Annals of Biology **41** (1) : 71-76, 2025 **http://agribioj.com** **Impact Score : 0.32 (Scopus)**

Impact of Minerals and PGR on Biochemical Parameters in Salt-Tolerant and Salt-Sensitive Wheat (*Triticum aestivum* L.) Cultivars under Saline Conditions

SEEMA GUPTA[1] AND SUSHILA SANGWAN*

Department of Botany, Government College, Hisar-125 001 (Haryana), India
(e-mail: drsushil2008@gmail.com; Mobile: 9466038245)

(Received: June 12, 2024; Accepted: August 31, 2024)

ABSTRACT

In arid and semi-arid regions, salinity remains a major problem restricting food production and the sustainability of agriculture. Salinity affects the plants at all the stages of their development. Sixteen possible combinations of seed priming treatments with plant growth regulators (GA_3 and CK) and minerals (K^+ and Ca^{2+}) were given to wheat grains and based on seedling germination and its growth the four most effective treatments (K^+, K^++GA_3, Ca^{2+}+GA_3 and K^++GA_3+CK) were selected for the biochemical analysis in leaves. All seed priming treatments were beneficial in mitigating the adverse effects of salinity. Out of the four treatments, seed soaking in K^++GA_3+CK was found to be the most effective in mitigating the salinity induced negative effect to all the biochemical parameters i.e. total soluble carbohydrates, proteins, free amino acids, nucleic acid and nitrate reductase, etc.

Key words: Salinity, wheat, growth regulators, minerals, seed priming, biochemical analysis

INTRODUCTION

Cereals are the most significant category of monocots that have provided us nutrition and other requirements for millions of years. Abiotic stressors, such as elevated temperatures, salt, drought, ultraviolet (UV) rays, contamination with heavy metals, etc., have a negative impact on the development and productivity of cereals. Plants use structural, physiological and biochemical mechanisms in combination with specific adaptive techniques to deal with stress, which are essential for their development and yield (Kosakivska *et al*., 2021). Saline soils have high concentrations of dissolved salts, particularly sodium chloride, which can hinder plants growth and productivity. These salts can accumulate in the soil due to a variety of both natural and artificial processes, such as irrigation with salted water, poor drainage and improper handling of soil practices.

Salinity is probably the most aggravating abiotic stress in food production, having a markedly negative impact on agricultural plants' physiology, morphology and biochemistry, including the absorption of both nutrients and water, germination, development, photosynthesis, metabolism of enzymes and production (Cisse *et al.,* 2019). An overview of cellular and physiological mechanisms in plant responses to salt was studied by Zelm *et al.* (2020).

Among cereals, globally wheat is ranked at the first position due to its domestication and contribution as the primary staple food crop (Iqbal *et al.,* 2021). Soil salinity poses a substantial impact upon wheat yield (Falcon *et al.,* 2022), since it affects over 6% of agricultural land globally (He *et al.,* 2016). Tetraploid wheat (Durum, Emmer) is more salt sensitive than hexaploid wheat (Bread wheat). Among cereals, sorghum and barley are the crops that are most suited to withstand salt stress, while maize and wheat are only somewhat suited (Kosakivska *et al.*, 2021). However, rice is most sensitive to salt, with substantial impact on both growth and grain yield (Alkharabsheh *et al*., 2021).

Excessive accumulation of cytoplasmic sodium (Na^+) is a direct outcome of salinity in the soil. This can lead to severe ionic toxicity, particularly in leaves where it can directly impede photosynthesis. In addition, osmotic and oxidative stress brought on by salt stress can further disrupt metabolic processes,

[1]Department of Botany, Fateh Chand College for Women, Hisar-125 001 (Haryana), India.

damage DNA and might result in cell death.

The findings demonstrated the detrimental effects of NaCl at concentrations between 100 and 200 mM on plant physiology, and the partial effectiveness of biochar in reducing the detrimental effects of salt on plant physiology. However, biochar was unable to prevent the buildup of Na. Similarly, biochar improved rather than decreasing the amount of free amino acids in lettuce leaves (Fadeli *et al.,* 2024). It appears that seed priming could be a promising strategy for enhancing salinity tolerance in future with changing climates. The factors responsible for salt tolerance in primed plants included accelerated physiological and biochemical processes like cell division and starch breakdown, α-amylase activity, better germination rate, lower Na^+ and higher K^+ accumulation, and generated antioxidative system (Pirasteh-Anosheh and Hashemi, 2020)

Phytohormonal engineering presents novel possibilities to boost yields and is regarded as a significant and effective strategy to mitigate the losses of cereals brought on by unfavourable external circumstances. Phytohormones played a key role in this complicated mechanism of salt tolerance. The source-sink connection, absorption of nutrients, growth and development of plants enhanced via phytohormone-mediated plant responses. Under conditions of salt stress, phytohormones caused particular gene expressions that changed a variety of plant processes (Khan *et al*., 2024).

Cytokinin controls plant development, physiological processes, yield and is also essential for responding to abiotic stressors including drought, salt and extreme heat or cold (Li *et al.,*2021). Role of cytokinin and auxin in controlling stress adaptations was also observed (Bielach *et al.,* 2017). Al-Harthi *et al.* (2021), Adam *et al.* (2021) and Attia *et al.* (2022) observed the mitigating effects of GA_3 on tolerance for salinity. Wheat plants have been shown to exhibit reduced auxin and limited cytokinin transduction under abiotic stress (Abhinandan *et al.,* 2018).

By altering vital plant functions, K^+ plays a critical part in reducing the effects of salt stress on plants. Additionally, the prerequisite characteristic for establishing a salt-tolerant mechanism is K^+ uptake and retention (Kumari *et al*., 2021).

Ionic toxicity and osmotic stress were inhibited by exogenous Ca^{2+} administration, which also increased proline and glycine betaine levels and decreased Na^+ uptake. As a result, the growth and yield qualities, RWC, SPAD value, and K^+, Ca^{2+} and Mg^{2+} uptake were all considerably enhanced by the Ca^{2+} treatment (Md. Moshiul Islam *et al.,* 2023).

MATERIALS AND METHODS

The Nuclear Seed Production Unit, Department of Plant Breeding, CCSHAU, Hisar, provided the seeds of wheat (cultivars WH-542 and HD-2329). NaCl, $MgCl_2$, $CaCl_2$ and $MgSO_4$ were dissolved in a milli-equivalent basis Na: Ca + Mg ratio of 1:3, Ca : Mg ratio of 1:3 and Cl:SO_4 ratio of 7:3, in distilled water to get the chloride dominated salinity of the desired level (9 and 12 dS/m). Prior to planting, sterilized seeds were treated/soaked with the appropriate PGR and nutrients for 18 h. Kinetin (0.4 mM), GA_3 (0.4 mM), KCl (20 mM), $CaCl_2$ (10 mM) and all feasible 16 combinations were used for the seed priming treatment. Based on seedling germination and early growth, the four most effective treatments (K^+, K^++ GA_3, Ca^{2+}+GA_3 and K^++GA_3+ CK) were chosen.

Saline solutions of desired levels were applied to each pot on soil saturation basis and seeds treated in four most effective treatments were grown in different saline levels i.e. S_0, S_9 and S_{12}. Observations were recorded 70 days after sowing. For different biochemical estimations 3rd leaf from top was used.

Total soluble carbohydrates, proteins, total free amino acids, free proline in leaves and nitrate reeducate activity were estimated by the standard methods. The residue left after carbohydrate was used for nucleic acid estimation.

RESULTS AND DISCUSSION

Carbohydrates in the cell fulfill dual function. On the one hand, it acts in the capacity of an osmotic regulant, while on the other it provides energy (ATP), reducing power (NADPH) and carbon skeletons for biosynthesis. It was observed that exposing the plants to salinity decreased accumulation of soluble sugars (Fig.1) and the reduction was more in HD-2329 than WH-542. The results clearly showed that at S_9 and S_{12} the reduction in soluble carbohydrate was 27.7 and 43% in HD-2329 in comparison to 17.8 and 30.8% in WH-542, respectively. Further, it was also observed that various seed priming treatments improved the total soluble carbohydrate content and out of them K^{+}+GA_3+CK treatment was found the most efficient and improvement in accumulation of total soluble carbohydrate. It was found more under saline conditions than under non-saline conditions in both the cultivars. However, it was more in HD-2329 than WH-542.

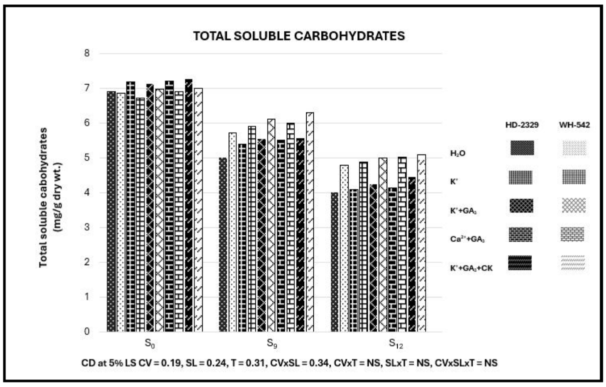

Fig. 1. Effect of seed priming treatments on total soluble carbohydrates in leaves (mg/g dry wt.) in two cultivars of wheat grown under various levels of salinity.

An increase in the concentration under salt stress in leaf sheath of salt tolerant wheat variety and low concentration in salt sensitive variety was observed by Tayyaba *et al.* (2022). The role of plant growth regulators in improving the soluble sugars carbohydrate content might be attributed to enhanced chl.a content and thus enhanced photosynthesis.

In plants, soluble protein is a crucial organic osmo-regulant that helps to minimize damage. Reduction in protein content under salinity accrued in both the cultivars (Fig. 2). However, this reduction was found more in HD-2329, the salt sensitive cultivar, where it was 37 and 66% at S_9 and S_{12} levels of salinity, while in WH-542 the reduction was of the level of 27 and 55% at S_9 and S_{12} levels of salinity, respectively. Various seed priming treatments improved the protein content, and it was found that in the combined treatment of K^{+}+GA_3+CK the protein content was enhanced by 4% under non-saline conditions and 18% under high salt stress i.e. S_{12} level. The decrease in protein synthesis may be one of the reasons for reduction in protein content and it may be due

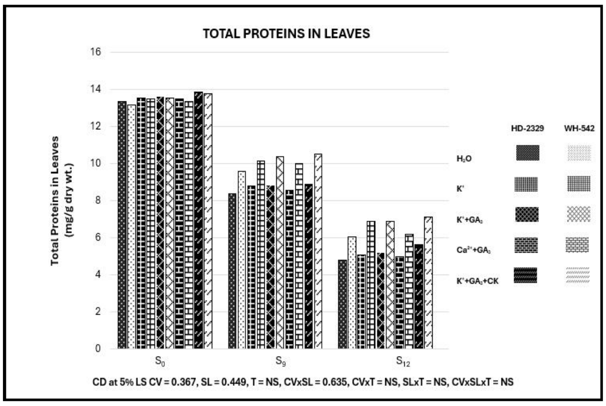

Fig. 2. Effect of seed priming treatments on total proteins in leaves (mg/g dry wt.) in two cultivars of wheat grown under various levels of salinity.

to the increase in polycations or due to loss in the capacity to regulate the ratio between polycations and polyanions of plants under salt stress, which is very important to mobilize protein synthesis. Another reason behind the decrease in protein content may be enhanced protein degradation, as under salt stress protease activity increases. This increase in protein concentration under the influence of various growth regulators may be due to increased protein synthesis either due to more supply of substrate or template availability (Zhang *et al.,* 2023).

An increase in free amino acid content under salinity was observed in both the cultivars (Fig. 3) and the increase was comparatively more in HD-2329 (86%) than WH-542 (62%). Seed priming treatments of K^{+}, K^{+}+GA_3, Ca^{2+}+ GA_3 and K^{+}+GA_3+CK were found effective in reducing the free amino acid content, but out of these a combination of K^{+} with GA_3+CK was found the most efficient. A considerable

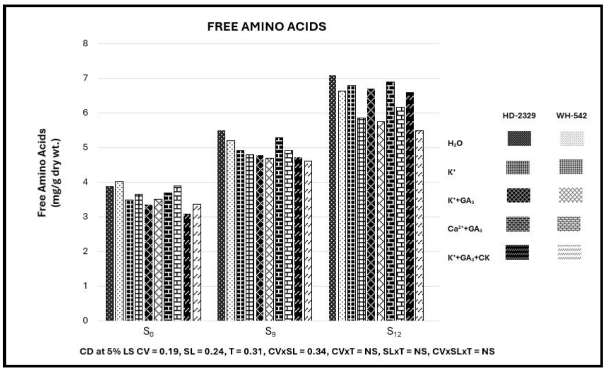

Fig. 3. Effect of seed priming treatments on free amino acids in leaves (mg/g dry wt.) in two cultivars of wheat grown under various levels of salinity.

increase in alanine, GABA, aspartic acid and asparagine contents was found in mycorrhizal roots under salt stress (Haque and Matsubra, 2018).

Proline is an important parameter to evaluate plant stress tolerance capability (Takahashi *et al.,* 2020). It is a highly water-soluble amino acid that protects cell membranes from the toxic effects of an excess of inorganic ions; in addition to its role as an osmolyte, it also helps the cells to alleviate oxidative stress in salt-affected plants (Rajashekar *et al.,* 2019). Proline accumulation in leaves significantly increased with the increasing levels of salinity (S_9 and S_{12}) in both the cultivars as compared to control (S_0). Cultivar WH-542 had free proline content than HD-2329 under non-saline conditions (Fig. 4). But, with the increasing level of salinity more accumulation of free proline was observed in HD-2329. In comparison to control (T_0), all the seed priming treatments considerably reduced the free proline content in leaves in both the cultivars and in all the salinity levels (S_0, S_9 and S_{12}). A combination of K^+, GA_3 and CK was found to be the most effective seed priming treatment. Interactive effects amongst the cultivars, salinity levels and seed priming treatments were non-significant, however, the individual effect was significant. Exogenous application of proline under saline conditions improved nutrient intake, absorption of water, biological fixation of nitrogen, antioxidant activity, Kz assimilation and decreased Na^+ and Cl^- absorption and translocation (Moukhtari *et al.,* 2020). In accordance with our findings, Dheeba *et al.* (2015) detected that exogenous GA decreased the level of proline content in salt-stressed plants.

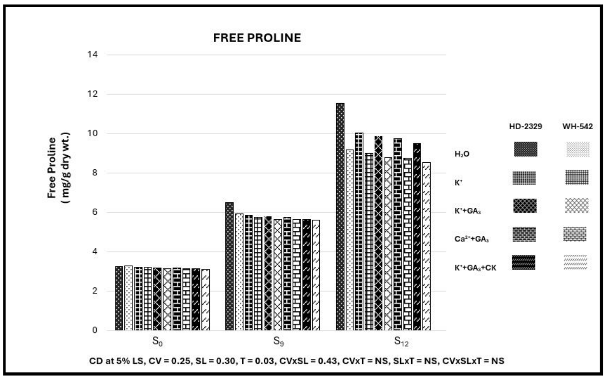

Fig. 4. Effect of seed priming treatments on free proline in leaves (mg/g dry wt.) in two cultivars of wheat grown under various levels of salinity.

Ribose nucleic acid (RNA) content was decreased by 32 and 58% in the leaves of wheat (HD-2329) at S_9 and S_{12} levels of salinity in comparison to control. This reduction was found comparatively less in WH-542. In contrast to this, the content of DNA increased with salinity and it was more in cv. HD-2329 than WH-542. Various seed priming treatments mitigated the adverse effects of salinity on nucleic acid but treatment K^++GA_3+CK was found most efficient in this respect (Figs. 5 and 6). DNA content increased with increasing level of salinity (Fig. 6). On comparison between two cultivars, HD 2329 had more DNA content under non saline as well as saline conditions. Although all the seed priming treatments were found effective in reducing the salt induced increase in DNA content, maximum reduction was with K^++GA_3+CK treatment. Interactions amongst cultivars (CV), salinity levels (SL) and seed soaking treatments (ST), (CVxSLxST) were non-significant.

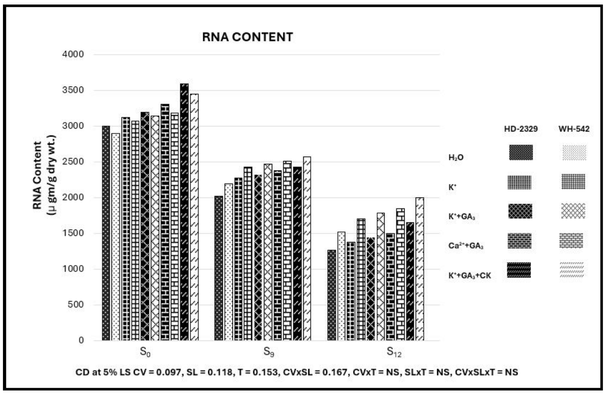

Fig. 5. Effect of seed priming treatments on RNA content in leaves (mg/g dry wt.) in two cultivars of wheat grown under various levels of salinity.

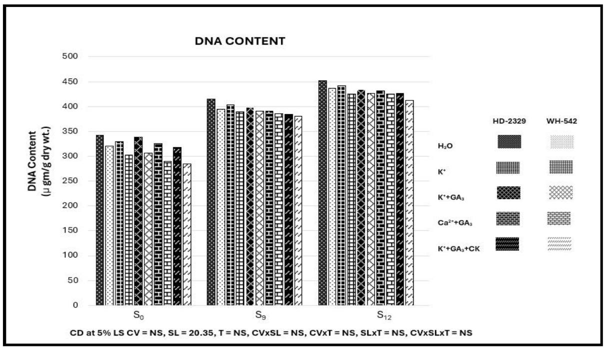

Fig. 6. Effect of seed priming treatments on DNA content in leaves (mg/g dry wt.) in two cultivars of wheat grown under various levels of salinity.

The activity of nitrate reductase (NR) significantly decreased with increasing level of salinity as compared to the plants grown under non-saline conditions in both the cultivars (Fig. 7). Of the cultivars studied, HD-2329 registered more nitrate reductase activity than WH-542 under non-saline conditions. However, salinization induced reduction was less in WH-542. Likewise, HD-2329 had comparatively low activity of NR under salinity (S_9, S_{12}) in contrast to WH-542. All the seed priming treatments with plant growth regulators and nutrients in combination significantly increased the activity of NR as compared to that when seeds were soaked in water (T_0) in both the cultivars. K^++GA_3+CK was found to be the most effective treatment.

Interaction amongst cultivars (CV), salinity levels (SL) and seed soaking treatments (ST), (CVxSLxST) was non-significant. According to Zhang *et al.* (2023), the effect of salt stress on the amount of nitrate-nitrogen and ammonium nitrogen was "low promotion and high inhibition". This could be because under saline conditions transport of nitrate was inhibited due to competitive absorption of Cl^- and NO^{3-} ions.

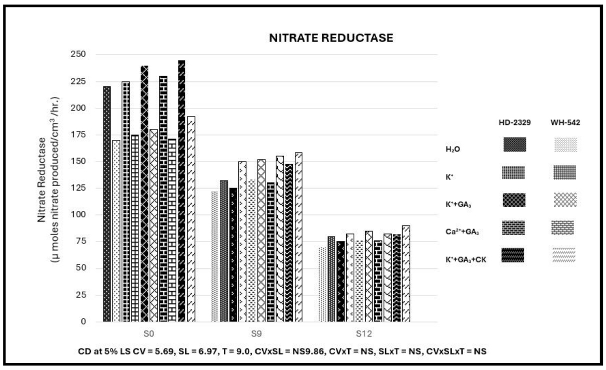

Fig. 7. Effect of seed priming treatments on nitrate reductase in leaves (mg/g dry wt.) in two cultivars of wheat grown under various levels of salinity.

CONCLUSION

The reduction in proteins was 37 and 66% in HD-2329, while in WH-542 it was 27 and 55% at S_9 and S_{12} levels of salinity, respectively. While 86 and 62% increase in free amino acids was observed at S_{12} levels of salinity in HD-2329 and WH-542, respectively, in comparison to S_0. Similarly, an increase of 2.5 folds for free proline content was observed in leaves of HD-2329 at S_{12} levels of salinity in comparison to control. RNA content in leaves was decreased by 32 and 58% in leaves of HD-2329 at S_9 and S_{12} levels of salinity. It was observed that pre-sowing seed priming treatments i.e. a combination of plant growth regulators and minerals had ameliorative/alleviating/mitigating effects on all the parameters studied during the course of this investigation. All the biochemical parameters showed decline in salt induced harmful effects to some extent. The efficacy of treatments was found in the order of K^++GA_3+CK > K^++GA_3 > Ca^{2+}+ GA_3 > K^+ alone. It was also found that WH-542 was comparatively more salt tolerant than HD-2329 as the salinity induced hazardous effects were more in HD-2329 than WH-542. Pre-sowing seed priming treatments were found beneficial in both the cultivars, however, the response of mitigation of deleterious effects of salinity was prominently observed in HD-2329 than WH-542. Different treatments of plant growth regulators and minerals were found to be effective under both the non-saline and saline conditions, but it was observed that under saline conditions the mitigation of salt induced deleterious effects was more than under non-saline conditions. This could be obviously ascribed to relatively more imbalance in mineral ions, growth regulators and various biochemical parameters, allocation and diversion of carbohydrates and other food material to tissue reparation mechanism, under salinity. Further, the exogenous application of growth regulators and minerals probably lead to streamline and offset the above adverse impact to some extent.

REFERENCES

Abhinandan, K., Skori, L., Stanic, M., Hickerson, N., Jamshed, M. and Samuel, M. A. (2018). Abiotic stress signaling in wheat–An inclusive overview of hormonal interactions during abiotic stress responses in wheat. *Front. Plant Sci.* **9**: 734. *doi: 10.3389/fpls.2018.00734.*

Adam, Y. A. A., Muhi, E. H. I., Guisheng, Nimir, E. A. N., Z., Aboagla, M. I. E., Xiurong, J., Guanglong, Z., Ebtehal, G. I. S., Mohamed, S. E. S. and Safiya B. M. E. (2021). Gibberellic acid and nitrogen efficiently protect early seedlings' growth stage from salt stress damage in sorghum. *Sci. Rep.* **11**:6672. *https:// doi. org/10.1038/s41598-021-84713-9*.

Al-Harthi, M. M., Bafeel, S. O. and El-Zohri, M. (2021). Gibberellic acid and jasmonic acid improve salt tolerance in summer squash by modulating some physiological parameters symptomatic for oxidative stress and mineral nutrition. *Plants (basel)* **10**: 2768. *https:// doi.org/10.3390/plants10122768.*

Alkharabsheh, H., Seleiman, M., Hewedy. O. A. and Ali, Nawab (2021). Field crop responses and management strategies to mitigate soil salinity in modern agriculture: A review. *Agronomy* **11**: 2299. *doi:10.3390/ agronomy11112299.*

Attia, H., Alamer, K., Algethami, B., Zorrig, W., Hessini, K., Gupta, K. and Gupta, B. (2022). Gibberellic acid interacts with salt stress on germination, growth and polyamine gene expression in fennel (*Foeniculum vulgare* Mill.) seedlings. *Physiol. Mol. Biol. Plants* **28**: 607-622. *https:// doi. org/ 10.1007/ s12298-022-01140-4.*

Bielach, A., Hrtyan, M. and Tognetti, V. B. (2017). Plants under stress: Involvement of auxin and cytokinin. *Int. J. Mol. Sci.***18**: 1427. *doi: 10.3390/ijms18071427*.

Cisse, A., Arshad, A., Wang, X., Yattara, F. and Hu, Y. (2019). Contrasting impacts of long-term application of biofertilizers and organic manure on grain yield of winter plant Bioler wheat in North China plain. *Agronomy* **9**: 312. *doi: 10.3390/ agronomy9060312.*

Dheeba, B., Selvakumar, S., Kannan, M. and Kannan, K. (2015). Effect of gibberellic acid on black gram (*Vigna mungo*) irrigated with different levels of saline water. *Res. J. Pharm. Biol. Chem. Sci*. **6**: 709-720.

Falcon, W. P., Naylor, R. L. and Shankar, N. D. (2022). Rethinking global food demand for 2050. *Pop. Dev. Rev.* **48**: 921-957. *doi: 10.1111/padr.12508*.

Fedeli , R., Vannini, A., Djatouf, N., Celletti, S. and Stefano Loppi (2024). Can lettuce plants grow in saline soils supplemented with biochar?. *Heliyon* **10**: e26526. *doi:10.1016/j.heliyon.2024.e26526*.

Haque, S. I. and Matsubra, Y. (2018). Relationship between salt tolerance and free amino acid content in mycorrhizal strawberry. *Plants J. JSATM* **25**: 43-50.

He, W., Goodkind, D. and Kowal, P. R. (2016). An aging world. In: *International Population Reports. Chapter 1. 2015.* Washington DC: U.S. Government Printing Office. pp. 2-3.

Iqbal, M. A., Junaid, R., Wajid, N., Sabry, H., Yassir, K. and Ayman, S. (2021). Rainfed winter wheat (*Triticum aestivum* L.) cultivars respond differently to integrated fertilization in Pakistan. *Fresenius Environ. Bull*. **30**: 3115-3121.

Khan, I., Chattha, M. U., Maqbool, R., Muqarrab Ali, Asif, M., Hassan, M. U. and Aslam, M. T. (2024). Perspectives of phytohormones application to enhance salinity tolerance in plants. *New Insights into Phytohormones*. Intech Open. *doi: 10.5772/intechopen. 1003714.*

Kosakivska, I. V., Vedenicheva, N. P., Babenko, L. M., Voyt, Enko , L. V., Romanenko, K. O. and Vasyuk, V. A. (2021). Exogenous phytohormones in the regulation of growth and development of cereals under abiotic stresses. *Mol. Biol. Rep.* **49**: 617-628. *doi: 10.1007/s11033-021-06802-2.*

Kumari, S., Chhillar, H., Chopra, P., Khanna, R. R. and Khan, M. I. R. (2021). Potassium: A track to develop salinity tolerant plants. *Plant Physiol. Biochem*. **167:** 1011-1023. *doi: 10.1016/j.plaphy.2021.09.031.*

Li, S. M., Zheng, H. X., Zhang, X. S. and Sui, N. (2021). Cytokinins as central regulators during plant growth and stress response. *Plant Cell Reports* **40**: 271-282. *doi: 10.1007/ s00299-020-02612-1.*

Md. Moshiul Islam, Khurshida Jahan, Arpita Sen, Tahmina Akter Urmi, Moynul Haque, Hayssam, M., Ali, Manzer, Siddiqui, H. and Yoshiyuki Murata (2023). Exogenous application of calcium ameliorates salinity stress tolerance of tomato (*Solanum lycopersicum* L.) and enhances fruit quality. *Antioxidants **12**: 558. https://doi.org/ 10.3390/antiox12030558.*

Moukhtari, A. E., Hourton, C. C., Farissi, M. and Savouré, A. (2020). How does proline treatment promote salt stress tolerance during crop plant development? *Front. Plant Sci.* **23**: 1127. *doi: 10.3389/fpls.2020. 01127.*

Pirasteh-Anosheh, H. and Hashemi, S. E. (2020). Priming, a promising practical approach to improve seed germination and plant growth in saline conditions. *Asian J. Agric. Food Sci.* **8**: 1. *doi:10.24203/ajafs.v8i1.6068.*

Rajasheker, G., Jawahar, G., Jalaja, N., Kumar, S. A., Kumari, P. H., Punita, D. L., Karumanchi, A. R., Reddy, P. S., Rathnagiri, P. and Sreenivasulu, N. (2019). Role and regulation of osmolytes and ABA interaction in salt and drought stress tolerance. In: *Plant Signaling Molecules.* Elsevier, The Netherlands. pp. 417-436.

Takahashi, Y., Zhang, J., Hsu, P. K., Ceciliato, P. H., Zhang, L., Dubeaux, G., Munemasa, S., Ge, C., Zhao, Y. and Hauser, F. (2020). MAP3 kinase-dependent SnRK2kinase activation is required for abscisic acid signal transduction and rapid osmotic stress response. *Nat. Com..* **11**: 12. *doi: 10.1038/s41467-019-13875-y*.

Tayyaba Naz, Muhammad Mazhar Iqbal, Javaid Akhtar, Saqib, M., Ali, M., Zafar, M. I., Dell, B., Rahul Datta, R., Ansari, M. J., Danish, S. and Fahad, S. (2022). Carbohydrate partitioning, growth and ionic compartmentalisation of wheat grown under boron toxic and salt degraded land. *Agronomy* ***12***: 740. *https://doi.org/10.3390/ agronomy12030740*

Zelm, E. V., Zhang, Y. and Testerink, C. (2020). Salt tolerance mechanisms of plants. *Ann. Rev. Plant Biol.***71**: 403-433. *doi: 10.1146/ annurev-arplant-050718-100005*.

Zhang, X., He, P., Guo, R. and Huang, K. (2023). Effects of salt stress on root morphology, carbon and nitrogen metabolism and yield of Tartary buckwheat. *Sci. Rep.* **13**: 12483. *doi: 10.1038/s41598-023-39634-0.*