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### RESEARCH ARTICLE

#### A REVIEW ON IMPROVEMENT OF SALT TOLERANCE IN RICE (ORYZA SATIVA L) BY INCREASING ANTIOXIDANT DEFENCE SYSTEMS USING EXOGENOUS APPLICATION OF PROLINE

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#### Abstract

Rice (*Oryza sativa* L) stands as a staple food crop globally particularly in the regions with warm climates. However, its cultivation faces significant challenges from abiotic stresses like salinity, which hinder crop growth and productivity. Salinity stress disrupts essential physiological processes, leading to reduced yields and compromised food security. In response to this pressing concern, this review investigates the potential of exogenous proline application to enhance salt tolerance in rice. The study delves into the intricate mechanisms underlying salinity stress in rice plants, highlighting its detrimental effects on growth, ion balance, and cellular metabolism. Salinity-induced oxidative stress, characterized by the accumulation of reactive oxygen species (ROS), exacerbates cellular damage, impairing plant growth and development. Proline, an amino acid known for its multifaceted roles in stress mitigation, emerges as a promising candidate for enhancing salt tolerance in rice. Exogenous application of proline demonstrates significant improvements in rice growth, yield and physiological attributes under saline conditions. The study elucidates the impact of exogenous proline on various aspects of rice physiology, including seed germination, water relations, and oxidative stress mitigation. By enhancing nutrient uptake, maintaining ion homeostasis, and fortifying antioxidant defences, proline offers a promising avenue for sustainable rice production in the face of escalating salinity stress. In conclusion, this study underscores the pivotal role of exogenous proline in augmenting salt tolerance in rice, offering insights into novel strategies for mitigating the adverse effects of salinity stress on crop yields.

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#### Introduction:-

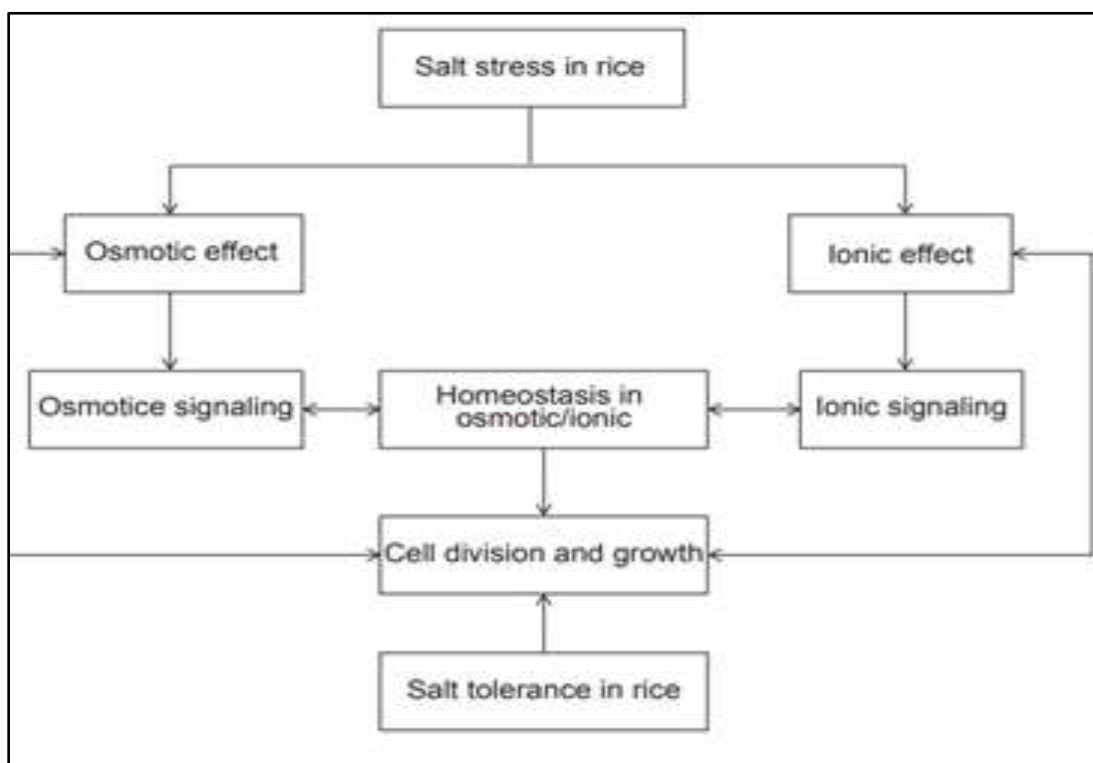
Rice (*Oryza sativa* L) is a grass plant that belongs to the family Poaceae and is an edible starchy cereal grain. It is mostly grown in warm regions of the world, particularly on the west coast of North America, Asia, Africa, and northern Italy. The world's most significant and widely farmed food crop is rice, which is also the most popular cereal. The huge rice farming has dependent on irrigation water since the state of the green revolution. Compared to other cereals, rice takes a lot of water to grow; around 3000-5000 liters are needed to create per kilogramme of rice (1). In India, rice is cultivated on 44 million hectares area accounting 20% of total rice production worldwide (2). In

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Asian nations like Nepal and Cambodia, the commencement of the rice growing season is commemorated with a Royal Ploughing Ceremony. The three biggest rice patrons are China, India, and Indonesia. Rice contains carbohydrates, fat, proteins, vitamins, minerals and water. The World Health Organization (WHO) conditionally advised supplementing rice with folic acid and vitamin A in 2018 and strongly advised reinforcing it with iron. Growth of rice depends on both biotic and abiotic environmental factors,

Among all the abiotic circumstances, the salinity is one of the major abiotic stresses which impose blockage in plant growth and production. Around 800 million hectares of land globally are affected by salinity. Plant phenotypic and physiological processes are intricately linked to soil salinity. Elevated salinity stress disturbs plant equilibrium and ion distribution in leaf cells as well as across the whole leaf (4). Older leaves accumulate NaCl as a result of prolonged saline exposure which affects the photosynthesis rate significantly (5). It also affects the overall metabolism of the plant and damages it (6). Salinity also effects the chloroplast activities. (7).



**Figure 1:-** Salt stress response in rice plant.

Reactive oxygen species (ROS) has been produced at a faster rate under salinity stress. Lipids, proteins and DNA are among the macromolecules damaged by ROS, which can also lead to cell death. Antioxidant enzymes, such as catalase (CAT) and peroxidase (POX), are known to provide stress protection by scavenging reactive oxygen species (ROS), which increases stress tolerance and prevents oxidative damage to the cellular structure (8). Because  $K^+$  participates in maintaining osmotic control and competes with  $Na^+$ , salt tolerance is directly correlated with  $K^+$  contents. Among all the cereals, rice is the most sensitive to salinity stress. Rice is relatively tolerant to soil salinity during germination and late vegetative growth. Rice has traditionally been grown in the saline soils of the coastal regions. A certain amount of salt is constantly present in the nearby rivers, canals, streams, and other water bodies. When the salinity of the soil exceeds  $EC \sim 4 \text{ dSm}^{-1}$ , it is said to be moderate for rice. In rice plants, too much salt lead to osmotic stress as well as ionic toxicity. When exposed to high salinities, rice plants exhibit distinct morphological and biochemical changes.



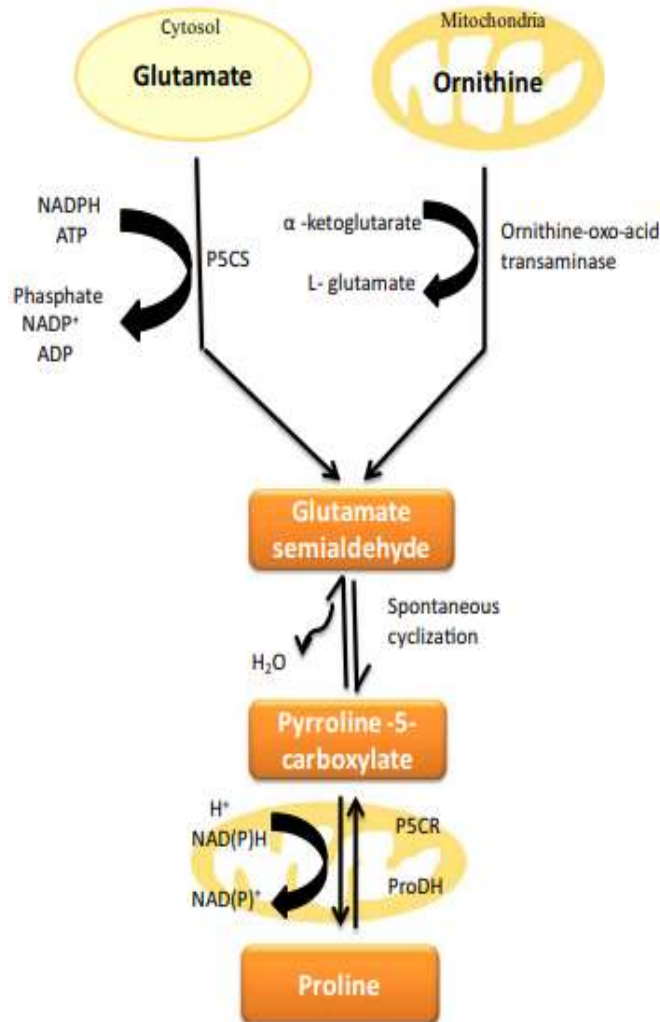
If proline supplied exogenously on plants at low concentrations it enhanced stress tolerance in rice. Proline when exogenously applied to roots of Arabidopsis resulted in a reduced level of ROS, indicating the ROS scavenging potential of proline (12). Exogenous application of proline provided osmoprotection and enhanced the growth of plants exposed to salt stress. There is various successful application of exogenous proline to improve stress tolerance. Proline application greatly raised  $K^+/Na^+$  in salt-tolerant and salt-sensitive cultivars (13). The amount of salt tolerance was correlated with the acquisition of proline and the enhanced activity of antioxidants CAT and APX (14). Proline increases nutrient absorption, maintains a greater  $K^+/Na^+$  ratio, and likely strengthens the rice's antioxidant defence system, all of which help rice become resistant to salt. The administration of proline affected the fresh weight of the shoots and did not tolerate the effects of salt stress (15).

#### **Role of salinity stress in rice :**

Salinity, an abiotic stressor, significantly constrains for crop production. Typically, saline soil exhibits a pH range of 7 to 8.5. The impact of salinity on plants is multifaceted and profoundly disrupts crop yield and production. Various factors contribute to poor plant performance under salt stress, including agronomic practices, enzyme activity, nutritional management, genetic traits, and hormonal imbalances. One of the most detrimental effects of salt stress is the accumulation of  $Na^+$  and  $Cl^-$  ions in plant tissues and soil. This accumulation disrupts ion balance within plants and soil, leading to physiological disorders. Additionally, high soil salt concentrations can impede water uptake by roots, leading to physiological drought, reduce plant osmotic potential, and disrupt cellular metabolic functions due to toxicity. It's estimated that salt stress has already caused a 20% reduction in crop production on irrigated lands globally, with projections indicating the potential loss of 50% of arable land by the mid-21st century due to this stressor. Salinity effect the plant mainly imparting two types of stresses – (i) Osmotic stress and (ii) Ionic stress. When it comes to coping with salinity stress, plants can be categorized into two main groups: Halophytes and Glycophytes, distinguished by their degree of tolerance to salt levels. Halophytes are capable of withstanding high concentrations of salt, reaching up to 400 mM NaCl, whereas Glycophytes thrive at lower concentrations. Excessive salt has detrimental effects on various essential metabolic processes in rice, such as cell wall integrity, plasmolysis, cytoplasmic stability, and ER function (16).

Rice is notably sensitive to salt, a concern underscored by the reduction in yield induced by salinity, posing a significant threat to food security. Particularly in coastal regions, rice cultivation has grappled with saline soil for generations. While rice can withstand limited exposure to saltwater without compromising growth and yield, excessive salinity triggers biochemical and physiological changes, inhibiting growth and causing yield losses. Despite management practices such as transplanting aged seedlings to mitigate salinity stress at the seedling stage, the flowering phase remains vulnerable. Unlike the seedling stage, where salt tolerance is independent of the flowering/reproductive stage, salinity poses a significant challenge during flowering. Various strategies, including the use of osmoprotectants, have been explored to counter salt stress in rice, with studies focusing on different types of osmoprotectants. Osmoprotectants, also known as compatible solutes, are small organic molecules characterized by a neutral charge and low toxicity at high concentrations. These compounds serve as osmolytes, aiding organisms in surviving extreme osmotic stress. They can be categorized into three chemical classes: betaines and related compounds, sugars and polyols, and amino acids. Upon encountering osmotic pressure, many floras accumulate specific organic solutes. This collection of compounds that work well together is a fundamental approach employed by plants to protect themselves from salinity. These solutes accumulate in the cytosol, reducing cytoplasmic water potential and functioning as osmoprotectants.

Proline, an indispensable imino acid crucial for primary metabolism in plants. It plays a pivotal role in maintaining the pH of cytosolic redox within cells and acts as an antioxidant or singlet oxygen quencher. Notably, there is a substantial accumulation of proline in salt-tolerant transgenic rice plants expressing P5CS (17). The synthesis of proline from glutamic acid occurs via  $\Delta^1$ -pyrroline-5-carboxylate (P5C) facilitated by two enzymes: P5C synthetase (P5CS) and P5C reductase. Conversely, proline is degraded back to glutamic acid via P5C through the actions of two enzymes: proline dehydrogenase and P5C dehydrogenase (18).

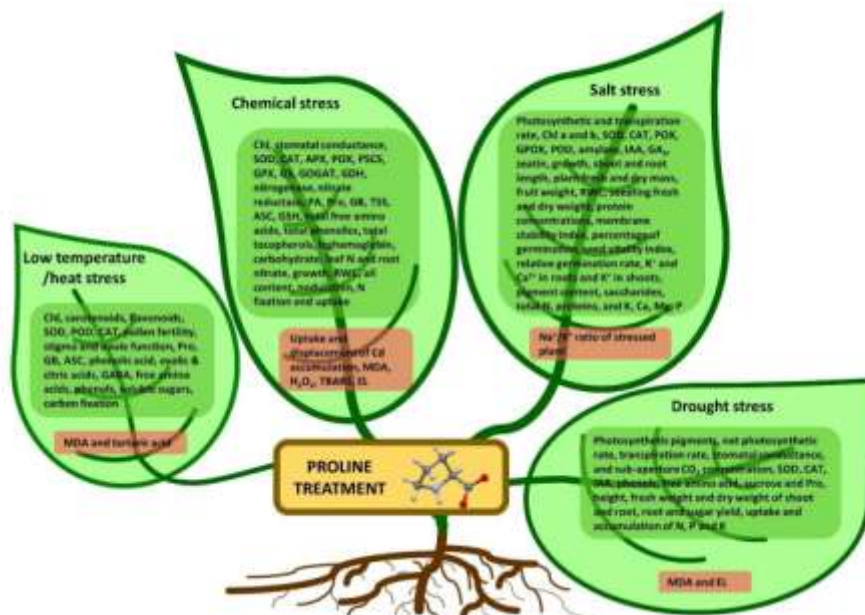


**Figure 4:-** Biosynthetic pathway of proline through Glutamate and ornithine in plants (adopted from Ahmad and Sharma 2008). P5CS pyrroline-5-carboxylate synthetase, P5CR pyrroline-5-carboxylate synthetase, Pro DH proline dehydrogenase.

#### Exogenous application of proline in rice:

Proline, a versatile imino acid, serves as both a signalling molecule initiating cascades of cellular communication and a key osmolyte, particularly prominent under diverse environmental stresses such as salinity (19). Its functions encompass the regulation of osmotic pressure within cells, prevention of protein denaturation, maintenance of membrane integrity, enzyme stabilization, and mitigation of toxic reactive oxygen species (ROS). Recognized as a crucial osmotic regulator in cells facing water scarcity, saline conditions, or heavy metal exposure, proline accumulation occurs in the cytosol in response to various biotic and abiotic stressors.

The utilization of proline had a significant impact on water relations in rice cultivars across both years of study. It enhanced water relations, likely by promoting increased water uptake. Notably, application of proline during the seedling and vegetative stages led to noteworthy improvements in water potential in the first year, with a similar trend observed in the second year. Additionally, foliar application of proline contributed positively to plant growth and yield characteristics. In wheat, exogenous proline application during the vegetative stage effectively mitigated the adverse effects of salt stress. This foliar proline treatment also enhanced the growth of salt-affected rice plants by bolstering photosynthetic capacity. Furthermore, under saline conditions, exogenous proline application significantly improved physiological attributes, including antioxidant activity such as catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA) contents. Moreover, proline application was associated with increased rice plant height, total tillers and panicle length also.



**Figure 5:-** The summarized physiological and biochemical changes observed under the influence of exogenous proline that have beneficial effects on the tolerance of stress factors in cultivated plants. Up-regulated processes and/or biochemicals are marked in green, down-regulated are marked in red.

#### Proline metabolism in salt stress:

Numerous researches indicate that exposure to saline stress initiates the activation of genes responsible for proline biosynthesis, ultimately leading to an accretion of proline (20). Application of proline from external sources can notably enhance plant tolerance to salt stress by modulating internal proline metabolism, a procedure that is in part made possible by the varying expression of particular proline-related genes. The foliar administration of proline to maize decreased P5CS activity while increasing PDH levels under salt stress conditions (21). The administration of proline exogenously considerably boosted the expression of P5CS and P5CR in salt-stressed rice (22).

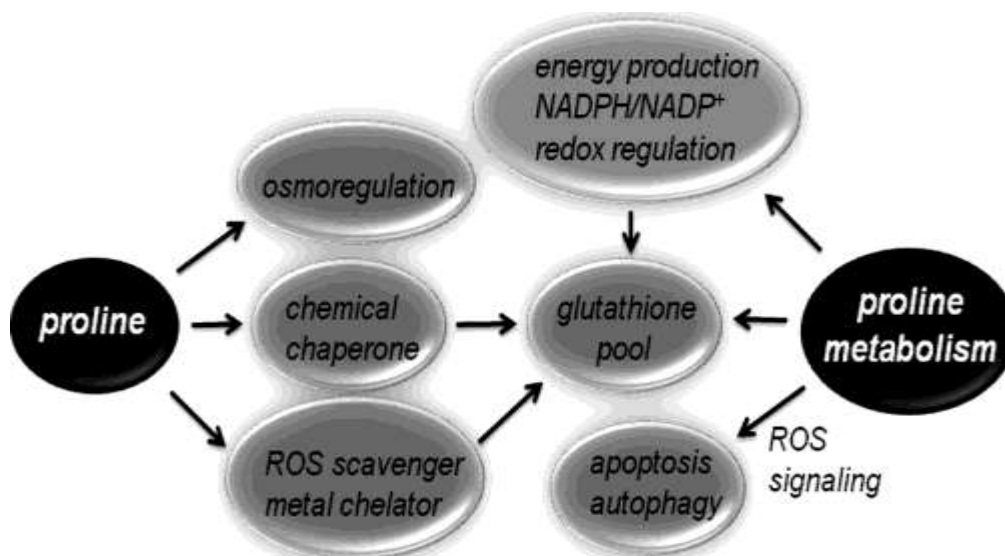
#### Impact of proline treatment on salt stressed crop plants:

Sprouting of seeds stands as a pivotal phase within the plant life cycle, as it is highly susceptible to abiotic stress factors (23). Exogenous application of proline demonstrates a favourable concentration-dependent impact on seed germination amidst salt stress conditions. The application of 1 mM proline effectively mitigates the adverse effects induced by 400 mM NaCl, although 100 mM proline fails to yield a notable effect. Likewise, treatment with 50 mM proline enhances the germination of two *Sorghum bicolor* cultivars under saline conditions (24).

In stressed plants, reactive oxygen species (ROS) are continuously generated as a result of incomplete oxygen reduction. Some ROS serve as secondary messengers, triggering tolerance to abiotic stresses. Proline is recognized as a molecular chaperone owing to its ability to scavenge ROS, stabilize proteins and other macromolecular complexes, and maintain cellular redox potential. When 10 mM proline is externally applied to salt-stressed rice seedlings, it reduces the activity of superoxide dismutase (SOD), peroxidase (POX), and catalase (CAT), while increasing the content of hydrogen peroxide ( $H_2O_2$ ).

#### Mechanisms of proline induced stress protection:

The precise molecular mechanisms underlying proline's protective role in cellular stress remain incompletely elucidated, though they are believed to hinge on its chemical properties and impact on redox systems such as the glutathione (GSH) pool. While proline's function in stress adaptation is commonly attributed to its osmolytic nature and capacity to mitigate water stress, challenging environmental conditions often disrupt intracellular redox equilibrium, necessitating additional mechanisms to counteract oxidative stress. Consequently, proline's protective mechanisms are thought to encompass the stabilization of proteins and antioxidant enzymes, direct scavenging of reactive oxygen species (ROS), maintenance of intracellular redox balance (e.g.,  $NADP^+/NADPH$  and  $GSH/GSSG$  ratios), and modulation of cellular signalling pathways via proline metabolism.



**Figure 6:-** Potential functions of proline and proline metabolism in stress protection (25).

### Conclusion:-

Salinity poses a significant challenge to crop yields in coastal regions of India. Application of exogenous proline has demonstrated marked improvements in growth, as well as increased grain and straw yields, while concurrently elevating the  $K^+/Na^+$  ratio and nutrient absorption in both rice varieties grown under saline conditions. Proline plays a pivotal role in enhancing rice tolerance to salinity by bolstering nutrient uptake, maintaining optimal  $K^+/Na^+$  ratios, and potentially fortifying antioxidant defence systems. Beyond field experimentation, gaining deeper insights into the biochemical and physiological functions of proline is essential for identifying and developing plants with enhanced tolerance mechanisms against salinity. The accumulation of proline and the enhanced activity of the antioxidant enzymes CAT and APX were related to the level of salinity tolerance. One of the most important challenges for plant physiologists is to find the right solutions and reduce the effects of stress. Numerous studies' findings demonstrate that applying exogenous proline to plants may decrease the harm that comes from environmental stress. Under both normal physiological parameters and stressful environments, plants acquire proline as a proteogenic amino acid. Additional functions of proline in plants include osmotic and energy supply, ROS scavenger, and stress reliever. Proline's function as an osmotic protector or growth signal is determined by the balance between its production and breakdown. It is still uncertain how proline content and abiotic stress tolerance in plants relate to one another. However, plant researchers agree that proline accumulation is beneficial to plants, especially after recovery from stress. Proline affects plant growth and production via its metabolic signal-mediating function. To increase plant stress tolerance, more investigation into the variables governing the expression of the enzymes involved in proline production and degradation will be helpful. Not only exogenous application of proline could be used for induction of stress response, but also molecular engineering modulating genes expression through gene modifications or gene editing. However based on regulations, which restrict applications of such organisms in particular country development of non-invasive techniques seems to be rationale choice. Additionally, it's essential for a deeper comprehension of the components of the regulatory network that govern how plants react to stress.

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### Conflicts of Interest:

The authors declare that there are no conflicts of interest.

### References:-

1. Bouman, B. A. M., Xiaoguang, Y., Huaqi, W., Zhiming, W., Junfang, Z., Changgui, W., & Bin, C. (2002, May). Aerobic rice (Han Dao): a new way of growing rice in water-short areas. In Proceedings of the 12th

- international soil conservation organization conference (Vol. 26, p. 31). Beijing, China: Tsinghua University Press. Doi: <https://doi.org/10.1016/j.fcr.2008.12.007>
2. Oo, A. Z., Win, K. T., & Bellingrath-Kimura, S. D. (2015). Within field spatial variation in methane emissions from lowland rice in Myanmar. *SpringerPlus*, 4, 1-11.10.1186/s40064-015-0901-2
  3. Jing, Q., Bouman, B. A. M., Hengsdijk, H., Van Keulen, H., & Cao, W. (2007). Exploring options to combine high yields with high nitrogen use efficiencies in irrigated rice in China. *European Journal of Agronomy*, 26(2), 166-177. Doi: <https://doi.org/10.1016/j.eja.2006.09.005>
  4. Yao MingZhe, Y. M., Wang Jian Fei, W. J., Chen Hong You, C. H., Zhai Hu Qu, Z. H., & Zhang Hong Sheng, Z. H. (2005). Inheritance and QTL mapping of salt tolerance in rice. Doi: <http://www.ricesci.org/CN/Y2005/V12/I1/25>
  5. Deinlein, U., Stephan, A. B., Horie, T., Luo, W., Xu, G., & Schroeder, J. I. (2014). Plant salt-tolerance mechanisms. *Trends in plant science*, 19(6), 371-379. Doi: <https://doi.org/10.1016/j.tplants.2014.02.001>
  6. Taffouo, V. D., Nouck, A. H., Dibong, S. D., & Amougou, A. (2010). Effects of salinity stress on seedlings growth, mineral nutrients and total chlorophyll of some tomato (*Lycopersicon esculentum* L.) cultivars. *African Journal of Biotechnology*, 9(33). Doi: <https://doi.org/10.1111/j.1365-3040.2009.02041.x>
  7. Miller, G. A. D., Suzuki, N., Ciftci-Yilmaz, S. U. L. T. A. N., & Mittler, R. O. N. (2010). Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant, cell & environment*, 33(4), 453-467. Doi: <https://doi.org/10.1111/j.1365-3040.2009.02041.x>
  8. Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in plant science*, 7(9), 405-410. Doi: [https://doi.org/10.1016/S1360-1385\(02\)02312-9](https://doi.org/10.1016/S1360-1385(02)02312-9)
  9. Aqsa Talat, A. T., Khalid Nawaz, K. N., Khalid Hussian, K. H., Bhatti, K. H., Siddiqi, E. H., Aneela Khalid, A. K., ... & Sharif, M. U. (2013). Foliar application of proline for salt tolerance of two wheat (*Triticum aestivum* L.) cultivars. Doi: [http://www.idosi.org/wasj/wasj22\(4\)13/14.pdf](http://www.idosi.org/wasj/wasj22(4)13/14.pdf)
  10. Farooq, M., Nawaz, A., Chaudhry, M. A. M., Indrasti, R., & Rehman, A. (2017). Improving resistance against terminal drought in bread wheat by exogenous application of proline and gamma-aminobutyric acid. *Journal of agronomy and crop science*, 203(6), 464-472. Doi: <https://doi.org/10.1111/jac.12222>
  11. Ali, Q., Ashraf, M., Shahbaz, M., & Humera, H. A. F. I. Z. A. (2008). Ameliorating effect of foliar applied proline on nutrient uptake in water stressed maize (*Zea mays* L.) plants. *Pak. J. Bot*, 40(1), 211-219. Doi: <http://www.pjbot.org>
  12. Cuin, T. A., & Shabala, S. (2007). Compatible solutes reduce ROS-induced potassium efflux in *Arabidopsis* roots. *Plant, cell & environment*, 30(7), 875-885. <https://doi.org/10.4161/psb.21949>
  13. Islam, M. T., Sharma, P. C., Gautam, R. K., Singh, D., Singh, S., Panesar, B., & Ali, S. (2011). Salt tolerance in parental lines of rice hybrids through physiological attributes molecular markers. *International Journal of Experimental Agriculture*, 2(1), 1-7.10.3329/ajmbr.v1i3.26465
  14. Elsayy, H. I., Mekawy, A. M. M., Elhity, M. A., Abdel-Dayem, S. M., Abdelaziz, M. N., Assaha, D. V., ... & Saneoka, H. (2018). Differential responses of two Egyptian barley (*Hordeum vulgare* L.) cultivars to salt stress. *Plant physiology and biochemistry*, 127, 425-435.10.1016/j.plaphy.2018.04.012
  15. Shahbaz, M., Mushtaq, Z., Andaz, F., & Masood, A. (2013). Does proline application ameliorate adverse effects of salt stress on growth, ions and photosynthetic ability of eggplant (*Solanum melongena* L.). *Scientia Horticulturae*, 164, 507-511. <https://doi.org/10.3389/fpls.2020.01127>
  16. Yeo, A. R., Caporn, S. J. M., & Flowers, T. J. (1985). The effect of salinity upon photosynthesis in rice (*Oryza sativa* L.): gas exchange by individual leaves in relation to their salt content. *Journal of Experimental Botany*, 36(8), 1240-1248. <https://doi.org/10.1093/jxb/36.8.1240>
  17. Greenway, H., & Munns, R. (1980). Mechanisms of salt tolerance in nonhalophytes. *Annual review of plant physiology*, 31(1), 149-190. 10.4236/nr.2015.66037
  18. Yoshida, Y., Kiyosue, T., Nakashima, K., Yamaguchi-Shinozaki, K., & Shinozaki, K. (1997). Regulation of levels of proline as an osmolyte in plants under water stress. *Plant and cell physiology*, 38(10), 1095-1102.10.1093/oxfordjournals.pcp.a029093
  19. Szabados, L., & Savaouré, A. (2010). Proline: a multifunctional amino acid. *Trends in plant science*, 15(2), 89-97.10.1016/j.tplants.2009.11.009
  20. Armengaud, P., Thiery, L., Buhot, N., Grenier-de March, G., & Savaouré, A. (2004). Transcriptional regulation of proline biosynthesis in *Medicago truncatula* reveals developmental and environmental specific features. *Physiologia plantarum*, 120(3), 442-450.10.1111/j.0031-9317.2004.00251.x
  21. de Freitas, P. A. F., de Souza Miranda, R., Marques, E. C., Prisco, J. T., Gomes-Filho, E. (2018). Salt Tolerance Induced by Exogenous Proline in Maize Is Related to Low Oxidative Damage and Favorable Ionic Homeostasis. *J. Plant Growth Regul.* 37, 911–924. DOI:10.1007/s00344-018-9787-x



22. Nounjan, N., Nghia, P. T., Theerakulpisut, P. (2012). Exogenous proline and trehalose promote recovery of rice seedlings from salt-stress and differentially modulate antioxidant enzymes and expression of related genes. *J. Plant Physiol.* 169, 596–604. DOI:10.1016/j.jplph.2012.01.004
23. Hubbard, M., Germida, J., Vujanovic, V. (2012). Fungal endophytes improve wheat seed germination under heat and drought stress. *Botany* 90, 137–149. <https://doi.org/10.1139/b11-091>
24. Nawaz, K., Talat, A., Hussain, K., II, Majeed, A. (2010). Induction of salt tolerance in two cultivars of Sorghum (*Sorghum bicolor* L.) by exogenous application of proline at seedling stage. *World Appl. Sci. J.* 10, 93–99 <https://doi.org/10.1139/b11-091>
25. Fig 4: Delauney, A. J., & Verma, D. P. S. (1993). Proline biosynthesis and osmoregulation in plants. *The plant journal*, 4(2), 215-223. DOI:10.1046/j.1365-313X.1993.04020215.x
26. Khanam M, Rahman MM, Islam MR, Islam MR (2001). Effect of manures and fertilizers on the growth and yield of BRR1 dhan30. *Pakistan Journal of Biological Sciences*, 4: 172-174. DOI: 10.3923/pjbs.2001.172.174
27. 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. DOI: 10.1016/j.jplph.2007.07.013
28. Abd El-Samad HM, Shaddad MAK, Barakat N (2011). Improvement of plants salt tolerance by exogenous application of amino acids. *Journal of Medicinal Plant Research*, 5: 5692-5699 DOI:10.17957/IJAB/15.1925
29. Ali, Q., Ashraf, M., Shahbaz, M., & Humera, H. A. F. I. Z. A. (2008). Ameliorating effect of foliar applied proline on nutrient uptake in water stressed maize (*Zea mays* L.) plants. *Pak. J. Bot.* 40(1), 211-219. DOI:10.3390/antiox12071438
30. Ahmad, P., & Prasad, M. N. V. (Eds.). (2011). *Abiotic stress responses in plants: metabolism, productivity and sustainability*. Springer Science & Business Media. DOI:10.1007/9781461406341
31. Hoque, M. A., Okuma, E., Banu, M. N. A., Nakamura, Y., Shimoishi, Y., & Murata, Y. (2007). Exogenous proline mitigates the detrimental effects of salt stress more than exogenous betaine by increasing antioxidant enzyme activities. *Journal of Plant Physiology*, 164(5), 553-561. DOI: 10.1016/j.jplph.2006.03.010
32. Mekawy, A. M. M., Abdelaziz, M. N., & Ueda, A. (2018). Apigenin pretreatment enhances growth and salinity tolerance of rice seedlings. *Plant Physiology and Biochemistry*, 130, 94-104. DOI: 10.1016/j.plaphy.2018.06.036
33. MH Abd-El Salam, K., AE El-Dalil, M., & KE Abd El Ghany, E. (2016). Mean performance and genetic variability of some grain quality characteristics of rice (*Oryza sativa* L.). *Alexandria science exchange journal*, 37(January-March), 76-84.1. DOI:10.21608/ASEJAIQJSAE.2016.1946
34. Islam, M. T., Sharma, P. C., Gautam, R. K., Singh, D., Singh, S., Panesar, B., & Ali, S. (2011). Salt tolerance in parental lines of rice hybrids through physiological attributes molecular markers. *International Journal of Experimental Agriculture*, 2(1), 1-7. DOI:10.3329/ajmbr.v1i3.26465
35. Zeng, L., & Shannon, M. C. (2000). Salinity effects on seedling growth and yield components of rice. *Crop science*, 40(4), 996-1003. DOI:10.3390/su15031804
36. Bhusan, D., Das, D. K., Hossain, M., Murata, Y., & Hoque, M. A. (2016). Improvement of salt tolerance in rice (*Oryza sativa* L.) by increasing antioxidant defense systems using exogenous application of proline. *Australian Journal of Crop Science*, 10(1), 50-56. DOI:10.58149/9e00-7x14
37. 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. <https://doi.org/10.1016/j.envexpbot.2005.12.006>
38. Yaqoob, H., Akram, N. A., Iftikhar, S., Ashraf, M., Khalid, N., Sadiq, M., ... & Ahmad, P. (2019). Seed pretreatment and foliar application of proline regulate morphological, physio-biochemical processes and activity of antioxidant enzymes in plants of two cultivars of quinoa (*Chenopodium quinoa* Willd.). *Plants*, 8(12), 588. DOI:10.3390/plants8120588
39. Munns, R. (2002). Comparative physiology of salt and water stress. *Plant, cell & environment*, 25(2), 239-250. <https://doi.org/10.1046/j.0016-8025.2001.00808.x>
40. Shereen, A., Ansari, R., Raza, S., Mumtaz, S., Khan, M. A., & Khan, M. A. (2011). Salinity induced metabolic changes in rice (*Oryza sativa* L.) seeds during germination. *Pak. J. Bot.* 43(3), 1659-1661. DOI:10.11591/.v5i2.4786
41. Fig 3: Csonka, L. N., & Hanson, A. D. (1991). Prokaryotic osmoregulation: genetics and physiology. *Annual review of microbiology*, 45(1), 569-606.
42. Fig:4; Ahmad, P., & Sharma, S. (2008). Salt stress and phyto-biochemical responses of plants. *Plant Soil Environ*, 54(3), 89-99.

43. MH Abd-El Salam, K., AE El-Dalil, M., & KE Abd El Ghany, E. (2016). Mean performance and genetic variability of some grain quality characteristics of rice (*Oryza sativa* L.). Alexandria science exchange journal, 37(January-March), 76-84.1 10.21608/ASEJAIQJSAE.2016.1946
44. Fig5: MH Abd-El Salam, K., AE El-Dalil, M., & KE Abd El Ghany, E. (2016). Mean performance and genetic variability of some grain quality characteristics of rice (*Oryza sativa* L.). Alexandria science exchange journal, 37(January-March), 76-84., <https://doi.org/10.3390/agriculture8110166>.