Vol. III, Issue 1, Jan - March 2022 *International Research Journal of Education and Innovation*
ISSN No: (ONLINE): 2710-043 ISSN No: ISSN (PRINT): 2710-0448
www.irjei.com DOI: https://doi.org/10.53575/irjei.v3.01.14(22)141-153

Role of Compatible Solutes in Alleviating Effect of Abiotic Stress in Plants

Dr. Munifa Jabeen
Department of Botany, Government College University, Faisalabad, Pakistan.
Email: munifa.jabeen63@gmail.com

Atiqa Jabeen
Government College University, Faisalabad, Pakistan.
Email: atiqajabeen1993@gmail.com

Professor Dr. Matloob Ahmad
(Corresponding Author) Dean Faculty of Arts & Social Sciences, The University of Faisalabad.
Email: dr.matloobahmad906@gmail.com

Received on: 14-01-2022 Accepted on: 15-02-2022

Abstract:

Plants face assorted of abiotic stresses such as, salinity, drought and heavy metals which produce ROS, and finally inhibit normal growth plant production. To stop cellular destruction due to oxidative stress, these abiotic stresses increase complex reactions in plants to avoid damage and boost their sustainability under severe stress situations. Plants produce several organic solutes known as osmoprotectant such as, polyamines, sugars, proline and glycine betaine (GB), to adjust the cellular mechanism and stable the membrane structure and proteins towards environmental stress. As well, they also defend the plant cells from oxidative stress by stopping the accumulation of damaging effect of ROS. In this review, we have deliberated the mechanisms of organic solutes as well as several functions in plants under abiotic stress situations. The organic solutes that are also known as osmolytes/osmoprotectants comprise soluble sugars, proline and glycine betaine.

Keywords: Abiotic stresses, organic solutes

1. Introduction

Plants face a variation of environmental stresses including, drought, heavy metal, heat salinity, light, cold and pesticide that impedes their cellular and physiological functioning (Sharma et al., 2018). Environmental stresses are deliberated as the main hazard to worldwide agronomy systems (Jabeen et al., 2019; Khan et al., 2015). Additionally, activities of anthropogenic also controlled to degradation of agricultural environment and plant yield caused by increased metal, ozone and drought stress (Sharma et al., 2019). These environmental strains cause enormous crop damage by decreasing yield by maximum 50%

in various plant crops recognized to the increased reactive oxygen species (ROS) that induce oxidative stress in plants (Shafi et al., 2009).
Environmental stresses decrease plant growth and development by affecting various physio organic mechanisms for example, hormonal indicating, antioxidant systems and photosynthesis (Jabeen et al., 2019; Saed-Moucheshi; 2019). These abiotic stresses induce compound responses in crop to inhibit growth and enhanced their survivability under drought stress situations. Plants modify several molecular and cellular changes in response to ecological strains that ultimately base their growth (Giri, 2011). Plants synthesized organic solutes or osmolytes to defend the photosynthetic machinery from numerous abiotic stresses. The most recognized organic solutes like, polyamines, sugars trehalose, mannitol, sorbitol, proline and glycine betaine (GB) These osmoprotectant get synthesized under several environmental strains and confer resistance to cell deprived of snooping through the cellular mechanism of the plant (Ozturk et al., 2021; Anjum et al., 2016). Accumulated sugars like mannitol, galactinol, and trehalose under stress has been stated commonly in plants, and some plant genetic factor plays a significant parts in bio-synthesis of osmolytes serving in the change of environmental stress tolerance in transgenic plants (Ozturk et al., 2021). Likewise, proline synthesis is very important mechanisms or reactions in several crop plants under many stresses (Begum et al., 2019). It is indispensible to comprehend the mechanisms that control altered mechanism and process essential ecological stress forbearance in plants. Plant growth regulators play a major role in several physio-logical and biochemical mechanisms in crop plants. Their role in reducing various stresses is serious in provided that lenience to plants under abiotic stress situation (Kaya et al., 2019; Sharma et al., 2019).

2. Role of organic solutes to confer abiotic stress resistance

Organic solutes are osmolytes to maintain cell integrity and increase cell potential without hampering the regular uptake. The major purpose of osmoprotectant is to adjust the osmotic potential. These compatible solutes aid the plants to tolerate severe stress throughout their life cycle (Sing et al., 2015). These organic metabolites alleviate the osmotic changes involving cells surrounds and cytoplasm (Ozturk et al., 2021). As well, they keep plants from injury through stopping the accumulation of ROS (Laxa et al., 2019). Compatible solutes are neutral compounds that protection the membranes and also proteins against several diseases and stress aspects on cellular function (Ozturk et al., 2021). These organic solutes comprise glycinebetain, proline, alanine betaine, sucrose, trehalose and polyols. These osmolytes protect the metabolism of the plant and is the central approach approved by vegetation to response strains. Several studies have proposed the influence of organic solutes in salinity (Masouleh et al., 2020; Wang et al., 2004), heavy metal (Sharma & Dietz, 2006) light, drought (Anjum et al.,2016) pesticide, osmotic (Conde et al., 2011) and heat stress (Sharma et al., 2019).

2.1. Drought stress

The shrinkage in water pressure of plants is very important signs of water scarcity in crop plant. It disturbs the plant physio-logical mechanism such as, photosynthesis and roots plant loss (Laxa et al., 2019). Din et al. (2011) detected a severe decline in chlorophyll content in plants upon response to water pressure, which aspects to the influence on enzymes activity connected to photosynthetic content (Ashraf & Karim, 1991). Plants must establish several mechanisms to lessen water pressure, one of them being production of compatible solutes or osmolytes in the plants in reaction to scarcity. A variety of dynamic organic solutes like pyrols, proline, sugar, glycinebetain, and organic metabolites get stored to stable the water potential in the shortage of water. Because of accumulation of organic molecules in tissue under drought pressure, the cell osmotic pressure becomes very harmful, which sources water endos-mosis into the cell tissue and stable the cell water potential. The osmotic adjustment is probable caused by the production of organic solutes. Likewise, proline osmolytes is the most significant under abiotic stresses included drought, salinity (Gupta & Thind, 2015). Alexieva et al. (2001) detected increased the level of proline in pea plant caused by scarcity. Also, Yamada et al. (2005) stated that production of proline in variety of Petunia hybrid under severe condition of stress. Various studies have proposed the part of proline in tolerance in case of water stress (Borgo et al., 2015). More accumulation of glycinebetain has been noticed in cotton plants improved to water stress (Cheng et al., 2018; Iqbal et al., 2005). Plants can endure to water stress mostly by osmotic change and stabilizing the antioxidant defense system that supports in molecules scavenging reactive oxygen species and provided that strength to the cell membranes organelles.

2.2. Salt stress

Salinity stress disturbs more than (1/3) of the land-living frame on World (Rajasheker et al., 2019). It is an ecological restraint that has two main elements: An ionic element and osmotic elements which are related to higher production of ions and decreased the outer osmotic potential of soil that positions hazard at greater absorption. Salinity stress reasons lipid breakdown, disturbs nutrient imbalance, water potential interrupts the activity of several enzymes, and produces reactive oxygen species that ultimately destruction of the photosynthetic machinery (Tang & Luo, 2018). Plants tolerate stress by storing low molecular weight organic solutes like polyamines, GB, and proline that helps to sustain the veracity of the membrane. These osmolytes boosts the germination rate, development, and growth thus inducing tolerance in reaction to salinity stress (Elhakem, 2020).

Several studies proposed that salinity stress lessens the enzymes accountable in biosynthesis of glycinebetain and proline (Sumithra et al., 2006; Ahmed et al., 2019) and their higher accumulation is related with more tolerance of stress. Proline accumulation is an adaptive mechanism towards salt stress (Elhakem, 2020). Proline adjusts stress reactive proteins, causing in perfection of plant adjustment against abiotic stress. Moreover, peroxidase and catalase enzymes activities were showed to be greater in Pancratium maritimum in proline under salinity stress (Khedr et al., 2003). It improved the enzymes that play an important role in antioxidative potential as known in *Nicotiana tobacum* (Hoque et al., 2008). Glycinebeyaine is an organic molecule that defends the plants towards disease and abiotic stress (Gupta & Thind, 2015). . It also protects the plant by alleviating proteins such as, RuBisCo and decreasing the production of ROS (Laxa et al., 2019). Lutts, (2000) detected that glycinebetain accumulation enhanced the endure capability and development of salted plants.

2.3. Temperature stress

Plants are exposed to an assortment of heat fluctuations in varying periods. Higher abiotic stresses effects in a reduced chlorophyll biosynthesis in crop cell (Reda, & Mandoura, 2011) which is a stress indicator in plastids (Li et al., 2010). Such as, temperature stress reduces the of 5-amino-levulinate-de-hydratase, an enzyme that protect in photosynthetic synthesis (Kumar et al., 1998). Also, chilling stress also disturbs crop production by damaging the bio-physiological mechanism of plants. Chilling stress makes higher levels reactive oxygen species, mainly hydrogen-peroxide (H_2O_2), which might be regulator for harmfully disturbing germination and growth expansion (Xu et al., 2007). Plants have established several stratagems to stand temperature fluctuations. Some of the apparatuses suitable in plants under temperature stress is the organic molecules accumulation that have a defensive part in crop plant. Kishor et al. (2005) stated that improved in plants growth and showing to cold stress caused by higher proline. Foliar treatment of proline at low application improved tolerance against cold stress (Hayat et al., 2012). Some works have described the role of glycinebetain in defending plants under disease temperature stress (Kawakami et al., 2008). Due to improvement in machinery, involved genes in biosynthesis of glycinebetaine are now moved in particular non-productive plants that aid plants endure under stresses. Such as, gene (cod-A) gives tolerance against chilling stress in *Oryza sativa*, which defends the plant from tissue injury, activity of ROS and enzymes (Sharma et al., 2019). The accumulation of ROS during the high temperature stress conditions destruct the photosynthetic appratus in chloroplast, but glycinebetaine sustained the action of amino acids and keeps the plant from abiotic environments.

2.4. Heavy metal stress

Contamination of heavy metals has occurred as a wide threat in adding to heat, salinity and drought strain (Kohli et al., 2018). Caused by rises in industrialization and urbanization, the treatment of heavy metals has improved at a disturbing level. The extreme production of reactive oxygen species in exposure to stress in plants (Tiwari & Lata, 2018) damaging the cellular, physiological and biochemical mechanisms in plants (Ali et al., 2021). Exposure of Pb decrease in plant pigment in *Coronopus didymus*, which influence to reduced chlorophyll or its breakdown in reaction to stress (Sidhu et al., 207). Thus, plants are involved in several mechanisms to purify heavy metals and endure heavy metal stress. Dhir et al. (2012) measured compatible solutes accumulation in *Salvinia natans* towards to heavy metals. They observed better concentration of mannitol, and glycinebetaine in reaction to Fe, Pb Cr, Mn, Zn, and Cu, (Ali et al., 2020). Also, Bhatti et al. (2013) detected that foliar applied glycinebetaine improved the resistance of wheat under heavy metal stress. They found that treatment of glycinebetaine higher in biomass, shoot and root length, photosynthetic pigments, and supported ROS hunting in adding to osmotic adjustments (Bhatti et al., 2013).

3. Organic solutes

Compatible osmoprotectant are organic solutes that maintain the osmotic potential and the cell integrity. The main role of organic solutes is to regulator osmotic adjustment. Organic solutes reform lipid membrane structure for increase the structure of crystalline or liquid required for proper source of energy and function of membrane. These organic solutes involve soluble sugars, organic acids, polyamines, lipids and amino acid (Dikilitas et al., 2018; Yadav, 2010). Organic solutes are natural osmolytes that protection the cells and proteins towards some stress aspects on cell s (Sharma structuret al., 2019).

Some of the most collective stress reactions in leaves are high accumulation of various forms of solutes (Serraj & Sinclair, 2002). Organic molecules or osmolytes are highly soluble compounds, low molecular-weight that are generally non-toxic at more cell absorptions. Mostly, they defend plants from anxiety or diseases during diverse ways, comprising de-toxification of ROS, involvement to cellular osmotic change, maintenance of membrane and protection of membrane stabality (Masouleh et al., 2020). Moreover, for several of these organic solutes also protect the plant structures from desiccation damage, they are usually discussed to as osmolytes/osmoprotectants. These organic solutes such as GB, proline, sucrose, pipecolate, trehalose alanine-betaine, (Ozturk et al., 2021).

3.1. Glycine betaine

Several quaternary ammonium-compounds recognized in plants, glycinebetaine happen most in large amounts in reaction to drought stress (Aamer et al., 2018). Glycinebetaine is mostly abundant in chloroplast where it shows a important part in protection of thylakoid membrane, thus retaining photosynthetic efficacy (Gupta & Thind, 2015). In plants, glycinebetaine is accumulated in chloroplast from serine to ethanolamine (Sharma et al., 2019). Choline is changed into betaine- aldehyde, with the presence of choline mono-oxygenase (CMO), which is then transfored into glycinebetaine by betaine-aldehyde-dehydrogenase (BADH). While other ways like, through N methylation glycine is also identified, the pathway from choline to glycine betaine has been known in all glycinebetaine producing plant species (Weretilnyk et al., 1989). Glycinebetaine is recognized to synthesized in reaction to stress in several plants, as well as spinach (*Spinacia oleracea*), sorghum (*Sorghum bicolor*) sugar beet (*Beta vulgaris*), barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) (Ashraf & Foolad, 2007).

3.2. Foliar application of GB on plants

Foliar applied glycinebetaine is proposed as a valued of stimulating tolerance in crop plants under abiotic environments with poor production (Kurepin et al., 2015). Various aspects define the effectiveness of foliar treatment of glycinebetaine including amount of glycinebetaine applied, treatment and forms of plant species on which is glycinebetaine applied (Ashraf & Foolad, 2007). Exogenous applications of glycinebetaine considerably lessen the destructive effects of toxicity of Cr on biomass and total chlorophyll in mung bean (Jabeen et al., 2016). A similar enhancement in amaranth, a significant increase was noted in biomass, growth, uptake nutrients and chlorophyll content under foliar applied glycinebetaine (Nusrat et al., 2014). Due to Cd stress, a noticeably improve the p physiochemical traits by decreasing the superoxide radicals, MDA and H_2O_2 contents in both wheat cultivars under the treatment of glycinebetaine (Duman et al., 2011). Exogenously applied of glycinebetaine increase the activities of antioxidant enzymes and lessen the stress induce under the Cr stress in wheat plant (Ali et al., 2015). Foliar applied thiamin significantly increased in both turnip cultivars under the shortage of water (Jabeen et al., 2020).

Foliar applied glycinebetaine during the chickpea bud stage exposed much superior

ISSN No: (ONLINE): 2710-043 ISSN No: ISSN (PRINT): 2710-0448
www.irjei.com DOI: https://doi.org/10.53575/irjei.v3.01.14(22)141-153

enhancement in flowering and pollen growth, active pollen sustainability, larger receptivity in stigma, tremendous growth in pollen tube, and wide feasibility of ovule in chilling stress situations. Instead, during pod filling, application of glycinebetaine showed in a higher in the yield, and its number of seeds, pods and seed biomass were considerably better after application. Glycinebetaine significantly induced the chilling stress in plants over active enhancement in total chlorophyll and leaf water contents for now, it also decline the abscisic acid and ROS (Cheng et al., 2018). Several plant species spinach (*Spinacia olerecea* L.) and barley (*Hordeum vulgare* L.) (Jagendorf & Takabe, 2001) produce supplementary glycinebetaine in several parts of chloroplasts than plants similar tobacco and rockcress (*Arabidopsis*) (Sulpice et al., 2003). Improving the activities of flower, pod formation and various yield attributes under the foliar application of glycinebetaine to chickpea plant, provides a moderately cold tolerance in the plants (Nayyar et al., 2005). When foliar applied glycinebetaine on plants, it proceeds successfully from the tomato leaves and formerly transfers it into several parts. Foliar applied glycinebetaine at the maximum level in plants plays a significant role towards cold high temperature. Maximum glycinebetaine is moved inside the cytosol. But, a small amount transfers to the chloroplasts and probably to several parts of cell. Consequently, photosynthesis is secure and cold tolerance is higher in glycinebetaine treated plants. It is needed to notice large quantities of glycinebetaine in the leaves like as the buds and apices in sprayed plants. Higher amount of glycinebetaine in these cells are supposed to be essential for plant protection and supporting growth rescue after cold stresses (Park et al., 2006). Then, the position of glycinebetaine production in cell parts of plants has the vital part on amount of its efficacy on the plant acceptance to pressures.

3.3. Proline

Proline is an amino-acids type that is extensively occurs in plants and regularly incorporated in excessive content in reaction to biotic and abiotic stresses. Proline is not only controlled in involvement as an organic solute, then moreover it alleviates several parts of cells including as membranes, hunts free radicals and defenses redox potential of cells towards environmental stresses (Kahlaoui et al., 2018; Ashraf & Foolad, 2007). Proline is the major source of energy that can involve to generation heat in the voodoo lily (Hare & Cress, 1997). Proline biosynthesis has been connected to ornithine or glutamate pathway and too particularly, linked to glutamate-glutamine metabolism and oxidative pentose phosphate pathway (Verslues & Sharma, 2010). Accumulation of proline in the cytoplasm by the glutamate pathway, glutamate changed into 1 pyrroline 5 carboxylate by 1 pyrroline 5 carboxylate synthetase, which is changed into proline by 1 pyrroline 5 carboxylate reductase. Instead, the ornithine pathway relates to proline accumulation in the mitochondrion from ornithine. Ornithine is converted into P5 C and glutamate-semialdehyde by ornithine-δ aminotransferase (OAT) and changed to proline. Also, ProDH, a fundamental enzyme in proline metabolism and catabolized proline into P5C (Trovato et al., 2008). The subsequent of bio-products are both proline synthetase, OAT and P5CS. This model confirms that the ProDH is the fundamental controller in the increasing proline production in plants (Szabados & Savoure, 2010).

ISSN No: (ONLINE): 2710-043 ISSN No: ISSN (PRINT): 2710-0448
www.irjei.com DOI: https://doi.org/10.53575/irjei.v3.01.14(22)141-153

3.4. Foliar application of proline on plant

Proline is an osmolyte that stores in qualified levels in plants under drought stress (Junior et al., 2018). It performs many roles in plants, as well as signaling, protein stabilizing, stress tolerance, radical hunting and helps as a nutrient tank (Hayat et al., 2012). Its foliar treatment is also active in the mitigation of drought stress. Some instructions have underlined its ameliorative effect in several abiotic stresses (Singh et al., 2018). Exogenously applied proline better the enzymes activity and the turgor potential of pea under nickel stress (Gajewska & Skłodowska, 2005). Likewise, foliar application of proline increased the higher biomass, antioxidant defense potential and photosynthetic activities of plants under Cd stress conditions (Hayat et al., 2021).

Foliar application of proline improved activities enzyme and greater chlorophyll content, absorption of nutrient and all growth traits under Cd stress in pigeon pea plants. Germination is the main sensitive phase in the plant cycle (Hubbard et al., 2012; Muhammad et al., 2021) as it is actual complex to environment stresses. In certain, salt stress causes osmotic stress that ion toxicity and bounds absorption water caused by the higher production of Na^+ and Cl^- (Farissi et al., 2011; Farissi et al., 2013). Treatment of proline improves the harmful effect of $NaCl^-$, but at the rate of 100 mM proline did not showed a significant effect. Also, foliar applied proline at the level of 50 mM significantly enhanced seed germination of both cultivars of *S. bicolor* under salinity stress (Nawaz et al., 2010). Then foliar application of proline at appropriate amount may improve the adverse influence of salinity stress by osmotic adjustment, and then comprehensive studies behind these records are still necessary to well know the involvement of molecular mechanism. Proline treated improved 1000-grain weight and grain yield of salt-stressed *T. aestivum* (El Moukhtari et al., 2020; Rady et al., 2019). Exogenously applied proline improved the number of seeds, 100-grain weight and total grain weight under stressed *Z. mays* (Alam et al., 2016). Over-all, foliar treatment of proline improved plant growth and production under salt stress however the primary mechanisms, almost certainly related to several compounds regulations, still remain intangible.

Proline can also save cell membranes and proteinsagainst salt stress and oxidative stress by increasing the several antioxidative activities (El Moukhtari et al., 2020). Such as tobacco growth, cells suspension under salinity stress was stimulated by foliar treatment of proline, which was suggested to be due to proline activity as a defender of membranes and enzymes (Okuma et al., 2004). In stressed soya-bean plant, a significantly improved in activities of POD and SOD under foliar treatment of proline(Hua & Guo, 2002). In barley under salt stress exogenouslly applied of proline caused in a reduction in NaCl production and an improved in growth (Lone et al., 1987). Such enhanced influences of proline were showed to be because of stabilization of membrane (Mansour, 1998).

3.5. Soluble sugars

Carbohydrates/sugars are very significant osmoprotectants to maintain plant metabolism and membrane structure during environmental stresses conditions (Masouleh et al., 2020). During abiotic stresses, soluble sugars have a role to stable the osmotic regulations and organize reactive oxygen species. Soluble carbohydrates are fundamentals in metabolic processes and protect some metabolic processes, as well as respiration, photosynthesis, and oxidative pathway from two scavenging structures and production of ROS. For example, mannitol to protect the chloroplastic organelle from poisonous harms due to free radicals during abiotic stress situations. Similarly, trehalose adjusts the mechanisms of ABA and carbon when plants are response to osmotic stress (Upadhyaya et al., 2013).

Conclusion

Several plant species produce naturally organic solutes or osmolytes as a major proline, glycinebetaine and soluble sugars when response to environmental strains. These solutes are to show very important roles in facilitate to osmotic adjustment and defending cellular mechanisms in strained plants. But, not all plants produce organic solutes proline and glycinebetaine in abundant levels to support avoiding severe impact of environmental pressures. These organic solutes stable the redox potential of cells by increasing the productivity of reactive ROS hunting and result in lessening of oxidative destruction to yield. Therefore, many methodologies have been considered to raise the amount of these complexes in plants under stress situations to enhance their strain tolerance. First, it is the use of short-cut method; foliar applied proline is a positive approach in increasing the plant production under abiotic stresses. The application of proline, GB, commercially, artificially has been used. Many studies reported proline, GB osmolytes proved to overcome the adverse influence of environmental experiments on stressed plants. Other method has been to genetically engineer plants that are proficient of manufacturing appropriate concentrations of these compounds in exposure to abiotic pressures. While several developments has been made in leading genes for the accumulation of these compounds in naturally low production or non producing plant species, production level in transgenic plants have often been lacking or low to increase plant stress tolerance. I conclude that, the positive effect of osmolytes in plants under water stress. Foliar applied proline and GB increased the plant crop production under stressed plant.

References

1. Aamer, M., Muhammad, U. H., Li, Z., Abid, A., Su, Q., Liu, Y., & Huang, G. (2018). Foliar application of glycinebetaine (GB) alleviates the cadmium (Cd) toxicity in spinach through reducing Cd uptake and improving the activity of anti-oxidant system. *Applied Ecology and Environmental Research*, *16*(6), 7575-7583.
2. Ahmed, S., Ahmed, S., Roy, S. K., Woo, S. H., Sonawane, K. D., & Shohael, A. M. (2019). Effect of salinity on the morphological, physiological and biochemical properties of lettuce (*Lactuca sativa* L.) in Bangladesh. *Open Agriculture*, *4*(1), 361-373.
3. Alam, R., Das, D. K., Islam, M. R., Murata, Y., & Hoque, M. A. (2016). Exogenous proline enhances nutrient uptake and confers tolerance to salt stress in maize (Zea mays L.). *Progressive agriculture*, *27*(4), 409-417.
4. Alexieva, V., Sergiev, I., Mapelli, S., & Karanov, E. (2001). The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant, Cell & Environment*, *24*(12), 1337-1344.
5. Ali, S., Abbas, Z., Seleiman, M. F., Rizwan, M., YavaŞ, İ., Alhammad, B. A., & Kalderis, D. (2020). Glycine betaine accumulation, significance and interests for heavy metal tolerance in plants. *Plants*, *9*(7), 896.
6. Ali, S., Chaudhary, A., Rizwan, M., Anwar, H. T., Adrees, M., Farid, M., & Anjum, S. A. (2015). Alleviation of chromium toxicity by glycinebetaine is related to elevated antioxidant enzymes and

suppressed chromium uptake and oxidative stress in wheat (*Triticum aestivum* L.). *Environmental Science and Pollution Research*, *22*(14), 10669-10678.

7. Anjum, S. A., Tanveer, M., Hussain, S., Shahzad, B., Ashraf, U., Fahad, S., & Tung, S. A. (2016). Osmoregulation and antioxidant production in maize under combined cadmium and arsenic stress. *Environmental Science and Pollution Research*, *23*(12), 11864-11875.
8. Ashraf, M. F. M. R., & Foolad, M. R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and experimental botany*, *59*(2), 206-216.
9. Ashraf, M., & Karim, F. (1991). Screening of some cultivars/lines of black gram (*Vigna mungo* L. Hepper) for resistance to water stress. *Trop. Agric*, *68*, 57-62.
10. Begum, N., Ahanger, M. A., Su, Y., Lei, Y., Mustafa, N. S. A., Ahmad, P., & Zhang, L. (2019). Improved drought tolerance by AMF inoculation in maize (*Zea mays*) involves physiological and biochemical implications. *Plants*, *8*(12), 579.
11. Bhatti, K. H., Anwar, S., Nawaz, K., Hussain, K., Siddiqi, E. H., Sharif, R. U., & Khalid, A. (2013). Effect of exogenous application of glycinebetaine on wheat (*Triticum aestivum* L.) under heavy metal stress. *Middle-East J Sci Res*, *14*(1), 130-137.
12. Borgo, L., Marur, C. J., & Vieira, L. G. E. (2015). Effects of high proline accumulation on chloroplast and mitochondrial ultrastructure and on osmotic adjustment in tobacco plants. *Acta Scientiarum. Agronomy*, *37*, 191-199.
13. Cheng, C., Pei, L. M., Yin, T. T., & Zhang, K. W. (2018). Seed treatment with glycine betaine enhances tolerance of cotton to chilling stress. *The Journal of Agricultural Science*, *156*(3), 323-332.
14. Cheng, C., Pei, L. M., Yin, T. T., & Zhang, K. W. (2018). Seed treatment with glycine betaine enhances tolerance of cotton to chilling stress. *The Journal of Agricultural Science*, *156*(3), 323-332.
15. Conde, A., Silva, P., Agasse, A., Conde, C., & Gerós, H. (2011). Mannitol transport and mannitol dehydrogenase activities are coordinated in *Olea europaea* under salt and osmotic stresses. *Plant and Cell Physiology*, *52*(10), 1766-1775.
16. Dhir, B., Nasim, S. A., Samantary, S., & Srivastava, S. (2012). Assessment of osmolyte accumulation in heavy metal exposed Salvinia natans. *International Journal of Botany*.
17. Dikilitas, M., Karakas, S., & Ahmad, P. (2018). Predisposition of crop plants to stress is directly related to their DNA health. In *Plant Microbiome: Stress Response* (pp. 233-254). Springer, Singapore.
18. Din, J., Khan, S. U., Ali, I., & Gurmani, A. R. (2011). Physiological and agronomic response of canola varieties to drought stress. *J Anim Plant Sci*, *21*(1), 78-82.
19. Duman, F., Aksoy, A., Aydin, Z., & Temizgul, R. (2011). Effects of exogenous glycinebetaine and trehalose on cadmium accumulation and biological responses of an aquatic plant (*Lemna gibba* L.). *Water, Air, & Soil Pollution*, *217*(1), 545-556.
20. El Moukhtari, A., Cabassa-Hourton, C., Farissi, M., & Savouré, A. (2020). How does proline treatment promote salt stress tolerance during crop plant development?. *Frontiers in plant science*, *11*, 1127.
21. Elhakem, A. (2020). Salicylic acid ameliorates salinity tolerance in maize by regulation of phytohormones and osmolytes. *Plant, Soil and Environment*, *66*(10), 533-541.
22. Farissi, M., Bouizgaren, A., Faghire, M., Bargaz, A., & Ghoulam, C. (2011). Agro-physiological responses of Moroccan alfalfa (*Medicago sativa* L.) populations to salt stress during germination and early seedling stages. *Seed Science and Technology*, *39*(2), 389-401.
23. Farissi, M., Ghoulam, C., & Bouizgaren, A. (2013). Changes in water deficit saturation and photosynthetic pigments of alfalfa populations under salinity and assessment of proline role in salt tolerance. *Agric. Sci. Res. J*, *3*(1), 29-35.
24. Gajewska, E., & Skłodowska, M. (2005). Antioxidative responses and proline level in leaves and roots of pea plants subjected to nickel stress. *Acta Physiologiae Plantarum*, *27*(3), 329-340.
25. Giri, J. (2011). Glycinebetaine and abiotic stress tolerance in plants. *Plant signaling &*

behavior, *6*(11), 1746-1751.
26. Gupta, N., & Thind, S. (2015). Improving photosynthetic performance of bread wheat under field drought stress by foliar applied glycine betaine. *Journal of Agricultural Science and Technology*, *17*(1), 75-86.
27. Gupta, N., & Thind, S. (2015). Improving photosynthetic performance of bread wheat under field drought stress by foliar applied glycine betaine. *Journal of Agricultural Science and Technology*, *17*(1), 75-86.
28. Hare, P. D., & Cress, W. A. (1997). Metabolic implications of stress-induced proline accumulation in plants. *Plant growth regulation*, *21*(2), 79-102.
29. Hayat, K., Khan, J., Khan, A., Ullah, S., Ali, S., & Fu, Y. (2021). Ameliorative Effects of Exogenous Proline on Photosynthetic Attributes, Nutrients Uptake, and Oxidative Stresses under Cadmium in Pigeon Pea (Cajanus cajan L.). *Plants*, *10*(4), 796.
30. Hayat, S., Hayat, Q., Alyemeni, M. N., Wani, A. S., Pichtel, J., & Ahmad, A. (2012). Role of proline under changing environments: a review. *Plant signaling & behavior*, *7*(11), 1456-1466.
31. Hayat, S., Hayat, Q., Alyemeni, M. N., Wani, A. S., Pichtel, J., & Ahmad, A. (2012). Role of proline under changing environments: a review. *Plant signaling & behavior*, *7*(11), 1456-1466.
32. Hoque, M. A., Banu, M. N. A., Nakamura, Y., Shimoishi, Y., & Murata, Y. (2008). Proline and glycinebetaine enhance antioxidant defense and methylglyoxal detoxification systems and reduce NaCl-induced damage in cultured tobacco cells. *Journal of plant physiology*, *165*(8), 813-824.
33. Hua, B., & Yuguo, W. (2002). Effect of exogenous proline on SOD and POD activity for soybean callus under salt stress. *Acta Agriculturae Boreali-Sinica*, *17*(3), 37-40.
34. Hubbard, M., Germida, J., & Vujanovic, V. (2012). Fungal endophytes improve wheat seed germination under heat and drought stress. *Botany*, *90*(2), 137-149.
35. Iqbal, N., Ashraf, M. Y., & Ashraf, M. (2005). Influence of water stress and exogenous glycinebetaine on sunflower achene weight and oil percentage. *International Journal of Environmental Science & Technology*, *2*(2), 155-160.
36. Jabeen, M., Akram, N. A., Ashraf, M., Alyemeni, M. N., & Ahmad, P. (2021). Thiamin stimulates growth and secondary metabolites in turnip (*Brassica rapa* L.) leaf and root under drought stress. *Physiologia Plantarum*, *172*(2), 1399-1411.
37. Jabeen, M., Akram, N. A., Ashraf, M., & Aziz, A. (2019). Assessment of biochemical changes in spinach (*Spinacea oleracea* L.) subjected to varying water regimes. *Sains Malaysiana*, *48*(3), 533-541.
38. Jabeen, N., Abbas, Z., Iqbal, M., Rizwan, M., Jabbar, A., Farid, M., & Abbas, F. (2016). Glycinebetaine mediates chromium tolerance in mung bean through lowering of Cr uptake and improved antioxidant system. *Archives of Agronomy and Soil Science*, *62*(5), 648-662.
39. Jagendorf, A. T., & Takabe, T. (2001). Inducers of glycinebetaine synthesis in barley. *Plant Physiology*, *127*(4), 1827-1835.
40. Júnior, D. F., Gaion, L. A., Júnior, G. S., Santos, D. M. M., & Carvalho, R. F. (2018). Drought-induced proline synthesis depends on root-to-shoot communication mediated by light perception. *Acta physiologiae plantarum*, *40*(1), 1-5.
41. Kahlaoui, B., Hachicha, M., Misle, E., Fidalgo, F., & Teixeira, J. (2018). Physiological and biochemical responses to the exogenous application of proline of tomato plants irrigated with saline water. *Journal of the Saudi Society of Agricultural Sciences*, *17*(1), 17-23.
42. Kawakami, A., Sato, Y., & Yoshida, M. (2008). Genetic engineering of rice capable of synthesizing fructans and enhancing chilling tolerance. *Journal of Experimental Botany*, *59*(4), 793-802.
43. Kaya, C., Ashraf, M., Wijaya, L., & Ahmad, P. (2019). The putative role of endogenous nitric oxide in brassinosteroid-induced antioxidant defence system in pepper (*Capsicum annuum* L.) plants under water stress. *Plant Physiology and Biochemistry*, *143*, 119-128.
44. Khan, M. I. R., Fatma, M., Per, T. S., Anjum, N. A., & Khan, N. A. (2015). Salicylic acid-induced abiotic

stress tolerance and underlying mechanisms in plants. *Frontiers in plant science*, *6*, 462.
45. Khedr, A. H. A., Abbas, M. A., Wahid, A. A. A., Quick, W. P., & Abogadallah, G. M. (2003). Proline induces the expression of salt-stress-responsive proteins and may improve the adaptation of *Pancratium maritimum* L. to salt-stress. *Journal of experimental botany*, *54*(392), 2553-2562.
46. Kishor, P. K., Sangam, S., Amrutha, R. N., Laxmi, P. S., Naidu, K. R., Rao, K. S.,& Sreenivasulu, N. (2005). Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance. *Current science*, 424-438.
47. Kohli, S. K., Handa, N., Sharma, A., Gautam, V., Arora, S., Bhardwaj, R., & Ahmad, P. (2018). Combined effect of 24-epibrassinolide and salicylic acid mitigates lead (Pb) toxicity by modulating various metabolites in *Brassica juncea* L. seedlings. *Protoplasma*, *255*(1), 11-24.
48. Kumar Tewari, A., & Charan Tripathy, B. (1998). Temperature-stress-induced impairment of chlorophyll biosynthetic reactions in cucumber and wheat. *Plant physiology*, *117*(3), 851-858.
49. Kurepin, L. V., Ivanov, A. G., Zaman, M., Pharis, R. P., Allakhverdiev, S. I., Hurry, V., & Hüner, N. P. (2015). Stress-related hormones and glycinebetaine interplay in protection of photosynthesis under abiotic stress conditions. *Photosynthesis research*, *126*(2), 221-235.
50. Laxa, M., Liebthal, M., Telman, W., Chibani, K., & Dietz, K. J. (2019). The role of the plant antioxidant system in drought tolerance. *Antioxidants*, *8*(4), 94.
51. Li, J., Pandeya, D., Nath, K., Zulfugarov, I. S., Yoo, S. C., Zhang, H., & Paek, N. C. (2010). Zebra-necrosis, a thylakoid-bound protein, is critical for the photoprotection of developing chloroplasts during early leaf development. *The Plant Journal*, *62*(4), 713-725.
52. Lone, M. I., Kueh, J. S. H., Wyn Jones, R. G., & Bright, S. W. J. (1987). Influence of proline and glycinebetaine on salt tolerance of cultured barley embryos. *Journal of Experimental Botany*, *38*(3), 479- Mansour, M. M. F. (1998). Protection of plasma membrane of onion epidermal cells by glycinebetaine and proline against NaCl stress. *Plant Physiology and Biochemistry*, *36*(10), 767-772.490.
53. Lutts, S. (2000). Exogenous glycinebetaine reduces sodium accumulation in salt-stressed rice plants. *International Rice Research Notes*, *25*(2), 39-40.
54. Masouleh, S. S. S., Aldine, N. J., & Sassine, Y. N. (2020). The role of organic solutes in the osmotic adjustment of chilling-stressed plants (vegetable, ornamental and crop plants). *Ornamental Horticulture*, *25*, 434-442.
55. Masouleh, S. S. S., Aldine, N. J., & Sassine, Y. N. (2020). The role of organic solutes in the osmotic adjustment of chilling-stressed plants (vegetable, ornamental and crop plants). *Ornamental Horticulture*, *25*, 434-442.
56. Muhammad, I., Shalmani, A., Ali, M., Yang, Q. H., Ahmad, H., & Li, F. B. (2021). Mechanisms regulating the dynamics of photosynthesis under abiotic stresses. *Frontiers in Plant Science*, *11*, 2310.
57. Nawaz, K., Talat, A., Hussain, K., & Majeed, A. (2010). Induction of salt tolerance in two cultivars of sorghum (Sorghum bicolor L.) by exogenous application of proline at seedling stage. *World Applied Sciences Journal*, *10*(1), 93-99.
58. Nayyar, H., Chander, K., Kumar, S., & Bains, T. (2005). Glycine betaine mitigates cold stress damage in chickpea. *Agronomy for sustainable development*, *25*(3), 381-388.
59. Nusrat, N., Shahbaz, M., & Perveen, S. (2014). Modulation in growth, photosynthetic efficiency, activity of antioxidants and mineral ions by foliar application of glycinebetaine on pea (*Pisum sativum* L.) under salt stress. *Acta physiologiae plantarum*, *36*(11), 2985-2998.
60. Okuma, E., Murakami, Y., Shimoishi, Y., Tada, M., & Murata, Y. (2004). Effects of exogenous application of proline and betaine on the growth of tobacco cultured cells under saline conditions. *Soil Science and Plant Nutrition*, *50*(8), 1301-1305.
61. Ozturk, M., Turkyilmaz Unal, B., García-Caparrós, P., Khursheed, A., Gul, A., & Hasanuzzaman, M. (2021). Osmoregulation and its actions during the drought stress in plants. *Physiologia*

Plantarum, *172*(2), 1321-1335.
62. Park, E. J., Jeknic, Z., & Chen, T. H. (2006). Exogenous application of glycinebetaine increases chilling tolerance in tomato plants. *Plant and cell physiology*, *47*(6), 706-714.
63. Rady, M. M., Kuşvuran, A., Alharby, H. F., Alzahrani, Y., & Kuşvuran, S. (2019). Pretreatment with proline or an organic bio-stimulant induces salt tolerance in wheat plants by improving antioxidant redox state and enzymatic activities and reducing the oxidative stress. *Journal of Plant Growth Regulation*, *38*(2), 449-462.
64. Rajasheker, G., Jawahar, G., Jalaja, N., Kumar, S. A., Kumari, P. H., Punita, D. L., ... & Kishor, P. B. K. (2019). Role and regulation of osmolytes and ABA interaction in salt and drought stress tolerance. In *Plant signaling molecules* (pp. 417-436). Woodhead Publishing.
65. Reda, F., & Mandoura, H. M. (2011). Response of enzymes activities, photosynthetic pigments, proline to low or high temperature stressed wheat plant (*Triticum aestivum* L.) in the presence or absence of exogenous proline or cysteine. *International Journal of Academic Research*, *3*(4), 108-115.
66. Saed-Moucheshi, A., Razi, H., Dadkhodaie, A., Ghodsi, M., & Dastfal, M. (2019). Association of biochemical traits with grain yield in triticale genotypes under normal irrigation and drought stress conditions. *Australian Journal of Crop Science*, *13*(2), 272-281.
67. Shafi, M., Bakht, J., Hassan, M. J., Raziuddin, M., & Zhang, G. (2009). Effect of cadmium and salinity stresses on growth and antioxidant enzyme activities of wheat (*Triticum aestivum* L.). *Bulletin of environmental contamination and toxicology*, *82*(6), 772-776.
68. Sharma, A., Kumar, V., Yuan, H., Kanwar, M. K., Bhardwaj, R., Thukral, A. K., & Zheng, B. (2018). Jasmonic acid seed treatment stimulates insecticide detoxification in *Brassica juncea* L. *Frontiers in plant science*, *9*, 1609.
69. Sharma, A., Shahzad, B., Rehman, A., Bhardwaj, R., Landi, M., & Zheng, B. (2019). Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. *Molecules*, *24*(13), 2452.
70. Sharma, S. S., & Dietz, K. J. (2006). The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. *Journal of experimental botany*, *57*(4), 711-726. *Physiology and Molecular Biology*, *30*(5), 496-502.
71. Sidhu, G. P. S., Singh, H. P., Batish, D. R., & Kohli, R. K. (2017). Appraising the role of environment friendly chelants in alleviating lead by Coronopus didymus from Pb-contaminated soils. *Chemosphere*, *182*, 129-136.
72. Singh, A., Sengar, K., Sharma, M. K., Sengar, R. S., & Garg, S. K. (2018). Proline metabolism as sensors of abiotic stress in sugarcane. *Biotechnology to enhance sugarcane productivity and stress tolerance*, 265-284.
73. Singh, M., Kumar, J., Singh, S., Singh, V. P., & Prasad, S. M. (2015). Roles of osmoprotectants in improving salinity and drought tolerance in plants: a review. *Reviews in Environmental Science and Bio/Technology*, *14*(3), 407-426.
74. Sulpice, R., Tsukaya, H., Nonaka, H., Mustardy, L., Chen, T. H., & Murata, N. (2003). Enhanced formation of flowers in salt-stressed Arabidopsis after genetic engineering of the synthesis of glycine betaine. *The Plant Journal*, *36*(2), 165-176.
75. Sumithra, K., Jutur, P. P., Carmel, B. D., & Reddy, A. R. (2006). Salinity-induced changes in two cultivars of *Vigna radiata*: responses of antioxidative and proline metabolism. *Plant Growth Regulation*, *50*(1), 11-22.
76. Szabados, L., & Savouré, A. (2010). Proline: a multifunctional amino acid. *Trends in plant science*, *15*(2), 89-97.
77. Tang, W., & Luo, C. (2018). Overexpression of zinc finger transcription factor ZAT6 enhances salt tolerance. *Open life sciences*, *13*(1), 431-445.
78. Tiwari, S., & Lata, C. (2018). Heavy metal stress, signaling, and tolerance due to plant-associated

microbes: an overview. *Frontiers in plant science*, *9*, 452.
79. Trovato, M., Mattioli, R., & Costantino, P. (2008). Multiple roles of proline in plant stress tolerance and development. *Rendiconti Lincei*, *19*(4), 325-346.
80. Upadhyaya, H., Sahoo, L., & Panda, S. K. (2013). Molecular physiology of osmotic stress in plants. In *Molecular stress physiology of plants* (pp. 179-192). Springer, India.
81. Verslues, P. E., & Sharma, S. (2010). Proline metabolism and its implications for plant-environment interaction. *The Arabidopsis Book/American Society of Plant Biologists*, *8*.
82. Wang, Y. M., Meng, Y. L., & Nii, N. (2004). Changes in glycine betaine and related enzyme contents in Amaranthus tricolor under salt stress. Journal of Plant
83. Weretilnyk, E. A., Bednarek, S., McCue, K. F., Rhodes, D., & Hanson, A. D. (1989). Comparative biochemical and immunological studies of the glycine betaine synthesis pathway in diverse families of dicotyledons. *Planta*, *178*(3), 342-352.
84. Xu, Z. S., Xia, L. Q., Chen, M., Cheng, X. G., Zhang, R. Y., Li, L. C., & Ma, Y. Z. (2007). Isolation and molecular characterization of the *Triticum aestivum* L. ethylene-responsive factor 1 (TaERF1) that increases multiple stress tolerance. *Plant molecular biology*, *65*(6), 719-732.
85. Yadav, S. K. (2010). Cold stress tolerance mechanisms in plants. A review. *Agronomy for sustainable development*, *30*(3), 515-527.
86. Yamada, M., Morishita, H., Urano, K., Shiozaki, N., Yamaguchi-Shinozaki, K., Shinozaki, K., & Yoshiba, Y. (2005). Effects of free proline accumulation in *petunias* under drought stress. *Journal of Experimental Botany*, *56*(417), 1975-1981.