

PHYSIOLOGICAL EFFECT OF SOME BIOREGULATORS ON WHEAT PLANTS GROWN UNDER SALT STRESS

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ABSTRACT

Wheat plants (*Triticum aestivum* L.cv. Sakha 8) were treated with some bioregulators (proline 10& 20ppm) , Salicylic acid (50 & 100 ppm) and paclobutrazol (25ppm)) with three salinity levels (0, 1000and 6000 ppm NaCl).

The results showed that, without bioregulators application, increasing NaCl levels increased root / shoot ratio to be 5 fold at 6000 ppm higher than control treatment. Adding the bioregulators obviously decreased the root/ shoot ratio under salt stress. Similar trend was detected for the grains yield (g) plant⁻¹ at harvest stage. Whereas, the highest level of salinity caused a decrease of grain yield (g) plant⁻¹ around 14 times lower than that obtained from control treatment. Meanwhile the bioregulator treatments reduces the harmful effect of salinity on grain yield (g) plant⁻¹. Paclobutrazol gave the best positive effect under the highest salinity level but proline at 20 ppm concentration gave the lowest positive effect on the grain yield (g) plant⁻¹ under the highest salinity level. Some physiological parameters such as total chlorophyll, total soluble sugars , free amino acids , indoles , phenols , proline, K and Na contents in leaves were tested as a physiological markers of wheat plants grown under salt stress. The results showed that, the K/Na ratio and indole content in leaves could be used as physiological markers for wheat plants grown under salt stress.

INTRODUCTION

In Egypt, wheat (*Triticum aestivum* L.) is considered one of the most important produced cereals. The importance of wheat lies in its vital nutritional and industrial commodity in which proteins and carbohydrates. Its cultivated area is extended to reach 3.06 million feddans in 2006 growing season, yielded about 8.274 million ton, with an average of 2.7 ton/fed (according to IAS 2006). However, that production doesn't meet our domestic needs. So, Egypt imports large amounts of wheat to face the great needs of the high population increment of people. So, according to the successive increase in population, the higher needs for agricultural products require maximum yields from the whole area including those salt-affected soils.

Alleviation of salt stresses can be achieved by different irrigation, fertilization, soil aeration, leaching practices or through use of nutrients, hormones, chemical and physical treatments and biological methods (El-Saidi, 1997)

The plant growth regulator, Paclobutrazol (PP₃₃₃), is a triazol derivative (Steffens *et al* 1985). Dawh *et al* (1998); El-Desouky and Atawia (1998) found that paclobutrazol reduced the harmful or negative effects of salinity on growth. Paclobutrazol has been reported to inhibit GA biosynthesis in plants (Dalziel and Lawrence, 1984). Treatment with paclobutrazol resulted in increase chlorophyll, soluble protein and mineral element concentration in leaf tissue (Wang and Steffens, 1987).

Lu and Lu (1999) showed that chlorophyll and proline contents of sorghum cv. Heike increased with PP₃₃₃ treatments under stress. Hassan (1989) found that application of kinetin decreased Na content and increased K content of wheat plants under saline condition of Wadi Sudr. Gadallah (1999) concluded that kinetin application helped wheat plants to grow successfully in the areas subjected to combined effects of salinity and oxygen deficiency.

Raskin (1992 a & b) identified salicylic acid (SA) as a new plant hormone. Application of SA led to an increase in fresh and dry weights of dry bean plants. Application of salicylic acid (SA) to plants has been shown to induce a variety of biological responses, such as, resistance to pathogens (Mills and Wood, 1984), production of pathogenesis-related proteins (Ohashi and Matsuoka, 1987). Leslie and Romani (1988) demonstrated that SA inhibited ethylene formation from ACC.

The mechanisms of salt tolerance still need further investigation either at the level of cell culture and / or molecules levels. Therefore, the purpose of the present investigation was use of plant biochemical regulators (PP₃₃₃, proline and SA) to minimize and alleviate the harmful effects of salinity in a tolerant plant (wheat) as well as increasing the salt tolerance and enhancing their growth. Furthermore, the determination of several salt stress marks such as, total chlorophylls, total soluble sugars, free amino acids, indoles, phenols and proline as response to salt stress.

MATERIALS AND METHODS

This study was performed in the greenhouse of Agric. Bot. Dept. of the Fac. of Agric. Ain-Shams University. Wheat (*Triticum aestivum* L.) grains cv. Sakha 8 kindly obtained from the Wheat Research Dept., Crop Research Institute, Sakha, Egypt.

Ten grains were sown per pot (35cm diameter) on November 20th and 25th for seasons 2002/2003 and 2003/2004 respectively. Fifteen days after sowing, the plants were thinned to four seedlings per pot. The pots were filled with washed sandy soil (15 kg sand per pot). Three levels of salinity (0, 1000 and 6000 ppm NaCl) after that, plant were fertilized with Arnon and Hoagland (1940) nutrient solution. Pots were arranged in complete randomized design. Each treatment has seven replicates. Thirty days after sowing aqueous solutions of 25 ppm paclobutrazol (PP₃₃₃), 10 & 20 ppm proline (Prol), 50 & 100 ppm salicylic acid (SA) and tap water (Control) were randomly sprayed onto plants. Tween 20 was used as a wetting agent at 0.05 ml / L.

Growth Parameters

Two samples were taken, 45 and 165 days (harvest samples) after planting. At the 1st sample date (45 days after sowing), shoot fresh weight and root fresh weight were recorded. At harvesting time (the 2nd sample), weight of 100 grains was evaluated.

Chemical Analyses

Chemical analyses were conducted in season 2002/2003 at 1st sample date for determination of total chlorophyll, total soluble sugar, free amino acid, indole, phenol, proline, K and Na contents in leaves. Total chlorophylls were determined in leaves spectrophotometrically using the method of Arnon (1949). Total soluble sugars were estimated according to Shales and Schales, 1945. Total amino nitrogen or free amino acids were determined according to methods of Plummer (1978). Phenols determination was carried out according to the method recorded by Daniel and George (1972). Indoles determination was carried out according to Larsen *et al.* (1962). The amount of proline was calculated from a standard curve as $\mu\text{g g}^{-1}$ fresh weight following the method of Bates *et al.*, (1973). The statistical analysis of data was done using SAS (2004).

RESULTS AND DISCUSSION

Data in tables (1) and (2) showed that the increasing salinity levels up to 6000ppm increased root/shoot ratio to reach 5 fold at 6000 compared with control (without salinity application) under no bioregulator treatments. Adding the bioregulators decreased root/ shoot ratio under salinity application to be lower than that obtained without bioregulator treatments. Similar trend was detected for weight of grain yield (g) plant⁻¹ at harvest stage. The highest level of salinity caused a decrease of grain yield around 14 time lower than that obtained from control. Meanwhile the bioregulator treatment reduces this harmful effect of salinity on grain yield (g) plant⁻¹ (Table 2). PP₃₃₃ gave the best positive effect under the highest salinity level but proline at 20 ppm concentration gave the lowest positive effect on the weight of grain yield (g) plant⁻¹ under the highest salinity level. These results were found to be agree with those obtained by Hamdy (1988); Ebad *et al* (1992); Hamdy *et al* (1993); Wanas (1996); El-Desouky and Atawia (1998); El-Deep (2003) and Abdel Ati *et al*; (2007). Concerning weight of grain yield (g) plant⁻¹ in the 2nd sampling data in table (2) showed that, saline water at the lowest level (1000 ppm) produced significant increase in weight of grain yield (g) plant⁻¹ as compared to the highest level of salinity. In general, PP₃₃₃ treatment obtained an increase in grain yield (g) plant⁻¹ comparing with the control and the other treatments. The previous results of grain yield (g) plant⁻¹ were found to agree with obtained results by Hamdy (1988); Ebad *et al* (1992); Hamdy *et al* (1993); Wanas (1996), El-Desouky and Atawia (1998); El- Deep (2003). However, pertinent to this is what already pointed out by Marschner (1995) that there are three major constraints for plant growth on saline substrates (1) water deficit (drought stress) arising from the low (more negative) water potential of the rooting medium; (2) ion toxicity associated with the excessive uptake mainly Cl⁻ and Na⁺; (3) nutrient imbalance by depression in uptake and/or shoot transport and impaired internal distribution of mineral nutrients. The same author, further indicated that, it is often not possible to assess the relative contribution of these three major constrains to growth inhibition at high substrate salinity as many factors are involved. These include ion concentrations and relations in the substrate, duration of exposure, plant

species, cultivar, and root stock (excluder or includer), stage of plant development, plant organ and environmental conditions. From our results, it could be concluded that, growth of plant shoot and root were obviously decreased by increasing salinity. However, the shoot system was more sensitive to salt stress than root system (Table 1). Consequently the root/shoot ratio was increased by increasing salinity levels. Adding plant growth regulators rebalance this ratio by decreasing the harmful effect of salinity on shoot. This may be reflect an exclusion behaviour for wheat plants under salt stress. An opposite trend was found by includer plants such as sugar beet whereas, the root/shoot ratio was decreased by increasing salinity stress (Eisa *et al* 2001).

Table 1. Effect of salinity and bioregulator treatments on root/ shoot ratio in the first sample

Na salinity levels (ppm)	0	1000	6000
Plant growth regulators			
Control	0.10	0.15	0.50
PP ₃₃₃ 25 ppm	0.13	0.18	0.23
Proline 10 ppm	0.15	0.10	0.17
Proline 20 ppm	0.13	0.10	0.19
SA 50 ppm	0.13	0.14	0.20
SA 100 ppm	0.13	0.160	0.19

Table 2. Effect of PP₃₃₃, SA and proline on weight of 100 grains under different salinity levels.

NaCl salinity levels (ppm)	0(ppm)	1000 (ppm)	6000 (ppm)	Means bioregulators
Bioregulators				
Control	1.6	1.20	0.11	0.97
PP ₃₃₃ 25 ppm	1.4	1.4	0.40	1.1
Proline 10 ppm	1.50	1.3	0.20	1.0
Proline 20 ppm	1.40	1.2	0.15	0.92
SA 50 ppm	1.55	1.3	0.20	1.02
SA 100 ppm	1.60	1.3	0.30	1.07
Means (salinity)	1.51	1.28	0.23	
LSD salinity	0.0542			
LSD bioregulators	0.00767			
LSD salinity x bioregulators	0.1329			

Physiological parameters

Data in figurs. (1-6) revealed some biochemical constituents such as total chlorophyll, total soluble sugars, free amino acids, indoles, phenols, proline and K/Na ratio were determined in leaves at the 1st sampling date. It was indicated that, the highest level of salinity individually or under saline application increased chlorophyll content as compared to fresh water. Chlorophyll content in the 1st sample was obviously increased under the highest concentration of salinity and recorded 22% more than that obtained in the control. In this respect, the results are in agreement with Joshi (1976); Tiku (1976); Ebad *et al* (1992); Reddy *et al* (1992); El-Desouky & Atawia (1998); El-Deep (2003); they found that application of saline water increased photosynthetic rate in wheat plants. In case of salinity on photosynthetic

pigments resulted depending upon the time of salinity application. Although there was clear evidence that chlorophyll content significantly decreased in early salt application but this reduction did not appear in late salt application (Eisa, 1999). A phenomenon which revealed that under late salinity the plant escaped from the deleterious effect of salinity on chlorophyll content. In salt tolerant species chlorophyll content increased (Lutts *et al* 1996) whereas in salt sensitive species it decreased (Salma *et al* 1994).

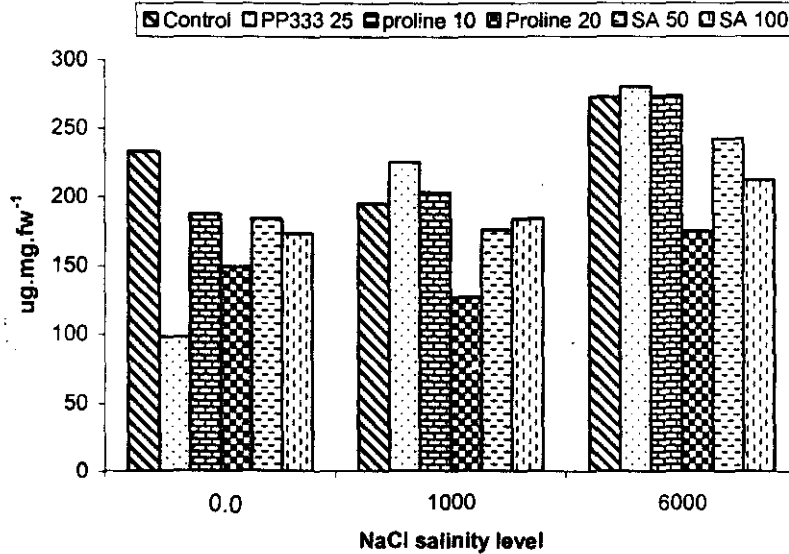


Fig. 1. Effect of PP₃₃₃, SA and proline on chlorophyll content under different salinity levels

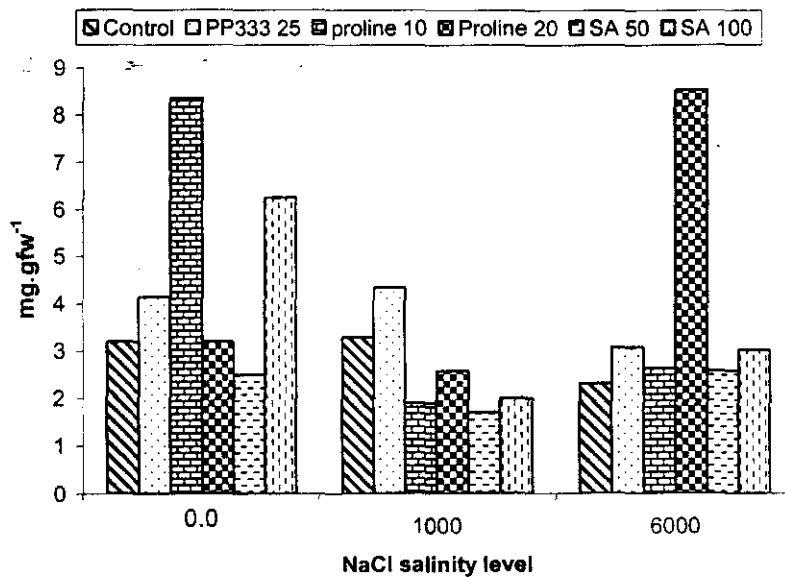


Fig. 2. Effect of PP₃₃₃, SA and proline on soluble sugars under different salinity levels

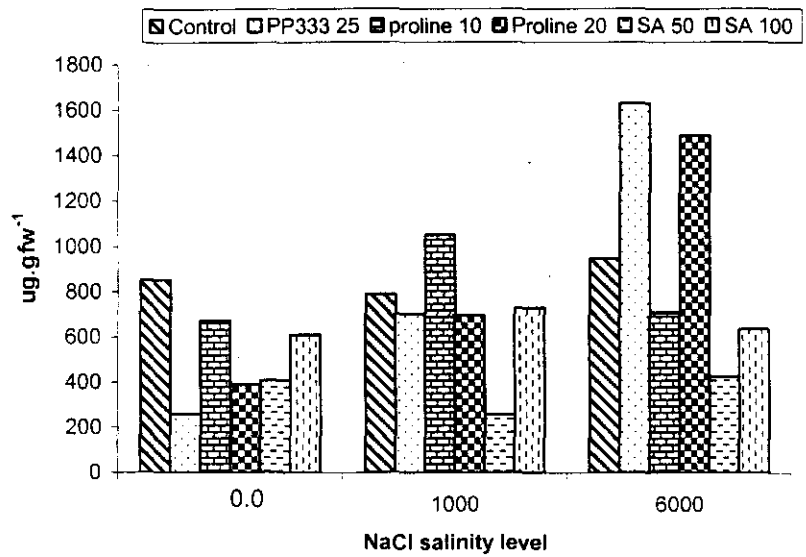


Fig. 3. Effect PP₃₃₃, SA and proline on free amino acid under different salinity levels

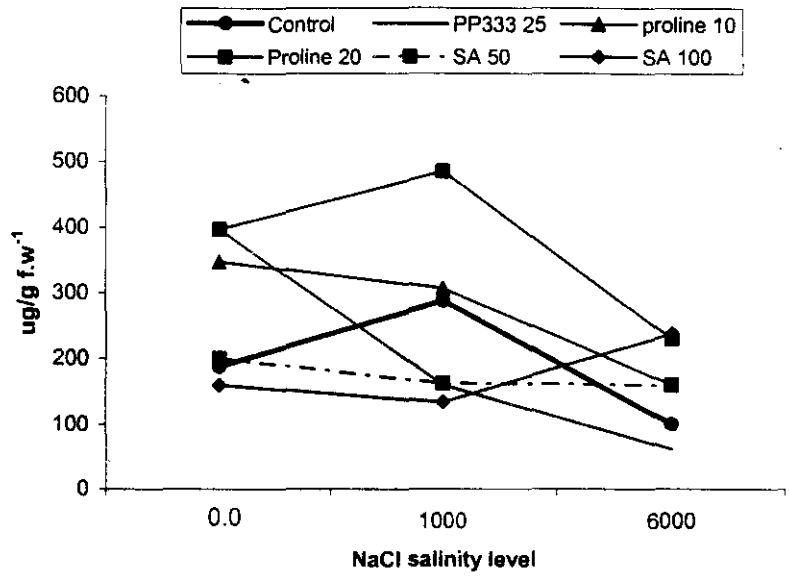


Fig. 4. Effect of PP₃₃₃, SA and proline on Indole content in wheat plants grown under different salinity levels

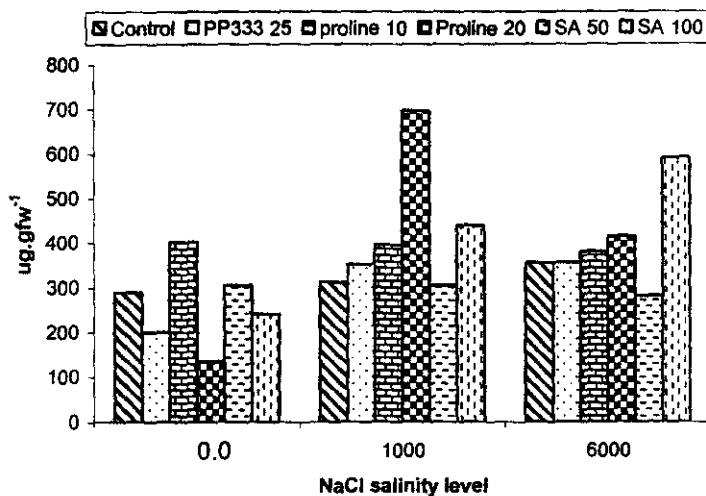


Fig. 5. Effect of PP₃₃₃, SA and proline on Phenoles content in wheat plants grown under different salinity levels

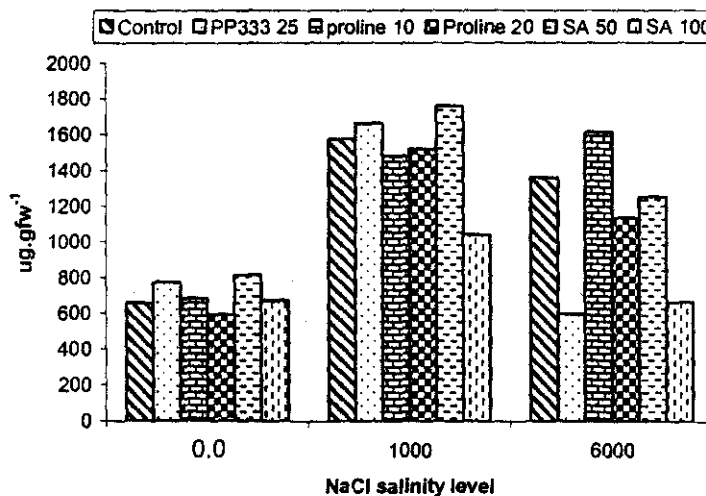


Fig. 6. Effect of PP₃₃₃, SA and proline on proline content in wheat plants grown under different salinity levels

Table 3. The effect of NaCl salinity and bioregulators application on K/Na ratio in leaves at first sample

Plant growth regulators	NaCl salinity levels (ppm)		
	0	1000	6000
Control	8.4	5.7	0.58
PP ₃₃₃ 25 ppm	10.1	5.4	1.12
Proline 10 ppm	8.1	4.9	0.90
Proline 20 ppm	7.6	6.1	0.92
SA 50 ppm	9.1	6.2	1.00
SA 100 ppm	8.8	5.9	0.89

As for the effect of bioregulator (PP₃₃₃, proline and salicylic acid) on total soluble sugars, data in fig. (2) showed that, total soluble sugars were insignificantly reduced in all treatments; similar trend was found in the highest level of saline water.

It could be noticed from fig. (3) that all treatments showed an insignificant value in free amino acids as compared to the control. But there are significant values in free amino acids between treatments particularly the low and high concentration of bioregulator (proline and salicylic acid).

The saline water at the highest level led to an increase in free amino acids comparing with fresh water and low saline level. The increase concentrations of total soluble sugars and free amino acids at higher level of salinity were associated with osmotic adjustment.

The data in fig (4) revealed that all treatments gave effects on indole content. Saline water induced a significant decrease in indole content comparing with fresh water. The biological activities of the endogenous phytohormones (cytokinin, gibberellin and auxin) were significantly reduced by excess salinity (El-Desouky and Atawia, 1998 and Ibrahim & Shehata, 2000).

Data in fig. (5) indicate that proline treatment at the rate 10 ppm significantly increased phenol content as compared to the control while the other treatments caused insignificant increase in phenol content. The treatments under saline water produced a significant increase in phenol content comparing with these treatments in fresh water. The increasing of salinity level tended to increase phenolic compounds. The previous results were found to be harmony with Ibrahim & Shehata (2000); Zaghlool and Ibrahim (2000). These phenolic compounds could be a cellular adaptive mechanism for scavenging oxygen free radicals during stress and this free radicals scavenger and others such as ascorbate could be readily oxidized in the system of tissue representing subcellular damages (Das *et al* 1990).

Regarding proline content fig (6), PP₃₃₃ treatment gave a significant increase in proline content comparing with control while SA 100 ppm led to a significant decrease. Saline water treatments resulted in significant increase as compared to fresh water. PP₃₃₃ treatment under the low salinity level stimulated a significant increase in proline content. These results were found to be agreement with (Lee *et al* 1994; Mumtaz *et al* 1995). PP₃₃₃ enhanced proline levels (Mumtaz *et al* 1995 and El- Deep, 2003). Proline accumulation is one of the most frequently reported modifications induced by water deficit and salt stress in plants, besides it is involved in stress resistance

mechanisms (Sakr *et al* 2007). Several functions are proposed for the accumulation of proline in tissues submitted to salt stress: (1): osmotic adjustment, (2): C and N reserve for growth after stress relief, (3): detoxification of excess ammonia, (4): stabilization of proteins and/or membranes, (5): and being a scavenger of free radicals, (6): improving the stability of some cytoplasmic and mitochondrial enzymes, (7): increases the solvation of protein, (8): proline plays an important role in the protection of enzymes or membranes against salinity (Ozdemir *et al* 2004 and Sakr *et al* 2008).

As for K/Na ratio in levels, data in table (2) showed a sharply decrease in K/Na ratio by increasing salinity levels. However, this decrease in K/Na ratio was partially improved by adding bioregulators. PP₃₃₃ gave the best rebalance for K/Na ratio among all other treatments. In a saline environment, plants take up excessive amount of Na⁺ and Cl⁻ as in halophytes resulted in high Na⁺/K⁺ ratio which may impair the selectivity of root membrane. The greater accumulation of Na⁺ in plant roots may be due to a regulatory mechanisms located within the roots that prevents translocation excessive cation, such as Na⁺ from the root to aerial parts, resulting in Na⁺ retention. The accumulation of Na⁺ in plant root may be due to the high mobility of Na⁺ in the phloem (Khan *et al* 1997 and Sakr *et al* 2008). Generally, from this work it could be concluded that, a significant increase in K/Na ratio and decreasing of indole contents could be good markers for salt tolerance in wheat plant (Lee *et al* 1994; Mumtaz *et al* 1995). PP₃₃₃ enhanced proline levels (Mumtaz *et al* 1995 and El-Deep, 2003).

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التأثير الفسيولوجي لبعض المنظمات الحيوية على القمح النامي تحت تأثير الإجهاد الملحي

إبراهيم زكى الشامي

قسم النبات الزراعي - كلية الزراعة - جامعة عين شمس - القاهرة - مصر

أجريت تجربة أصص في كلية الزراعة جامعة عين شمس خلال موسمي ٢٠٠٢/٢٠٠٣، ٢٠٠٣/٢٠٠٤ على نباتات القمح (صنف سخا ٨) لدراسة تأثير الإجهاد الملحي ومحاولة تخفيف هذا الأثر باستخدام بعض المنظمات الحيوية.

استخدمت ثلاث مستويات ملوحة (صفر، ١٠٠٠، ٦٠٠٠ جزء في المليون من كلوريد الصوديوم) وعملت النباتات ببعض المنظمات الحيوية (برولين ١٠، ٢٠ جزء في المليون وحمض السليسيليك ٥٠، ١٠٠ جزء في المليون والباكلوبيوتريازول ٢٥ جزء في المليون).

أشارت النتائج إلى أن زيادة الملوحة إلى مستوى ٦٠٠٠ جزء في المليون قد أدى لزيادة نسبة المجموع الجذري/ المجموع الخضري لتصل لحوالي ٥ مرات عند مقارنتها بنباتات الكنترول بينما أظهر التأثير عكس ذلك على محصول الحبوب بالجذم/ نبات حيث أن التركيز الأعلى من الملوحة (٦٠٠٠ جزء في المليون) أدى لانخفاض في محصول الحبوب لحوالي ١٤ مرة عند المقارنة بالكنترول. وأدت المعاملة بالمنظمات الحيوية إلى تقليل الأثر الضار للإجهاد الملحي على النباتات وكان الباكلوبيوتريازول (٢٥ جزء في المليون) هو الأكثر تأثيراً في تخفيف أثر الملوحة.

قدرت بعض المظاهر الفسيولوجية في الاوراق كدلائل على الإجهاد الملحي مثل محتوى الكلوروفيل الكلي ، السكريات الذائبة ، الاحماض الامينية الحرة ، الاندولات ، الفينولات ، البوتاسيوم والصوديوم في الاوراق . أكدت النتائج أن زيادة نسبة الصوديوم الى البوتاسيوم وانخفاض نسبة الاندولات هي أفضل الدلائل الفسيولوجية على حدوث الإجهاد الملحي لنبات القمح النامي تحت ظروف الإجهاد الملحي.