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Alleviation of salt stress effects using phytohormones and mineral nutrients in wheat seedlings (*Triticum aestivum* L.)

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ABSTRACT: Wheat is one of the major crops all over the world. In India it is used in preparation of lots of end products. Climate change, depleting ground water and increasing salinity is posing a threat to the production of wheat. Although wheat is a moderately saline tolerant crop in salinity affected areas, still the mitigation of salinity effect is necessary. Salt stress, a major abiotic stress has enormous negative effects on morphological, physiological, and biochemical attributes of the plant including its germination, growth, water uptake, photosynthesis, nutrient uptake, enzymatic activities and yield. The present study we have conducted experiments to alleviate the effect of salinity using phytohormones and/or minerals in wheat crop. Studies in seedlings of wheat variety WH-542 show decrease in many morphological, physiological and biochemical parameters under chloride salinity and this reduction increases with increase in salinity. Pre-sowing seed soaking treatments with growth regulators (GA₃ and CK) and minerals ((K⁺ and Ca²⁺) in their various combinations were found to alleviate the effects caused by salt stress. Seed soaking in 'K⁺+ GA₃+ CK' combination was observed as the most effective treatment.

KEYWORDS: Wheat, Salt stress, Chloride salinity, Phytohormones, Minerals.

I. INTRODUCTION

In world's grain production, wheat occupies the first position. It is consumed as staple food by more than 36% of world population. Wheat is the major staple food crop, providing almost half of all calories in the region of North Africa and West and Central Asia. Globally wheat is cultivated in an area about 220 million hectares. Maximum area under wheat is in India (14%), followed by Russia (12.43%), China (11.14%) and the USA (6.90%). However, China is the major producer of wheat with a record production of 136 million tonnes, followed by India (98.51mt), Russia (85mt) and the USA (47.35mt)⁽¹⁾. To meet 2050 demands its production must increase at a rate of 2% annually⁽²⁾. The productivity of wheat is often adversely affected by various types of abiotic stress conditions. Salt stress is one of the major stresses among abiotic stress. It is estimated that 50% of all arable land worldwide will be affected by salt stress by 2050⁽³⁾. This stress may cause a loss of more than US\$12 billion annually due to reduced crop productivity⁽⁴⁾. Salt stress is having enormous negative effects on morphological, physiological, and biochemical attributes of the plant including its germination, growth, water uptake, photosynthesis, nutrient uptake, enzymatic activities and yield.

In India about 4.5 million hectares salt affected area is under wheat cultivation posing a major threat for canal irrigated areas⁽¹⁾. Solutions to the problem of salinity like soil amendments and proper drainage are not substantial. As a result there is a significant reduction in the wheat yield occur. Under salt stress, plants are affected adversely at every step of their growth, development and in attaining maturity. Salinity severely limits plant metabolism by causing oxidative stress, water deficit, ion toxicity, and the nutritional deficiencies⁽⁵⁾. Due to salt stress, the plant has to spend extra energy on ion transport, tissue reparation mechanism and osmotic adjustment⁽⁵⁾. In wheat salinity adversely affect the growth and productivity due to less nutrient uptake, increased reactive oxygen species and low photosynthetic rate⁽⁷⁾. Recently, investigations have focused more on the mechanisms of salt tolerance in plants^(8,9). Some researchers have suggested the role of plant growth regulators for reducing or eradicating the negative effects of salinity^(10, 11,12). The repressive effect of salinity on seed germination and plant growth could be related to reduced endogenous levels of phytohormones^(13, 14). Many researchers have shown the positive effects using plant growth regulators in alleviating salinity effect in various crops^(15,16,17,18).



Wheat is a moderately sensitive crop towards salinity. Many studies in wheat have shown the positive effect of plant growth regulators and minerals in alleviating salt stress. The Auxins were reported to increase the germination percentage, shoots DW and maintained ion homeostasis under salt stress by⁽¹⁹⁾. Shaddad et al⁽²⁰⁾ showed alleviation of salinity effect through seed priming with GA₃ and observed increase in grain weight and grain quality. Foliar application of GA₃ was found to confer salt stress tolerance by Tabatabaei⁽²¹⁾. Along with plant growth regulators, plant nutrients play positive roles in alleviating effects of salt stress by many researchers. Exogenous application of K⁺ enhanced salt stress tolerance in wheat seedlings was observed by El-Lethyet al⁽²²⁾ and Kausar and Gull⁽²³⁾. Foliar application of phosphorus (P) in alleviating salt-induced damage was reported by Khan et al⁽¹⁶⁾. Application of CaSO₄ was found to increase plant growth, water status and K and Ca uptake under salt stress condition by Zaman et al⁽²⁴⁾. Tian et al⁽²⁵⁾ also noted positive effect of Ca(NO₃)₂ in reducing the effect of salinity in wheat seedlings. Salt stress alleviation was also noticed using calcium and sulphur by Singh⁽²⁶⁾. However, potassium did not show any salinity lessening effect. The pre-sowing seed soaking in plant growth regulators (GA₃) has been shown to be beneficial in the mitigation of salt stress effects⁽²⁷⁾.

II. METHODOLOGY

Seeds of cultivar WH-542 were obtained from Nuclear Seed Production unit, Department of Plant Breeding, CCS HAU, Hisar. Seeds were germinated in petridishes under controlled conditions in BOD incubator maintained at 27± 1°C in dark. Chloride dominated salinity of desired level (9dSm⁻¹ and 12dS m⁻¹) was prepared by dissolving mixtures of salts like NaCl, MgCl₂, CaCl₂ and MgSO₄ in distilled water, where Na: Ca+Mg ratio was 1:1 and Ca:Mg ratio was 1:3 and Cl:SO₄ ratio was 7:3 on milliequivalent basis. Before sowing sterilized seeds were given seed priming treatments of desired plant growth regulators and nutrients. Seed priming treatment was done with Kinetin (0.4 mM), GA₃ (0.4 mM), KCl (20 mM) and CaCl₂ (10 mM) and their all possible combinations. On the basis of germination and early seedling growth, four most effective treatments were selected for all morphological, physiological and biochemical studies. Seed soaking time was 18 hours. 10 healthy seeds were placed in each petridish lined with Whatman filter paper and containing 10 ml of specific solution (S₀-Control, S₁-9dSm⁻¹ and S₂ -12dS m⁻¹)

III. EXPERIMENTAL RESULTS

Wheat seeds were sown as per the experimental details given above. Morphological, physiological and biochemical data were recorded for wheat seeds sown under various treatments. Data obtained from these experiments has been represented in following paragraphs

Morphological parameters: Observations regarding germination and other morphological parameters were recorded at the three levels of salinity used i.e. S₀, S₁ and S₂ and also for the treatments given for alleviation of salt stress. The germination percentage observed was 92.5%, 74.5% and 60% at S₀, S₁ and S₂ levels of salinity i.e. germination was reduced to 60% under high salt stress. For alleviating salinity effect, pre-sowing seed soaking treatments with Kinetin, GA₃, KCl and CaCl₂ were found very effective in improving germination percentage (fig. 1).

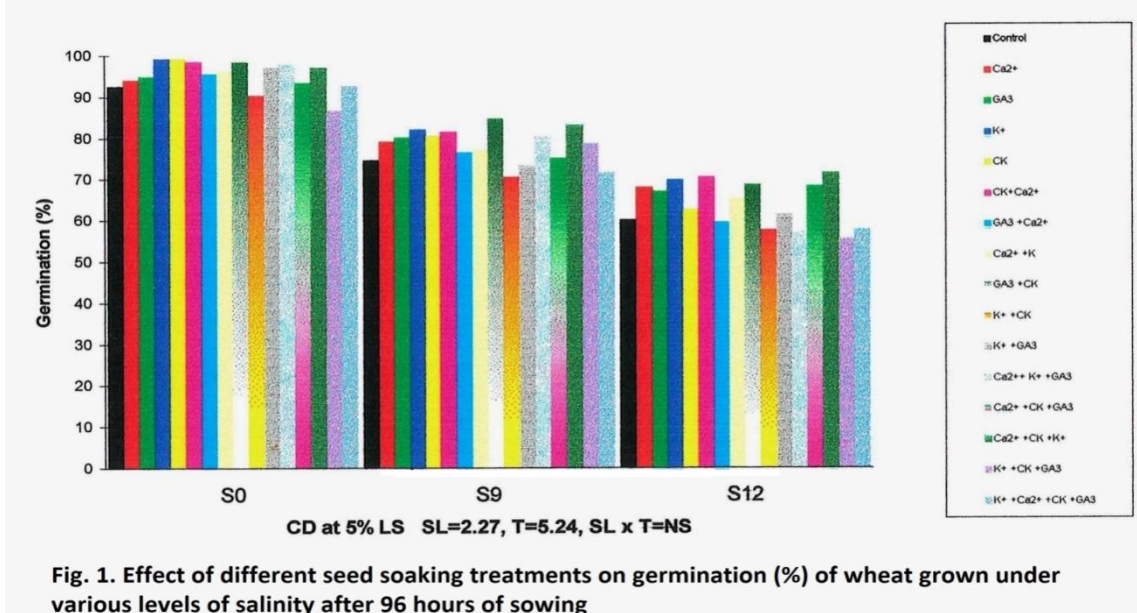


Fig. 1. Effect of different seed soaking treatments on germination (%) of wheat grown under various levels of salinity after 96 hours of sowing

For the observed parameters like radicle and coleoptile length, significant reduction in radicle and coleoptile length was observed at the tune of 33% and 17% respectively in 96 hours old seedlings under low salinity. At higher level of salinity i.e. at S₂ the reduction was more pronounced (54% and 43% respectively). However, when growth regulators were used in conjunction with mineral nutrients, the enhancement in length of radicle (fig.2) and coleoptile (fig. 3) was observed showing mitigation of salinity treatment.

There was 68% reduction in fresh weight of radicle and 55% in coleoptile, similarly, 58% and 33% reduction in dry weight of radicle and coleoptile respectively was observed. Pre-sowing seed soaking treatment also improved fresh weight of radicle. Of all the treatments, 'K⁺' and 'K⁺ + GA₃' were found most ameliorative as shown in fig. 4.

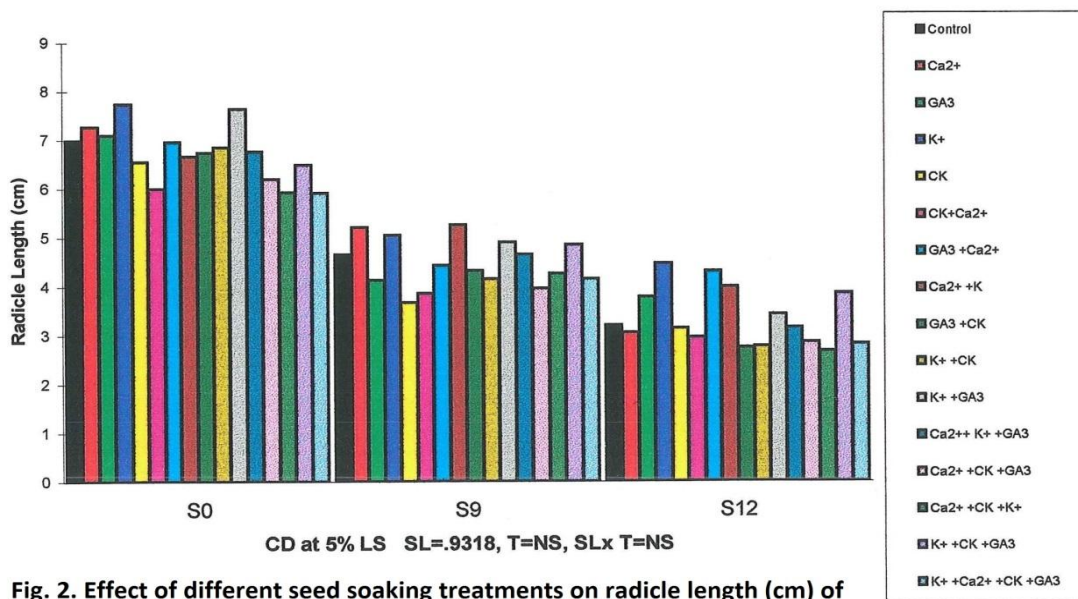


Fig. 2. Effect of different seed soaking treatments on radicle length (cm) of wheat grown under various levels of salinity at 96 hours of sowing

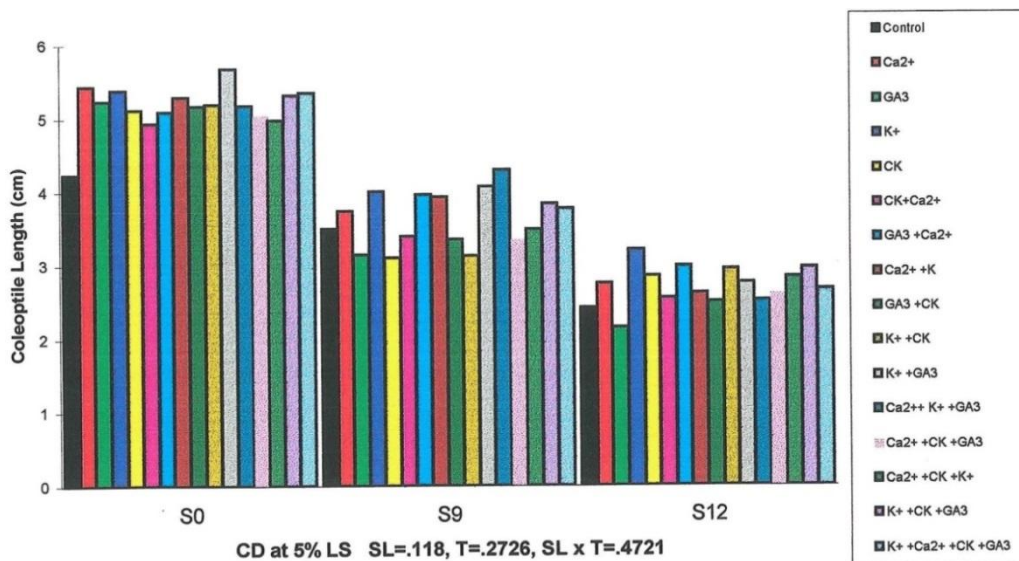
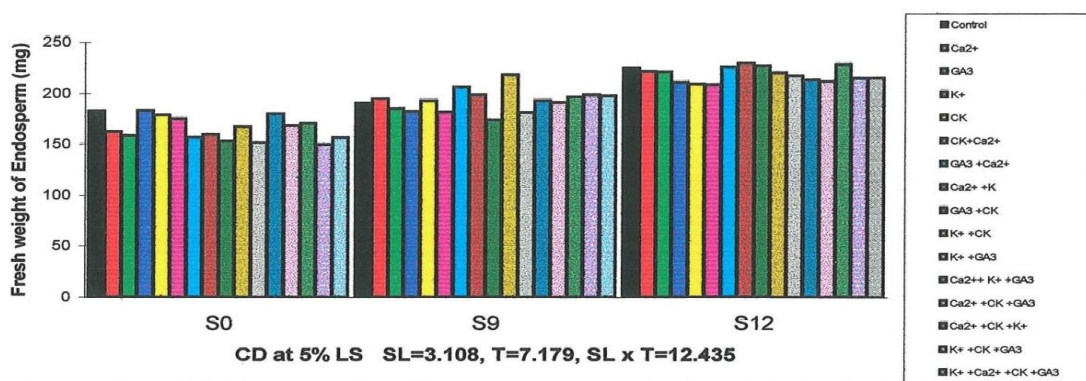
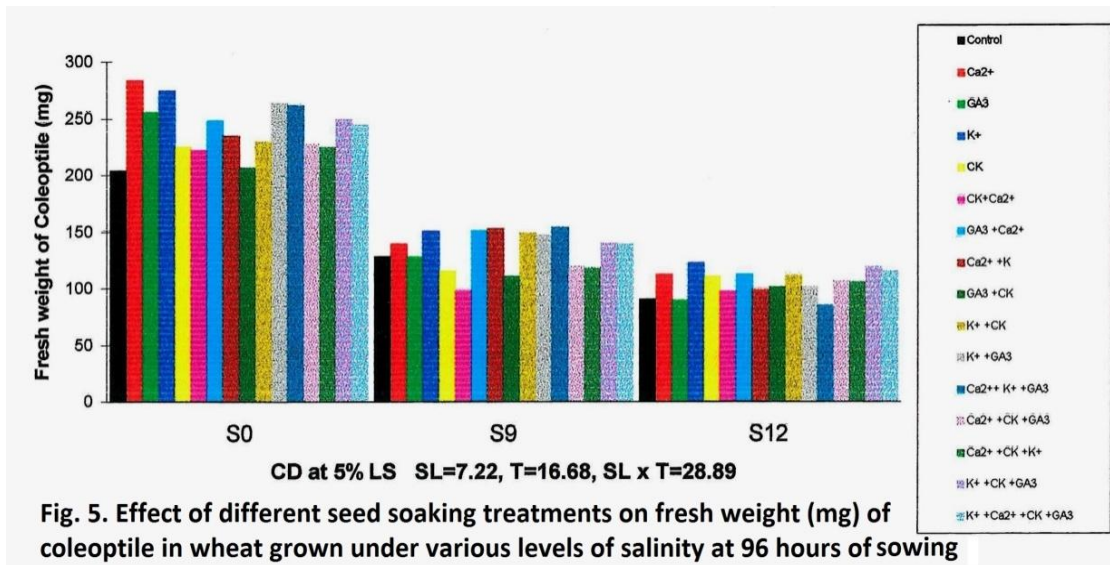


Fig. 3. Effect of different seed soaking treatments on coleoptile length (cm) of wheat grown under various levels of salinity at 96 hours of sowing.

Similarly, reduction in fresh weight of coleoptile due to salinity was observed and this parameter was also found to be improved under some ameliorative treatments (K^+ and K^+ + GA_3 + CK) as represented in fig. 5. A significant increase in fresh weight of endosperms was observed under S_1 and S_2 salinity as compared to control and this salt induced increase was significantly reduced with ' K^+ + GA_3 ' treatment. (fig.6). Likewise 50% reduction in dry weight of radicle was observed under S_2 level of salinity, which was most effectively improved by K^+ treatment, while in case of dry weight of coleoptile, ' K^+ + GA_3 + CK' was found as the most effective treatment (fig. 7). Salinity induced enhancement in dry weight was significantly reduced by pre-soaking treatments due to better mobilization of food reserve (fig.8).



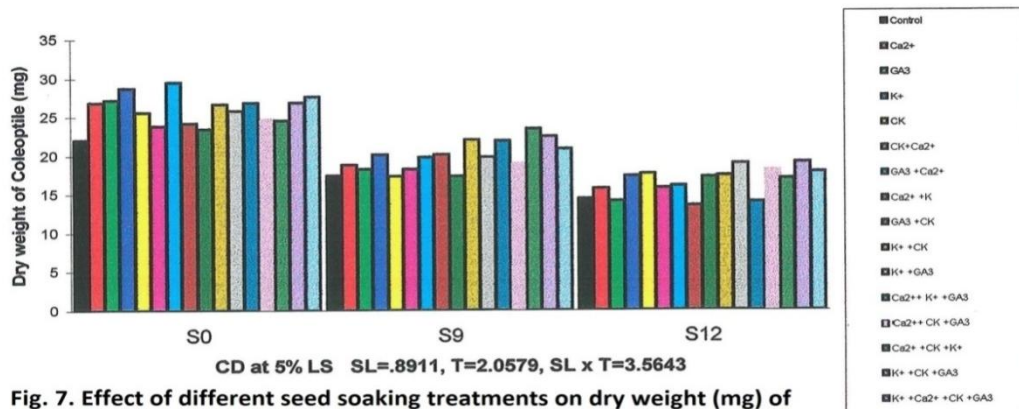


Fig. 7. Effect of different seed soaking treatments on dry weight (mg) of coleoptile of wheat grown under various levels of salinity at 96 hours of sowing

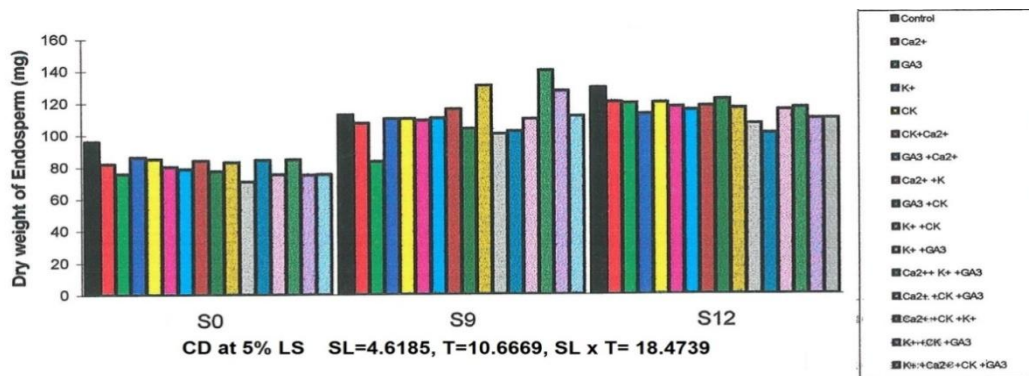


Fig. 8. Effect of different seed soaking treatments on dry weight (mg) of endosperm of wheat grown under various levels of salinity at 96 hours of sowing

Physiological parameters: Salinity showed deleterious effect on physiological parameters also. Relative water content (RWC) of 96hours old seedlings show decline with increase in salinity levels (radicle, 9% and 13%; coleoptile, 9% and 11% and endosperm, 2% and 5% under low and high salinity levels respectively as shown in fig. 9).

In radicle the most effective treatment was $Ca^{2+} + GA_3$, while in coleoptile and endosperm, ' $K^+ + GA_3 + CK$ ' was found most efficient. Osmotic potential Ψ_s of radicle, coleoptile and endosperm becomes more negative with the increase of salt stress. To enhance the osmotic potential of radicle, coleoptile and endosperm, pre-soaking treatment with ' $K^+ + GA_3 + CK$ ' was found most beneficial (fig. 10).

Biochemical parameters: For various biochemical parameters also salinity exhibited its effect. In case of α -amylase activity, 35% increase at S_2 level of salinity was observed. Further enhancement in the α -amylase activity takes place with pre-soaking seed treatments. Maximum increase was observed in ' $K^+ + GA_3 + CK$ ' i.e. 28.8%, 33.8% and 26.4% at S_0 , S_1 and S_2 levels of salinity (fig.11).

Similarly, increase in total soluble content was found to increase with increasing salinity level and with presoaking seed treatments. The increase was 52%, 25% and 21% at S_2 salinity level in radicle, coleoptile and endosperm respectively (fig.12).

For the parameter free amino acids, it observed steep rise with rise in salinity in the seedling (fig. 13). However, treatment with growth regulators and minerals decrease the content may be due to the synthesis of more proteins. Total protein content decreased with increase in salinity, the reduction was 23%, 22% and 10.5% at low level of salinity, while at S_2 the decrease was 38%, 41% and 32% in coleoptile, radicle and endosperm respectively (fig.14). All the treatments were found beneficial. At S_0 level of salinity, the increase in protein content was enhanced by ' $K^+ + GA_3 + CK$ ' to the tune of 13%, 12% and 9% in coleoptile, at S_1 it was 12%, 17% and 18% in radicle, and at S_2 15%, 8% and 16% in coleoptile, radicle and endosperm respectively (fig. 15).

In case of total chlorophyll (Chl-a + Chl-b), 3% and 11% reduction was observed at S_1 and S_2 salinity levels respectively (fig. 16). However the reduction in chl-a/ chl-b was 12% and 43% at S_1 and S_2 salinity levels. The roles of

various seed priming treatments were found positive and out of all the 4 treatments ‘K⁺+ GA₃+ CK’ were found to reduce the effect of salinity.

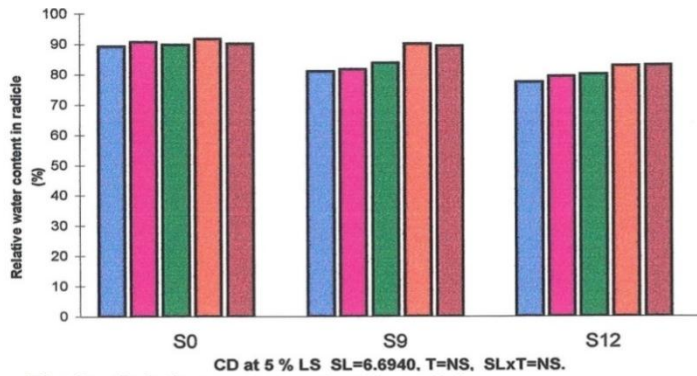


Fig. 9a. Relative water content in radicle

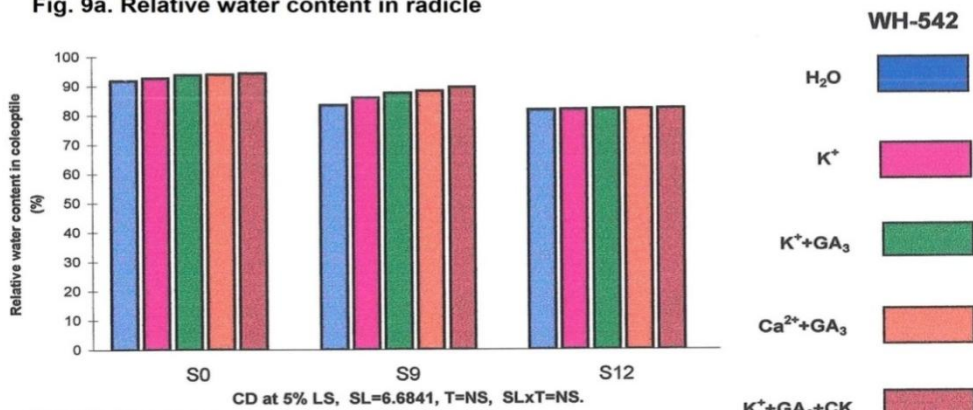


Fig. 9b. Relative water content in coleoptile

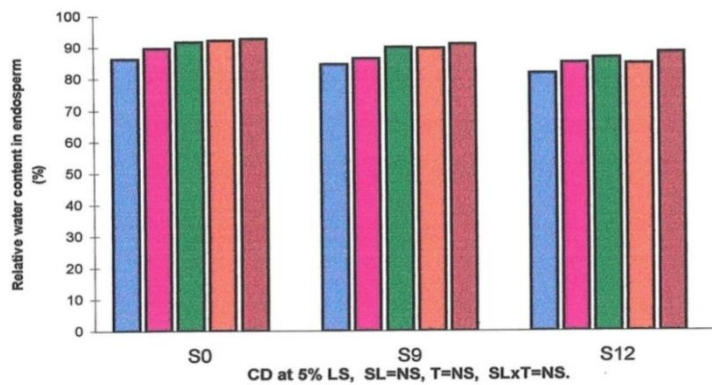


Fig. 9c. Relative water content in endosperm

Fig. 9. effect of different presowing seed soaking treatments on relative water content (%) in radicle, coleoptile and endosperm of wheat grown under various levels of salinity at 96 hours

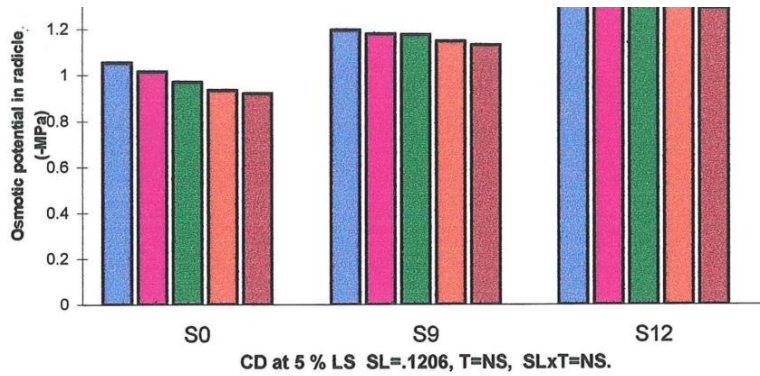


Fig. 10 a. Osmotic potential in radicle

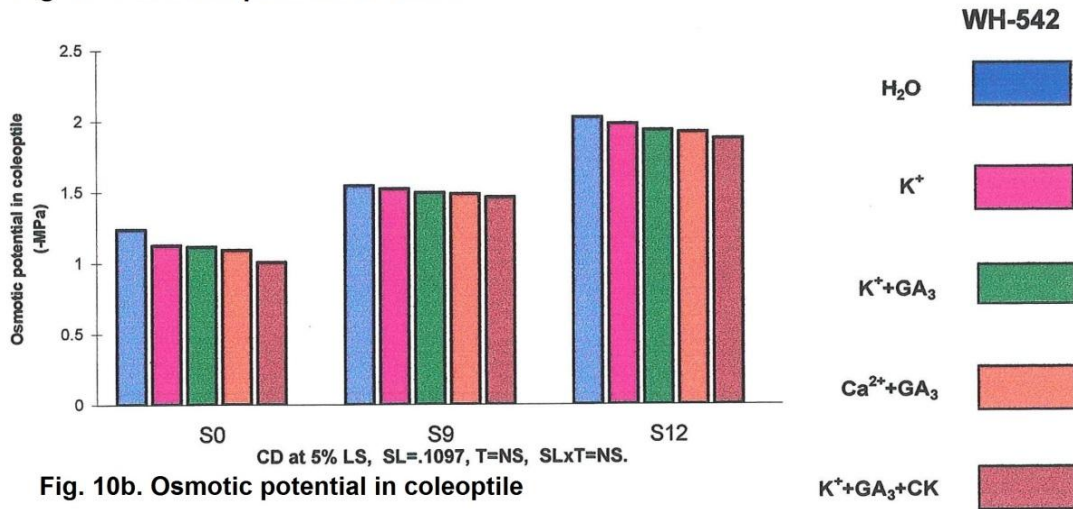


Fig. 10b. Osmotic potential in coleoptile

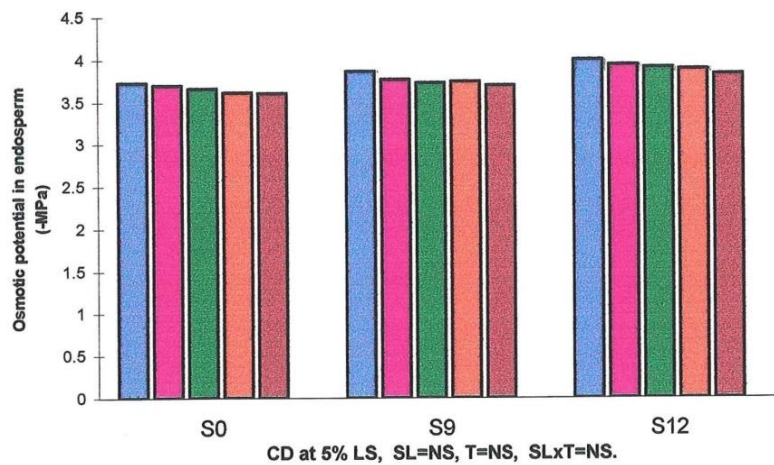


Fig 10c. Osmotic potential in endosperm

Fig. 10. Effect of different presowing seed soaking treatments on osmotic potential (-MPa) in radicle, coleoptile and endosperm of wheat grown under various levels of salinity at 96 hours

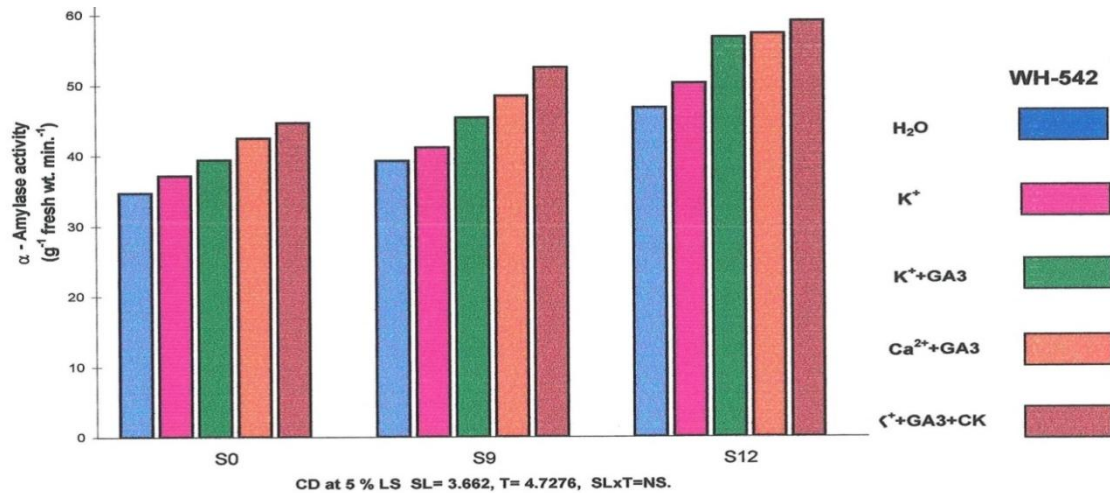


Fig. 11. Effect of seed priming treatments on α -amylase activity (g⁻¹ fresh wt. min⁻¹) after 12 hours of sowing of wheat grown under various levels of salinity

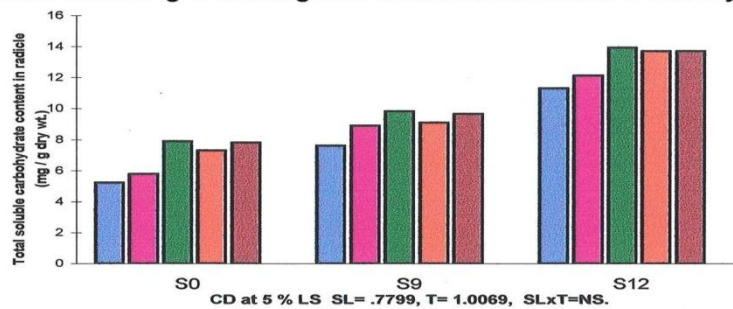


Fig. 12a. Total soluble carbohydrate content in radicle

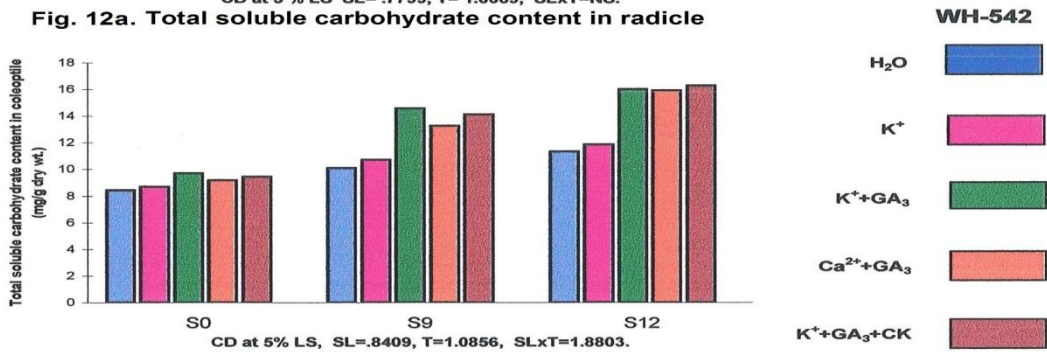


Fig. 12b. Total soluble carbohydrate content in coleoptile

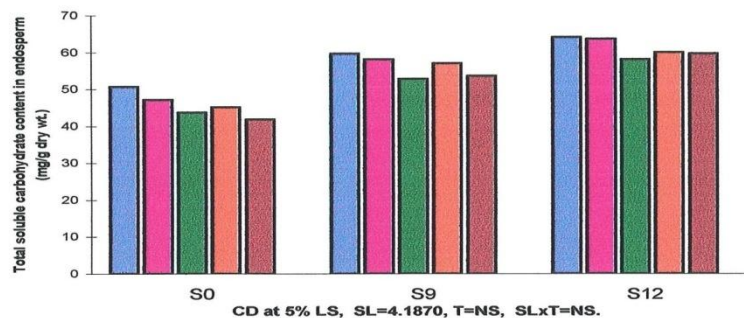


Fig. 12c. Total soluble carbohydrate content in endosperm

Fig. 12. Effect of presowing seed soaking treatments on total soluble carbohydrate content (mg/g dry wt.) in radicle, coleoptile and endosperm of wheat grown under various levels of salinity at 96 hours.

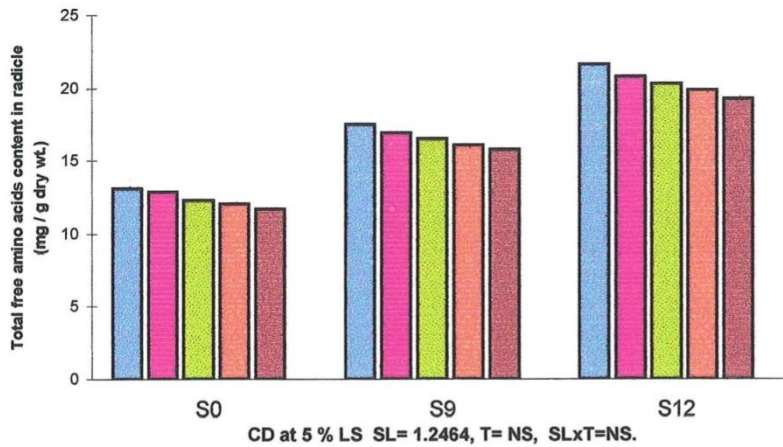


Fig. 13a. Total free amino acids content in radicle

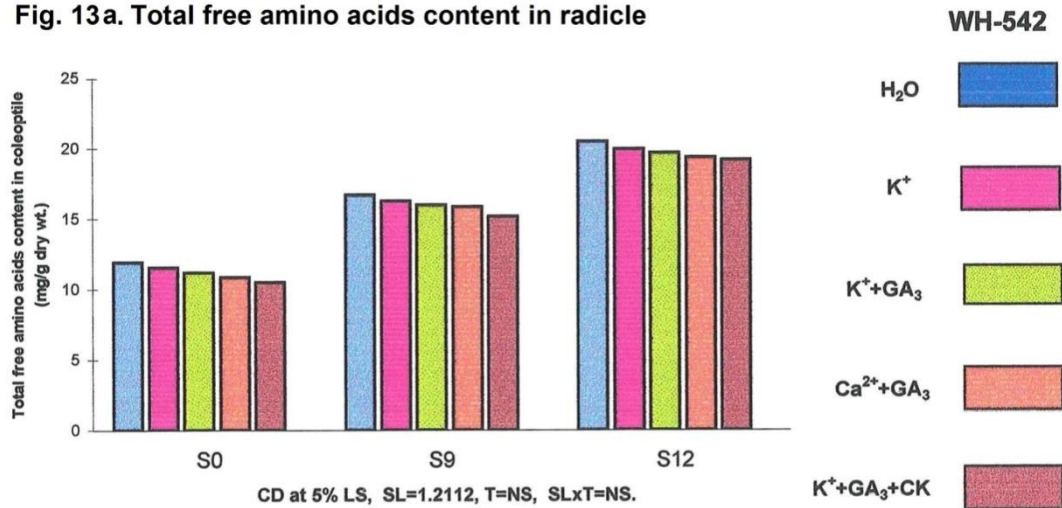


Fig. 13b. Total free amino acids content in coleoptile

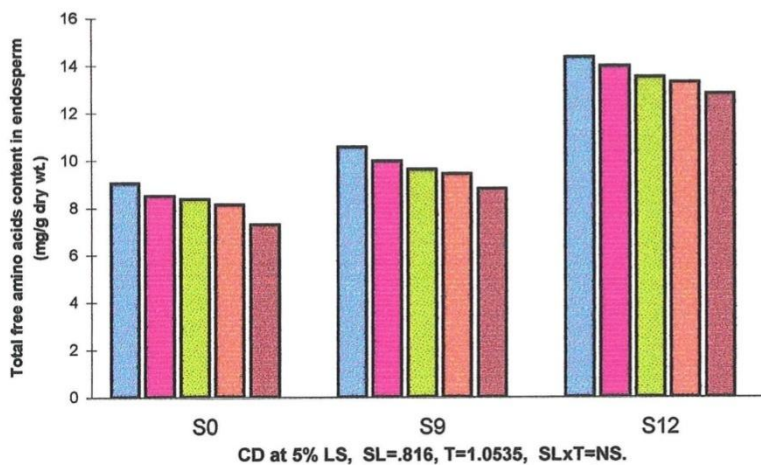


Fig. 13c. Total free amino acids content in endosperm

Fig. 13. Effect of different presowing seed soaking treatments on total free amino acids content (mg/g dry wt.) in radicle, coleoptile and endosperm of wheat grown under various levels of salinity at 96 hours

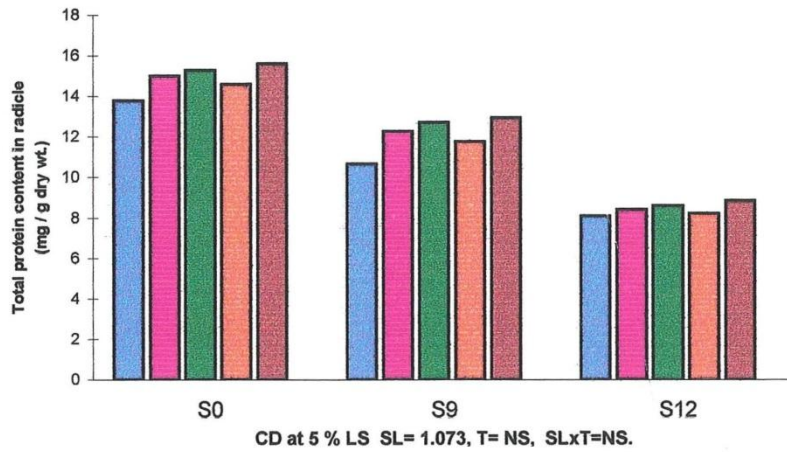


Fig. 14a. Total protein content in radicle

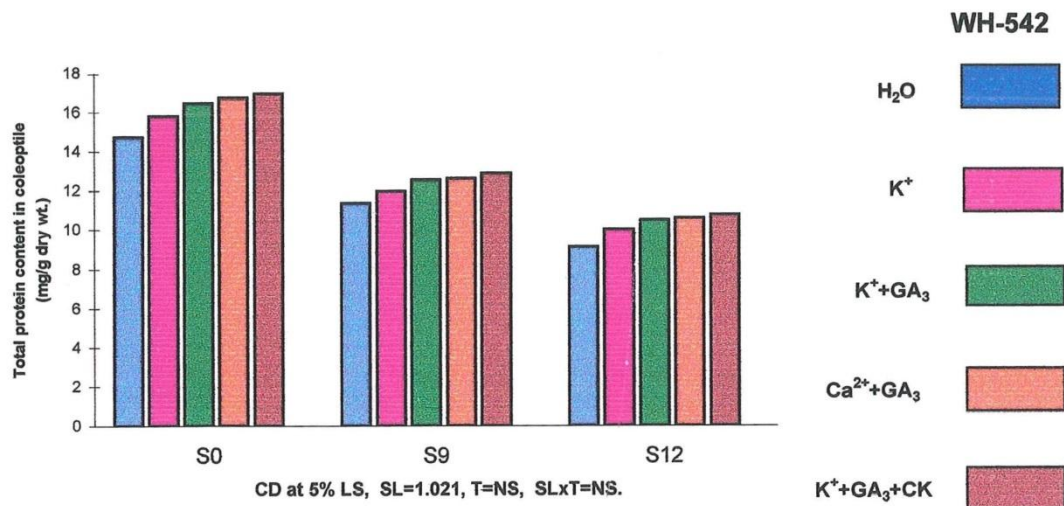


Fig. 14b. Total protein content in coleoptile

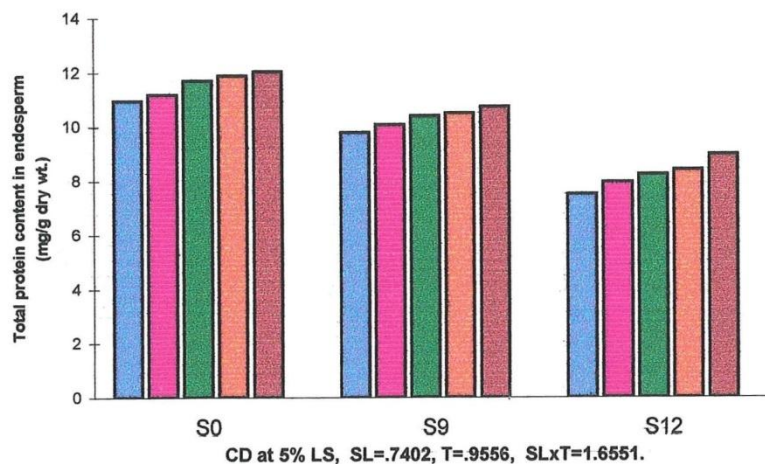


Fig. 14c. Total protein content in endosperm

Fig. 14. Effect of different presowing seed soaking treatments on total protein content (mg/g dry wt.) in radicle, coleoptile and endosperm of wheat grown under various levels of salinity at 96 hours.

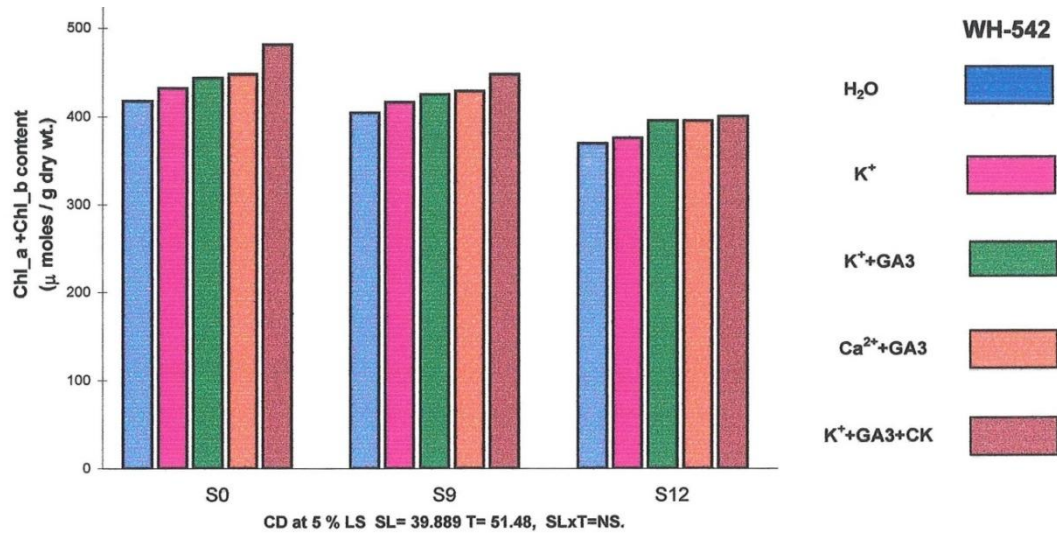


Fig. 15. Effect of seed priming treatments on chl-a + chl-b content (µ moles/g dry wt.) in coleoptile of wheat grown under various levels of salinity at 96 hours in dark plus 48 hours in light.

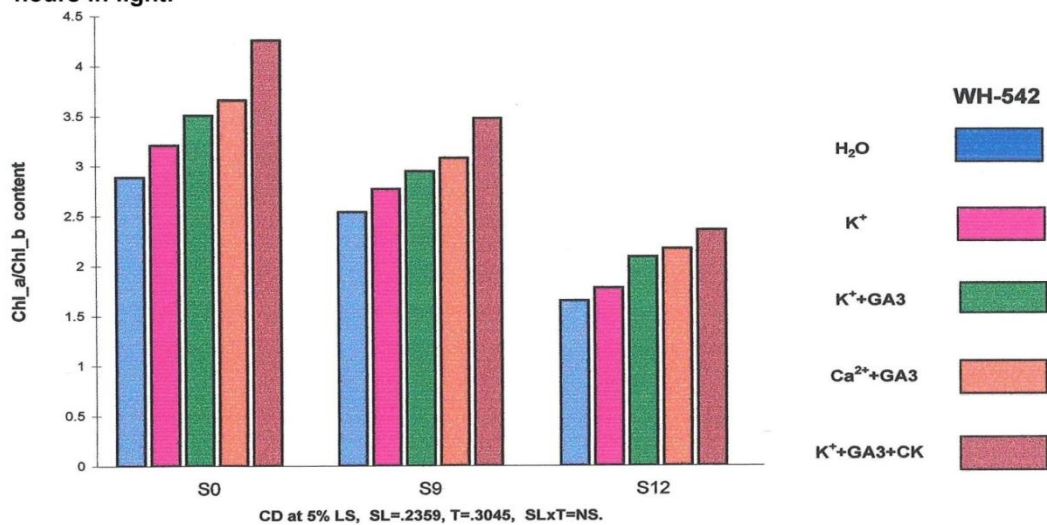


Fig. 16. Effect of seed priming treatments on chl-a/chl-b content in coleoptile of wheat grown under various levels of salinity at 96 hours in dark plus 48 hours in light.

An increase of 112% in Ethylene production was also observed at S₂ level of salinity. This salt induced ethylene production was found to be reduced by the use of seed soaking treatments (fig 17). Rate of respiration in wheat seedlings show decline with increase in salinity. Pre-sowing seed soaking treatments increased the rate of respiration mainly under non-saline conditions but ‘K⁺+ GA₃+ CK’ was found most efficient under saline and non-saline conditions (fig. 18).

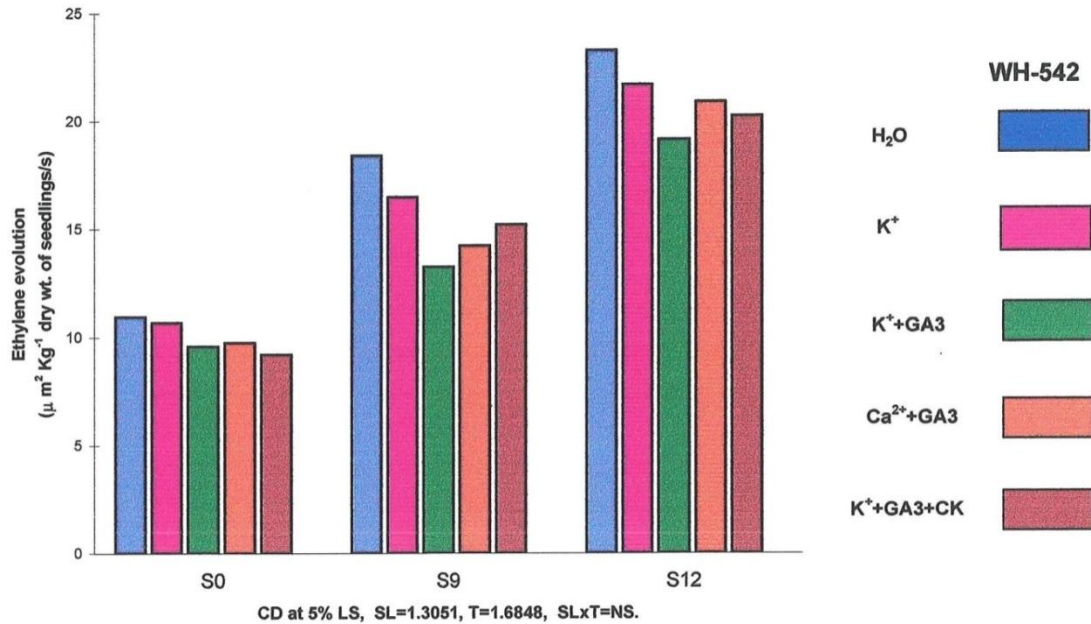


Fig. 17. Effect of seed priming treatments on ethylene evolution ($\mu\text{m}^2 \text{Kg}^{-1}$ dry wt. of seedling/s) of wheat grown under various levels of salinity after 7 days of sowing

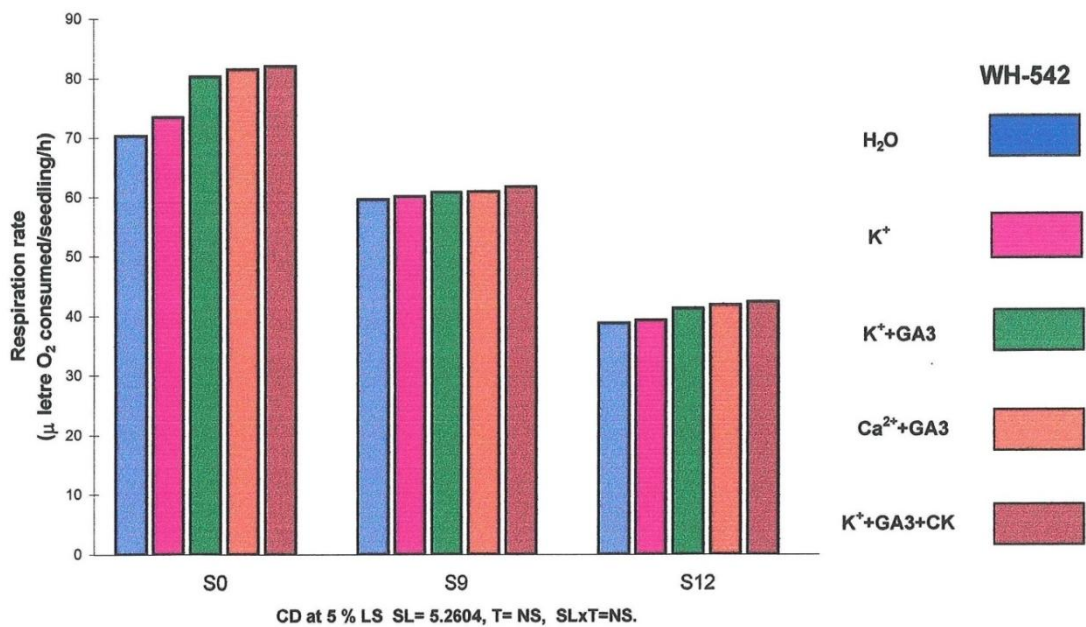


Fig. 18. Effect of seed priming treatments on respiration rate (μ litre O₂ consumed/seedling/ hour) of wheat grown under various levels of salinity after 4 days of sowing.

IV. DISCUSSION & CONCLUSION

Wheat is one of the major crop all over the world, particularly in the Asian region. In India it is used in preparation of a lots of food items. After the green revolution its production in India increased tremendously. Change in the climate and the injudicious use of underground water will cost its impact on the production of wheat in future. Along with this salinity is also posing a threat to wheat production. Although wheat is a moderately saline tolerant crop and many salt resistant varieties are also being produced, still amelioration of salt stress in wheat crop is a good tool to enhance its

production in salinity affected areas. Salt stress is one of the major stress among abiotic stresses. Salt stress is having enormous negative effects on morphological, physiological, and biochemical attributes of plant including germination, growth, water uptake, photosynthesis, nutrient uptake, enzymatic activities, and yield. At lower level of salinity the seed germination is mainly reduced due to ionic toxicity while at higher salinity, it is reduced by osmotic along with ionic toxicity⁽²⁷⁾.

Many studies have been undertaken by researchers to study the mitigation of the above salinity induced attributes. Here we have undertaken this study at the seedling stage. Effect of salinity at 2 levels and alleviation of stress using plant growth regulators and/or with minerals have been studied using many parameters. For many parameters Kinetin along with Gibberellins and minerals have been seen to reduce the effect of salinity. For most of the parameters it was found in our studies that plant growth regulators alone and along with minerals were effective in alleviating the effect of salt stress. Similar results were also observed by many researchers. Shaddad et al⁽²⁰⁾ showed alleviation of salinity effect through seed priming with GA₃ and observed increase in grain weight and grain quality. Foliar application of GA₃ was found to confer salt stress tolerance by⁽²¹⁾. The positive role of Cytokinin restoring the chlorophyll content was observed by Jain⁽²⁹⁾. Kumar⁽³⁰⁾ reported that Gibberellins and Cytokinin in combination were helpful in increasing water status in wheat under salt stress. Kumar⁽³⁰⁾ also suggested that GA₃ and CK antagonized the salinity induced ethylene production. Some other researchers have also suggested the role of plant growth regulators in alleviating negative effects of salinity^(10, 11, 12, 15, 17, 18, 31). The repressive effect of salinity on seed germination and plant growth could be related to reduced endogenous levels of plant growth regulators^(13, 14). Mitigation of salt stress by using Potassium was reported by El-Lethy et al⁽²²⁾ and Kausar and Gull⁽²³⁾, by using Phosphorus reported by Khan et al⁽¹⁶⁾ and by using Calcium was reported by Zaman et al⁽²⁴⁾ and Tian et al.⁽²⁵⁾ Salt stress alleviation was also noticed using calcium and sulphur by Singh⁽²⁶⁾. Our results are in accordance with the previous reports by many researchers. Our studies suggest the use of phytohormones and minerals at the most initial stage of wheat crop.

V. ACKNOWLEDGEMENT

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