older leaves with the time.

Ionic stress: When there is more accumulation of salt ions in the plants and expulsion of Na^+ ions are not enough for maintainñ intracellular Na^+ levels, then there is an ionic response in plants. This effect acts in the delayed phase when there is an aggravation of salt ions up to toxic levels. It is quite specific in nature, which helps the halophytes to expel the high concentration of salt ions up to toxic levels within the cells. There is internal compartmentalization of ions leading to their sequestration in vacuoles to decrease the injurious effect of these ions on plants. This compartmentation process efficiently carried out in halophytes while in glycophytes there is the failure of establishment of high tissue tolerance in prolonging saline conditions.

Ion related signaling: In saline stress, there is an increment in activities of salt ions especially Na⁺ ions in soil water that is present around plant root cells. Due to these active gradients, salty ions the passive passively comes in the cytoplasm of the cell. This is prolonged and of continuous nature, as it is dependent on parameters of intracellular salt ions, which shows increment during saline conditions and along with senescent of various parts of plants. Due to the accumulation of Na⁺ level at the cellular level, it persuades the initiation of Ca²⁺ signalling, which activates of sodium ions expulsion interactively via SOS1/SOS2/SOS3 (salt overly sensitive) pathways.

Mitigating effects of Trichoderma

Application of Trichoderma: Trichoderma sp. are microbes, which live inside plants and live in a symbiotic relationship with them. They extensively applied as biofertilizers for crop's growth and biocontrol agents for various infected plants. In saline conditions, beneficial effects of Trichoderma harzianum (TH) visualized. As there is decreased yield up to one-fifth due to adverse effect on growth, metabolic processes and oil percentage in Mustard crops. The higher loss around 30% in crop yield when more salt is applied. With the application, there is an increase in dry weight content and yield ranging from 11 to 16 per cent in comparison to those in which alone salt is applied. While there is also a significant increase in oil content about 19-24%, which was adverse, affected due to salt application. TH also improves the Chl, 'a' which significantly dropped up to its half content. There is further supplement in proline content up to 70%, which was nearly 60% in salinity stress. TH also increased the mineral absorption through roots and shoots, which declined due to application of NaCl.

Shoresh *et al.* conclude Trichoderma strains can enhance plant tolerance to biotic and abiotic stresses such as drought and salinity through enhanced root growth, nutritional uptake and by inducing protection against oxidative damage. Mastouri *et al.* (2012) state "Enhanced resistance of colonized water deficit plants by *Trichoderma harzianum* (TH) T22 is explained partly due to higher capacity to scavenge ROS and recycle oxidized ascorbate and glutathione, a mechanism that is expected to enhance tolerance to abiotic stresses." However, specific knowledge of mechanisms used by TH controlling multiple plant stress factors is still lacking and needs to study. Effect on oil content (%) under NaCl stress in the presence and absence of *Trichoderma harzianum (TH)* in *Brassica juncea* seedlings. Values are means \pm SE (n = 5). Improvement in Oil Content in Trichoderma Improved the Oil Production in Mustard Plants Under NaCl Stress. The effect of TH on the oil content of Mustard plants with the application of NaCl at a concentration of 100 mM and 200mM, there is a significant decrease in oil content up to 25% and 19% in comparison to the control. While in TH treated plants there is an improvement in the content of oil up to 30% at 100mM of NaCl + TH and 23% at 100mM of NaCl + TH. Therefore, it proved with the above experiment that TH shows defensive role to mustard in response to saline stress and helps mustard to elevate the oil content in it.

Refurbishment of Chlorophyll by application of TH in the condition of Saline stress

There is decrement of photosynthetic pigment Chl'a' and Chl'b' under salt application of 100mM up to 1.33 mg g⁻¹ fresh weight and 0.82 mg g⁻¹ fresh weight leading total chlorophyll 1.95 mg g⁻¹ fresh weight as compared to control which has a content of Chl'a'1.75 mg g⁻¹and Chl 'b' 0.95 mg g⁻¹ fresh weight. The decrement continues with further application of NaCl up to 200 mM, which significantly drops up to 0.84 mg g⁻¹ in Chl 'a' and up to 0.71 mg g⁻¹ fresh weight. The beneficial effect of TH is observed in terms of both Chl 'and Chl 'b' content in the mustard plant which elevates their amount up to 1.38 mg g⁻¹ fresh weight and 0.73 mg g⁻¹ fresh weight in Chl 'which increases the total chlorophyll content up to 2.31 mg g⁻¹ fresh weight under 100 mM salt application. There is also increment in the application of TH along with 200mM salt application in which Chl 'a' content increases up to 0.99 mg g^{-1} fresh weight and in Chl 'b' content inclines up to 0.81 mg g^{-1} fresh weight leading to overall chlorophyll amount up to 1.80 mg g⁻¹ fresh weight. Therefore, TH plays a very beneficial role in mustard plants by refurbishing of both Chl 'a' and Chl 'b' leading to overall inclination in chlorophyll component which is beneficial in carrying out the photosynthetic activity.

Consequences of application of TH on proline constituent in NaCl stress

As depicted in the table, increment of proline content with application of NaCl 100mM up to 42.26%. The increment continues with further application of NaCl up to 200 mM, which significantly drops up to 59.12%. The positive effect of TH observed in terms of proline content in the mustard plant, which elevates its amount further up to 56.25% in 100 mM salt application and 70.37% in 200mM salt application. TH plays a very beneficial role in mustard plants by elevating the content of proline, which leads to the decrement of osmotic potential in the cytoplasm, which facilitates absorbing capacity of water of plants. Proline also helps in scavenging ROS molecules.

Mitigating effects of Vesicular-Arbuscular Mycorrhiza

Application of Vesicular-Arbuscular Mycorrhiza (VAM): VAM sp. is microbes, which live inside plants extensively applied as biofertilizers for crop's growth and biocontrol agents for various infected plants. In saline conditions, beneficial effects of VAM visualized. As there is a decreased yield of more than 20% due to the adverse effect on growth, metabolic processes and oil percentage in Mustard crops. The higher loss around 30% in crop yield seen when more salt is applied. By applying VAM With the application of VAM there is increase dry weight content and yield ranging from 11 to 16 per cent in comparison to those in which alone salt is applied. The more beneficial effect of VAM observed more along with the application of FYM and vermicompost. While there is, also a significant increase in oil content as it leads to increment in glucoside constituent in the seed, which produces more oil after its hydrolysis and esterification about 18-26%, which adversely affected due to salt application. VAM has the capability of production of growing substances, which make more availability of N, and P as it properly solubilizes and mineralizes them properly. VAM enhanced proper nutrient uptake of many macronutrients like N, P, S, K and Zn as it makes available nutrients in a balanced way during all growth stages. This increment in nutrient uptake leads to enhancement in photosynthetic rate in mustard plants as proper nutrient uptake leads to increment in dry matter production and a higher amount of nutrients in seeds. The protein content also surges with applying VAM, as more N is available to plants, which resulted in absorbing and utilizing the plant nutrients properly. VAM also increased the mineral absorption through roots and shoots, which declined due to application of NaCl. It also resulted in improving the physiochemical properties of soil, which includes decrement in bulk density, as there is more humus formation, which helps in improving water holding capacity and increment in biomass synthesis. The increment in the retention of moisture, cation exchange capacity and hydraulic conductivity which is due to better aggregating of soil, increased microbial activity and enhancement in no. of stable soil structures. This leads to more availability of moisture to the plants, which is much beneficial in salt stress conditions.

Mitigating effects of Rhizobacteria

Application of Rhizobacteria: Rhizobacteria shows mutualistic effects in leguminous plants also have beneficial effects in non-leguminous plants mustard by improving the physio-chemical properties of soil which includes decrement in bulk density as there is more humus formation which helps in improving water holding capacity and increment in biomass synthesis. The increment in the retention of moisture, cation exchange capacity and hydraulic conductivity which is due to better aggregating of soil, increased microbial activity and enhancement in many stable soil structures. This leads to more availability of moisture to the plants, which is much beneficial in salt stress conditions. However, this change is quite less significant in the rhizobacterial application as compared to mycorrhizal and trichoderma application, which help more significantly. Rhizobacteria lead to the formation of IAA (Indole Acetic Acid) which helps the mustard plants to cope with salt stress as this helps in regulating growth during the life cycle of plants. It also leads to the growth of more adventitious roots, which helps in proper absorption of nutrients from the soil. There is the utilization of soil exudates and help in the production of metabolites that alleviates the salt stress. It also has important in breeding of soils and efficient biocontrol agents. During salt stress, there is the production of Osmolytes to counter the effect of stress, which varies according to the degree of stress. There is also the production of Osmoprotectant, which plays both roles of Osmoprotectant and energy source. Under high osmotic stress they act as an Osmoprotectant and in an opposite condition where there is low osmotic stress, they act as a source of energy. There are also many beneficial effects on applying rhizobacteria in mustard plants in various significant parameters like improvement in emergence% seedling, length of roots and vigour index of seedlings. The increment in the

retention of moisture, cation exchange capacity and hydraulic conductivity which is due to better aggregating of soil, increased microbial activity and enhancement in no. of stable soil structures. This leads to more availability of moisture to the plants, which is much beneficial in salt stress conditions.

Mitigating effects of cycocel

There are many beneficial effects on treating cycocel before sowing in mustard plants in various significant parameters like improvement in emergence% seedling, length of roots and vigour index of seedlings while there is a decrement in some insignificant parameters like harvesting index.

The significant parameters like improvement in emergence% seedling, length of roots and vigour index of seedlings which causes stimulation in an increment of germinating and root growth hormones while alleviation in seedling and shoot length due to its dwarf character of cycocoel. There is a clear distinction in cycocel soaked and neon-soaked seeds in terms of leaf area and its index. Seeds which are treated by cycocel by soaking in it show greater leaf area and its index which help to cope with osmotic stress. There is also a beneficial effect in the rate of photosynthesis at all stages, life cycles of plants, which is due to increment in leaf area and leading to increment chlorophyll pigment. This increment significantly persuaded by an increment in water use efficiency at growth stages of plants. There is also decrement in transpiration loss of water, which caused due to decrement in conductivity and opening of stomata and lowering proline amount in cells, which helps in more streamlining of efficient use of water.

In mustard, Cycocel helps in efficiently combating salinity as in its presence leads to decrement in up taking of sodium ions, which leads to decrement in Na/K ratio in stem and roots at all stages of plant's growth. Its importance is tackling the saline conditions is also observed due to inhibition of synthesis of gibberlines. Cycocel also leads to increment in the number of amino acids and other metabolites, which attributes to more oil and protein content.

Mitigating effects of Benzyl adenine

Application of benzyl adenine: In mustard plants, there is a clear distinction in benzyl adenine soaked and non-soaked seeds in terms of leaf area and its index. Seeds which are treated by benzyl adenine by soaking in it show greater leaf area and its index which help to cope with osmotic stress. There are also many other beneficial effects on treating benzyl adenine before sowing in various significant parameters like improvement in emergence% seedling, length of roots and vigour index of seedlings while there is a decrement in some insignificant parameters like harvesting index. There is also a beneficial effect in the rate of photosynthesis at all stages, life cycles of plants, which is due to increment in leaf area and leading to increment chlorophyll pigment. This increment significantly persuaded by an increment in water use efficiency at growth stages of plants. There is also decrement in transpiration loss of water, which caused due to decrement in conductivity and opening of stomata and lowering proline amount in cells, which helps in more streamlining of efficient use of water. The significant parameters like improvement in emergence seedling, length of roots and vigour index of seedlings, which cause stimulation in an increment of germinating, and root growth hormones while alleviation in seedling and shoot length due to its dwarf character of benzyl adenine. There is a clear distinction in benzyl adenine soaked and non-soaked seeds in terms of leaf area and its index. Seeds which are treated by benzyl adenine

by soaking in it show greater leaf area and its index which help to cope with osmotic stress. In mustard, Benzyl adenine helps in efficiently combating salinity as in its presence leads to decrement in up taking of sodium ions, which leads to decrement in Na/K ratio in stem and roots at all stages of plant's growth. Its importance is tackling the saline conditions is also observed due to inhibition of synthesis of gibberellins. Benzyl adenine also leads to increment in the number of amino acids and other metabolites, which attributes to more oil and protein content.

Conclusion

The salinity stress does not only hinder the growth and development of plants but also affect some physiological and metabolic activities as it affects osmolyte and ionic concentrations in plants. To prevent the crops from these losses various mitigation strategies adopted to combat the harmful effects of saline stress. In this trichoderma, mycorrhiza and rhizobium plays a positive role in reducing the harmful effects of salinity stress. Various methods like Nitric oxide application and salicylic acid also helps in reduction of saline stress in them applied in proper dose and manner. Rhizobium having mutualistic effects in leguminous plants also have beneficial effects in non-leguminous plants mustard. It reduces the adverse salinity effects through increasing water availability to the in root zones and proper supply of their minerals and helpful nutrients. Like rhizobium, mycorrhiza is a fungus, which contributes to counteract the negative impacts of saline stress on crops. It resides in rhizosphere area of soil where it forms filamentous network associating it with plant roots which provides a proper supply of minerals and nutrients and get accessed to carbs which are properly transported from roots to this is mycorrhiza. With the proper application of Trichoderma, even under saline conditions mustard have more H₂O content as well as stomata's conductance, increase in photosynthetic rate. Besides, the amount of proline in these salt applied plants was higher when treated with Trichoderma, while peroxide amount decreased. There also positive mitigating effects of cycocel and benzyl adenine observed which contribute towards alleviation of saline stress conditions. It is achieved through various ways, which include increasing water use efficiency, increment insignificant factors improvement in emergence% seedling, length of roots and vigour index of seedlings.

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Author Contributions

P.K. designed the study, established the biochemical protocols, S.B performed the experiments and collected the data analyzed and interpreted the data. S.B. wrote the paper.

Conflict of Interest Statement

We wish to confirm that there are no known conflicts of interest associated with this publication.

References

1. Ahmad P, Hashem A, Abd-Allah EF, Alqarawi AA, John R, Egamberdieva D *et al.* Role of *Trichoderma harzianum* in mitigating NaCl stress in Indian mustard (*Brassica juncea* L) through antioxidative defence system. Frontiers in plant science. 2015; 6:868.

- 2. Hashem A, Abd_Allah EF, Alqarawi AA, Al-Huqail AA, Wirth S, Egamberdieva D *et al.* The interaction between arbuscular mycorrhizal fungi and endophytic bacteria enhances plant growth of Acacia gerrardii under salt stress. Frontiers in microbiology. 2016; 7:1089.
- 3. Hashem A, Abd_Allah EF, Alqarawi AA, Wirth S, Egamberdieva D. Comparing the symbiotic performance and physiological responses of two soybean cultivars to arbuscular mycorrhizal fungi under salt stress. Saudi journal of biological sciences. 2019; 26(1):38-48.
- Egamberdieva D, Wirth SJ, Shurigin VV, Hashem A, Abd_Allah EF. Endophytic bacteria improve plant growth, the symbiotic performance of chickpea (*Cicer arietinum* L.) and induce suppression of root rot caused by Fusarium solani under salt stress. Frontiers in Microbiology. 2017; 8:1887.
- 5. Silva LVD, Oliveira SBRD, Azevedo LAD, Rodrigues AC, Bonifacio A. Coinoculation With Bradyrhizobium And Trichoderma Alleviates The Effects Of Salt Stress In Cowpea. Revista Caatinga. 2019; 32(2):336-344.
- Alenazi MM, Egamberdieva DILFUZA, Ahmad P. Arbuscular mycorrhizal fungi mitigate NaCl induced adverse effects on *Solanum lycopersicum* L. Pak. J Bot. 2015; 47(1):327-340.
- 7. Egamberdieva D, Wirth S, Jabborova D, Räsänen LA, Liao H. Coordination between Bradyrhizobium and Pseudomonas alleviates salt stress in soybean through altering root system architecture. Journal of Plant Interactions. 2017; 12(1):100-107.
- 8. Meena KK, Sorty AM, Bitla UM, Choudhary K, Gupta P, Pareek A *et al.* Abiotic stress responses and microbemediated mitigation in plants: omics strategies. Frontiers in plant science. 2017; 8:172.
- Hashem A, Abd_Allah EF, Alqarawi AA, Egamberdieva D. Arbuscular mycorrhizal fungi and plant stress tolerance. In-Plant Microbiome: Stress Response, 2018, 81-103. Springer, Singapore.
- Bencherif K, Dalpé Y, Hadj-Sahraoui AL. Arbuscular Mycorrhizal Fungi Alleviate Soil Salinity Stress in Arid and Semiarid Areas. In Microorganisms in Saline Environments: Strategies and Functions, Springer, Cham, 2019, 375-400.
- 11. Singh M, Singh A, Prasad SM, Singh RK. Regulation of plant metabolism in response to salt stress: an omics approach. Acta Physiologiae Plantarum. 2017; 39(2):48
- 12. Egamberdieva D, Wirth S, Li L, Abd-Allah EF, Lindström K. Microbial cooperation in the rhizosphere improves liquorice growth under salt stress. Bioengineered. 2017; 8(4):433-438.
- 13. Sarwat M, Hashem A, Ahanger MA, Abd_Allah EF, Alqarawi AA, Alyemeni MN *et al.* Mitigation of NaCl stress by arbuscular mycorrhizal fungi through the modulation of osmolytes, antioxidants and secondary metabolites in mustard (*Brassica juncea* L.) plants. Frontiers in plant science. 2016; 7:869.
- 14. Jahan B, Al-Ajmi MF, Rehman MT, Khan NA. Treatment of nitric oxide supplemented with nitrogen and sulfur regulates photosynthetic performance and stomatal behaviour in mustard under salt stress. Physiologia Plantarum. 2020; 168(2):490-510.
- 15. Yasmeen R, Siddiqui ZS. Ameliorative effects of *Trichoderma harzianum* on monocot crops under hydroponic saline environment. Acta physiological Plantarum. 2018; 40(1):4.

- Yasmeen R, Siddiqui ZS. Physiological responses of crop plants against *Trichoderma harzianum* in a saline environment. Acta Botanica Croatica. 2017; 76(2):154-162.
- 17. Zhang S, Gan Y, Xu B. Application of plant-growthpromoting fungi Trichoderma longibrachiatum T6 enhances tolerance of wheat to salt stress through the improvement of the antioxidative defence system and gene expression. Frontiers in plant science. 2016; 7:1405.
- López-Carrión AI, Castellano R, Rosales MA, Ruiz JM, Romero L. Role of nitric oxide under saline stress: implications on proline metabolism. Biologia Plantarum. 2008; 52(3):587.
- 19. Wani AS, Ahmad A, Hayat S, Tahir I. Epibrassinolide and proline alleviate the photosynthetic and yield inhibition under salt stress by acting on the antioxidant system in mustard. Plant physiology and biochemistry. 2019; 135:385-394.
- 20. Khoshbakht D, Asgharei MR. Influence of foliar-applied salicylic acid on growth, gas-exchange characteristics, and chlorophyll fluorescence in citrus under saline conditions. Photosynthetica. 2015; 53(3):410-418.
- 21. Yousuf PY, Ahmad A, Ganie AH, Iqbal M. Salt stressinduced modulations in the shoot proteome of *Brassica juncea* genotypes. Environmental Science and Pollution Research. 2016; 23(3):2391-2401.
- 22. Hashem A, Abd_Allah EF, Alqarawi AA, Al-Huqail AA, Shah MA. Induction of osmoregulation and modulation of salt stress in Acacia gerrardii Benth. By arbuscular mycorrhizal fungi and Bacillus subtilis (BERA 71). BioMed research international, 2016.
- 23. Hashem A, Abd_Allah EF, Alqarawi AA, Aldubise A, Egamberdieva D. Arbuscular mycorrhizal fungi enhances salinity tolerance of Panicum turgidum Forssk by altering photosynthetic and antioxidant pathways. Journal of Plant Interactions. 2015; 10(1):230-242.
- 24. Nadeem SM, Ahmad M, Zahir ZA, Kharal MA. Role of phytohormones in stress tolerance of plants. In Plant, soil and microbes. Springer, Cham, 2016, 385-421.
- 25. Zhang F, Wang Y, Liu C, Chen F, Ge H, Tian F *et al. Trichoderma harzianum* mitigates salt stress in cucumber via multiple responses. Ecotoxicology and environmental safety. 2019; 170:436-445.
- 26. Fatma M, Masood A, Per TS, Rasheed F, Khan NA. Interplay between nitric oxide and sulfur assimilation in salt tolerance in plants. The Crop Journal. 2016; 4(3):153-161.
- 27. Saxena B, Shukla K, Giri B. Arbuscular mycorrhizal fungi and tolerance of salt stress in plants. In Arbuscular mycorrhizas and stress tolerance of plants (pp. 67-97). Springer, Singapore, 2017.
- 28. Fariduddin Q, Khan TA, Yusuf M, Aafaqee ST, Khalil R. RAE. Ameliorative role of salicylic acid and spermidine in the presence of excess salt in *Lycopersicon esculentum*. Photosynthetica. 2018; 56(3):750-762.
- 29. Latef AAHA, Hashem A, Rasool S, Abd_Allah EF, Alqarawi AA, Egamberdieva D *et al.* Arbuscular mycorrhizal symbiosis and abiotic stress in plants: a review. Journal of Plant Biology. 2016; 59(5):407-426.
- 30. Sharma DK, Singh A. Current trends and emerging challenges in the sustainable management of salt-affected soils: a critical appraisal. In Bioremediation of salt-affected soils: an Indian perspective (pp. 1-40). Springer, Cham, 2017.

- Sharma PC, Singh A. Reviving the Productivity of Saltaffected Lands: Technological Options, Constraints and Research Needs. In Research Developments in Saline Agriculture (pp. 591-627). Springer, Singapore, 2019.
- Waśkiewicz A, Gładysz O, Goliński P. Participation of phytohormones in adaptation to salt stress. In Plant Hormones under Challenging Environmental Factors (pp. 75-115). Springer, Dordrecht, 2016.
- 33. Ramírez JIS, Maiti R. Research Trends in Abiotic Stress Resistance of Crops. In Bioresource and Stress Management (pp. 131-163). Springer, Singapore, 2016.
- 34. Yasmeen T, Tariq M, Iqbal S, Arif MS, Riaz M, Shahzad SM *et al.* Ameliorative Capability of Plant Growth Promoting Rhizobacteria (PGPR) and Arbuscular Mycorrhizal Fungi (AMF) Against Salt Stress in Plant. In Plant Abiotic Stress Tolerance (pp. 409-448). Springer, Cham, 2019.
- 35. Husen A, Iqbal M, Sohrab SS, Ansari MKA. Salicylic acid alleviates salinity-caused damage to foliar functions, plant growth and antioxidant system in Ethiopian mustard (*Brassica carinata* A. Br.). Agriculture & amp; Food Security. 2018; 7(1):44.
- 36. Rasool S, Hameed A, Azooz MM, Siddiqi TO, Ahmad P. Salt stress: causes, types and responses of plants. In Ecophysiology and responses of plants under salt stress (pp. 1-24). Springer, New York, NY, 2013.
- 37. Aggarwal M, Sharma S, Kaur N, Pathania D, Bhandhari K, Kaushal N *et al.* Exogenous proline application reduces phytotoxic effects of selenium by minimising oxidative stress and improves growth in bean (*Phaseolus vulgaris* L.) seedlings. Biological trace element research. 2011; 140(3):354-367.
- Hossain MA, Mostofa MG, Fujita M. Heat-shock positively modulates oxidative protection of salt and drought-stressed mustard (*Brassica campestris* L.) seedlings. J Plant Sci Mol Breed. 2013; 2(1):2.
- 39. Bharucha U, Patel K, Trivedi UB. Optimization of indole acetic acid production by Pseudomonas putida UB1 and its effect as plant growth-promoting rhizobacteria on mustard (*Brassica nigra*). Agricultural research. 2013; 2(3):215-221.
- 40. Ananthi T. Influence of intercropping systems mycorrhizal inoculation and fertilizer levels on the productivity of maize based cropping system, 2013.
- 41. Natwadia S. Physiology of Salt Tolerance in Indian mustard [*Brassica Juncea* (L.) Czern and Coss (SKNAU) (Doctoral dissertation, SKNAU).
- 42. Sharma, K. C. Physiology of Taramira (Eruca stiva) Mill under Salinity Stress (SKNAU) (Doctoral dissertation, SKNAU).