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Effects of Salicylic Acid on the Growth and Some Physiological Characters in Salt Stressed Wheat (*Triticum aestivum* L.)

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Abstract: This study was conducted to determine the effects of seed soaking in salicylic acid (10^{-2} mol/L, 10^{-4} mol/L, 10^{-6} mol/L and control) on the growth and some physiological characters in wheat (*Triticum aestivum L.*) under salinity (8 ds m^{-1}) and non salinity conditions. NaCl reduced the emergence percentage, the growth parameters (shoot and root dry weight), K^+/Na^+ ratio, osmotic potential and photosynthetic pigments (Chl *a, b* and carotenoids) contents in wheat seedlings. The emergence percentage was not changed, in contrast, shoot and root dry weight of seedlings, K^+/Na^+ ratio, photosynthetic pigments (Chl *a, b* and carotenoids) contents and osmotic potential were increased by salicylic acid treatments under non salinity condition. Seed soaking in SA increased the emergence percentage, osmotic potential, shoot and root dry weight, K^+/Na^+ ratio, photosynthetic pigments (Chl *a, b* and carotenoids) contents in the salinity stressed wheat seedlings. These results were indicated that SA has positive effects on plant growth in salinity and non salinity conditions. But the effects of SA was higher on emergenge percentage, K^+/Na^+ ratio and osmotic potantial in salinity condition compared to non salinity condition was obtained in this study.

Key Words: Salicylic acid, salinity, wheat

Salisilik Asitin Tuz Stresindeki Buğdayın (*Triticum aestivum* L.) Büyümesi ve Bazı Fizyolojik Özellikleri Üzerine Etkileri

Öz: Bu çalışma tohuma uygulanan salisilik asitin (10^{-2} mol/L, 10^{-4} mol/L, 10^{-6} mol/L ve kontrol) tuzlu (8 ds m^{-1}) ve tuzsuz koşullarda buğdayın büyümesi ve bazı fizyolojik özellikleri üzerine etkilerini belirlemek için yürütülmüştür. Tuz buğday fidelerinde çıkış oranı, büyüme değerleri (toprak altı ve toprak üstü kuru ağırlıklar), K^+/Na^+ oranı, ozmotik potansiyel ve fotosentetik pigment (Klo *a, b* and karotenoidler) içeriklerini azaltmıştır. Tuzsuz şartlarda salisilik asit uygulamasının çıkış oranını değiştirmediği, aksine fidelerin toprak altı ve toprak üstü kuru ağırlıkları, K^+/Na^+ oranı, fotosentetik pigment içerikleri (Klo *a, b* and karotenoidler) ve ozmotik potansiyelini arttırmıştır. Tuz stresindeki fidelerde ise salisilik asit uygulaması ile çıkış oranı, ozmotik potansiyel, toprak altı ve toprak üstü kuru ağırlıkları, K^+/Na^+ oranı ve fotosentetik pigment (Klo *a, b* and karotenoidler) içerikleri artmıştır. Bu sonuçlar salisilik asitin tuzlu ve tuzsuz şartlarda bitki büyümesini olumlu yönde etkilediğini göstermektedir. Fakat tuzlu şartlarda salisilik asitin çıkış oranı, K^+/Na^+ oranı ve ozmotik potansiyele olan etkilerinin tuzsuz şartlardaki uygulamasına nazaran daha yüksek olduğu belirlenmiştir.

Anahtar Kelimeler: Salisilik asit, tuzluluk, buğday

Introduction

Salinity is one of the main problems that negatively affect soil fertility and limit plant production (Richards 1954). Soil salinity affects osmotic stress, decreasing water availability, ionic stress, changes in the cellular ionic balance (Kirst 1989). The effect of salinity were determined at seedling stage of wheat range from reduction in germination percentage, fresh and dry weight of shoots and roots to the uptake of various nutrient ions (Afzal et al. 2005).

Salicylic acid (SA) is recognized as an endogenous signal molecule, mainly involved environmental stress tolerance in plants. Seed soaking have been shown to enhance stand establishment in non saline areas (Khan 1992) and have potential in saline areas as well (Basra et al. 2005). Plants produce proteins in response to abiotic and biotic stress and many of these proteins are induced

Plants produce proteins in response to abiotic and biotic stress and many of these proteins are induced by phytohormones such as ABA (Jin et al. 2000) and salicylic acid (Hoyos and Zhang 2000). SA is synthesized by many plants (Raskin et al. 1990) and is accumulated in the plant tissues under the impact of unfavorable abiotic factors, contributing to the increase

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of plants resistance to salinization (Ding et al. 2002, Kang and Saltveit 2002). In addition, SA-induced increase in the resistance of wheat seedlings to salinity (Shakirova and Bezrukova 1997). Thus the detrimental effects of high salts on the early growth of wheat seedlings may be alleiveted by treating seeds with the proper concentration of a suitable hormone (Darra et al. 1973).

The aim of the present work was to study the character of the changes induced by SA in the growth of wheat plants under salinity and non salinity conditions.

Materials and Methods

The pot trial was conducted in greenhouse during October-November 2004 with using a complete randomized design containing of non salinity and sodium chloride (NaCl) (0 and 8 ds m^{-1}) (Rhoades et al. 1992) and four SA, (S0:0 mol/L, S1:10^{-6} mol/L, S2:10^{-4} mol/L, S3:10^{-2} mol/L) with 3 replications. The seeds of local variety, Tir, were used as experimental material. In this study home bred, Tir, line was selected as the test plant because of the current concern for high yielding in Van Region. Seeds were surface sterilized then rinsed with sterilized water and air-dried.

Salicylic acid was dissolved in distilled water and the pH was adjusted at 6.5 with NaOH. The seeds were soaked with different SA levels for 12 h in the dark at 22 ^{o}C.

Soil Description: The soil samples were air dried and sieved (< 2 mm stainless steel mesh) before determination of chemical properties. The soil samples used in the pots were dried, powdered and mixed thoroughly. The texture of the soil based on sand clay silt, total oganic matter 2.1 %, total salt 0.025 %, pH 7.50, total nitrogen 0.8 %, available phosphorus 15.8 mg P kg^{-1} dry soil, exchangeable potassium 150.5 mg K kg^{-1} dry soil.

Each plastic pot (14 x 17 cm) with the closed bottom end was filled-up with 2 kg air-dried soil and kept inside glass greenhouse under natural light. Soil was allowed to equilibrate in the greenhouse for 1 week before sowing the seeds. All pots were fertilized with 100 $mgNkg^{-1}$ (AS) and 100 $mgPkg^{-1}$ (TSP). The respective amount of SA was soaked with the seed before sowing. Pre-soaked ten seeds were sown in each pot. The seedlings were irrigated with non salinized tap water every 2-3 days, maintaining the soil moisture content at 15 % (w/w) by weighing. The pots were randomly arranged in a greenhouse and rearranged several times during the growth period. Samples were collected after 21 days of emergence. After harvest, the seedlings were separated into shoots and roots, and rinsed thoroughly with deionized water. The dry weight (DW) of shoots and roots were recorded after dried at 70 ^{o}C for 48 h (Bray 1963).

The determining of leaf osmolality, extracts were prepared from leaves by using Jones and Turner’s (1978) method. The osmolality (-MPa) was determined by using a Vapour–pressure osmometer (Vapro Osmometer).

Chlorophyll Extraction and Measurement: Chlorophyll concentration was determined from fully expanded leaves. A leaf sample of 0.1 g was ground and extracted with 5 mL of 80% (v/v) acetone in the dark (Arnon 1949). The mixture was filtered and absorbancies (Jenway 6105 UV/VIS, Spectrophotometer) were determined at 645, 663 and 450 nm. Concentration of chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoids were estimated by the equations of Witham et al. (1971).

Extraction and measurement of K^{+}/Na^{+} ratio: Dried plant samples were finely ground in a stainless steel miller. Shoot samples were wet digested with a HNO_3 and $HClO_4$ acid mixture and analyzed by a flame photometer (SOLAAR AA Series, Thermo Electron Corporation).

The data were statistically analyzed by COSTAT and MSTATc computer programme and comparative analyses of the means were performed by LSD.

Results and Discussion

Emergence percentage: Variance analysis results of emergence percentage are shown in Table 1. According to Table 1, salt ($P<0.01$) and salicylic acid ($P<0.05$) affected emergence percentage significantly. In addition, an interaction was obtained between salicylic acid and salt significantly ($P<0.05$). Emergence percentage was reduced by salinity compared to non salinity condition. Increasing of SA levels caused increases in emergence under salinity condition. But under non salinity condition, emergence percentage was not affected by SA levels. On the contrary, emergence percentages were drastically increased by SA under salinity conditions compared to non treatment of SA. (Figure 1). The seeds pretreated with S3 exhibited higher emergence percentage than untreated seeds. Shakirova et al. (2003), El-Tayeb (2005) and Afzal (2005) observed that SA treatment increases emergence percentage under salinity conditions.

Table 1. Summary of variance analysis

Source	df	Emergence Percentage	Shoot Dry Weight	Root Dry Weight	Chl a	Chl b	Carotenoids	K^+/Na^+ Ratio	Osmotic Potential
		M S							
Main Effects									
Salicylic Acid	3	147.16*	213.98**	115.36**	6.640**	2.633**	3.283**	0.493**	0.082**
Salt	1	3901.50**	2626.08**	555.84**	54.77**	1.966**	3.944**	4.690**	0.277**
Interaction									
SAXSalt	3	96.05*	0.293	17.318	0.500	0.195	0.156	0.183**	0.238**
Error	16	29.41	18.63	18.603	0.223	0.162	0.173	0.021	0.005
Total	23								

**P<0.01, *P<0.05

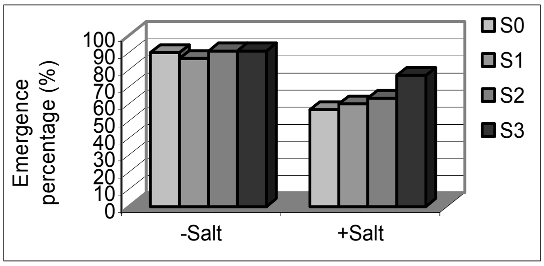

Figure 1. Effects of SA on emergence percentage under salinity and non salinity

Table 2. Effects of salicylic acid on the growth parameters of wheat

SA	Shoot dry weight (mg $seedling^{-1}$)			Root dry weight (mg $seedling^{-1}$)		
	-Salt	+Salt	Av.	-Salt	+Salt	Av.
S0	45.7	24.2	34.5 c	25.2	12.3	18.7 b
S1	49.2	28.6	38.9 bc	29.7	18.8	24.2 a
S2	52.1	31.6	41.9 b	30.3	20.4	25.3 a
S3	59.6	38.5	49.1 a	31.8	26.9	29.4 a
Mean	51.6 a	30.7b		29.1 a	19.6 b	
SA (LSD:0.05)			5.283			5.279
Salt (LSD:0.05)			3.735			3.732
SAXS(LSD:0.05)			ns**			ns

*The same letters are not statistically significant in columns (p<0.05); **ns, non significant

Shoot dry weight and root dry weight: Variance analysis results of shoot dry weight and root dry weight are shown in Table 1. According to Table 1, salt (P<0.01) and salicylic acid (P<0.01) influenced shoot dry weight and root dry weight significantly.

Dry weights of shoot and roots decreased drastically under salinity condition compared with non salinity condition. These results are similar to those reported by and Afzal (2005) who found that dry weight was reduced by salt stress in wheat. Dry weights of seedlings was decreased due to salinity stress but seedlings raised from seeds primed with SA improved dry weight of seedlings as compared to non treatment of SA under non salinity and salinity conditions (Table 2). Maximum shoot and root dry weights were recorded in seedlings raised from seeds primed with S3 treatments. Salt tolerance was increased in seeds subjected to S3 level of SA as indicated by shoot and dry weight. This may indicate that, treatment of seed with SA exhibited an significant increase in salt tolerance. These results was similar to the studies of El-Tayeb (2005) reported that SA pretreatment increased dry weight in the stressed barley seedlings. In an another work Gutierrez Coronado et al. (1998), observed significant effect of SA on soybean increaeses in shoot growth, root growth and plant height. Khodary (2004) who reported that SA increased the fresh and dry weight of shoot and roots of salt stressed maize plants.

Photosynthetic pigments (chlorophyll *a, b* and carotenoids) contents: Variance analysis results of photosynthetic pigments (chlorophyll *a, b* and carotenoids) are shown in Table 1. According to Table 1, salt (P<0.01) and salicylic acid (P<0.01) affected photosynthetic pigments (chlorophyll *a, b* and carotenoids) significantly.

Under the influence of salinty the photosynthetic pigments greatly decreased. Dela-Rosa and Maiti (1995), observed that chlorophyll biosynthesis decreased salt conditions of sorghum plants. El-Tayeb (2005) found that Chl *a, b* and carotenoids decreased significantly in NaCl treated plants in comparison to controls of barley plants.

SA treatments were increased pigments content of the plants under non salinity and salinity condition (Table 3). In bean plants, foliar spray with saliciylic acid, increased Chl *a, b* and carotenoids under normal field conditions (Türkyılmaz et al. 2005). Pancheva et al. (1996), showed that long-term treatment (7 days) of barley seedlings with SA decreased the rate of

Table 3. Effects of salicylic acid on photosynthetic pigment contents of wheat

SA	Chl a (mg g^{-1} fresh weight)			Chl b (mg g^{-1} fresh weight)			Carotenoids (mg g^{-1} fresh weight)		
	-Salt	+Salt	Average	-Salt	+Salt	Average	-Salt	+Salt	Average
S0	10.29	7.10	8.19 c *	4.03	3.31	3.67 c	2.02	1.31	1.66 c
S1	10.30	7.20	8.75 c	4.13	4.07	4.10 bc	2.49	2.02	2.25 b
S2	11.14	7.85	9.49 b	4.88	4.27	4.57 b	2.83	2.00	2.42 b
S3	12.38	8.88	10.63 a	5.66	4.77	5.21 a	4.06	2.82	3.44 a
Meann	10.78 a	7.75 b		4.67 a	4.10 b		2.85 a	2.04 b	
Salysilic Acid (LSD:0.05)			0.578			0.493			0.510
Salt (LSD:0.05)			0.409			0.348			0.360
SAXSalt (LSD:0.05)			ns**			Ns			ns

*The same letters are not statistically significant in columns (p<0.05); **ns, non significant

photosynthesis (chlorophyll content) whereas short-term treatment (2 hours) did not effect chlorophyll content as compared with unterated control plants. Zhou et al. (1999) reported that photosynthetic pigments were increased in corn with SA application. Moreover, Khan et al. (2003) showed that SA increased photosynthetic rate in corn and soybean.

The seeds pretreated with 10^{-2} mol/L SA solution exhibited higher chlorophyll content. These results are in agreement with those obtained by other author, showing that SA significantly inreased the pigment content under salt stress (El-Tayeb 2005)

Shoot K^+/Na^+ ratio: Variance analysis results of shoot K^+/Na^+ ratio are shown in Table 1. According to Table 1, salt (P<0.01) and salicylic acid (P<0.01) affected shoot K^+/Na^+ ratio significantly. In addition, an interaction was obtained between salicylic acid and salt significantly (P<0.01).

Salinity reduced K^+/Na^+ ratio in the shoots compared to non salinity condition. Salt stress induced decrease in the K^+/Na^+ ratio is inimical to cellular biochemical processes. In addition to this, potassium provides necessary osmotic potential for water uptake by plant cells (Claussen et al. 1997). Thus, K^+ uptake is pivotal for cell turgor and maintenance of biochemical processes under salinity. In plants, Na^+ competes with K^+ uptaken under salinity conditions (Chinnusamy and Zhu 2003). Previous studies have shown that high K^+/Na^+ ratio show a positive relationship with salt tolerance (Ashraf et al. 1997).

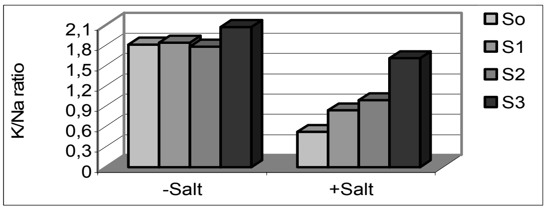

Figure 2. Effects of SA on K^+/Na^+ under salinity and non salinity conditions

SA treatments increased K^+/Na^+ ratio in the plant shoots under salinity condition. However K^+/Na^+ ratio was the highest in 10^{-2} mol/L seed applied SA (Figure 2). Hamada and Al-Hakimi (2001), observed positive effects of SA in the Na, K, Ca and Mg content of wheat plants grown under salinity. SA application inhibited Na accumulation in salinity condition. This main indicate that, seed pretreatment with SA induced a reduction sodium absorption and toxicity, which is further reflected in low membranes injury, high water content and dry matter production (El-Tayeb 2005). Also SA incrases K^+ concentrations of plants under salinity condition. Günes et al (2005) found SA treatments stimulated N accumulation in plants. And P, K, Mg and Mn concentrations of SA received plants were increased in the stress conditions.

Osmotic potential: Variance analysis results of osmotic potential are shown in Table 1. According to Table 1, salt (P<0.01) and salicylic acid (P<0.01) affected osmotic potential significantly. In addition, an interaction was obtained between salicylic acid and salt significantly (P<0.01).

The osmotic potential was decreased by the influence of salinity stress. Osmotic potential was increased by application of SA levels. The highest osmotic potential was obtained by the seeds pretreated with 10^{-2} mol/L SA solution. Comparing to effect of four different SA concentrations during osmotic potential, that SA application was more effective at 10^{-2} mol/L than the others. The effects of SA was higher on osmotic potantial in salinity condition compared to non salinity condition was obtained in this study (Figure 3). Chinnusamy and Zhu (2003) have suggested that plant survival depends on maintaining a positive turgor, which is indispensable for expansion growth of cells and stomatal opening. A decrease in water availability under soil salinity causes osmotic stress, which leads to decreased turgor. Szepesi et al. (2005) reported that salinity reduced osmotic potential but the SA pretreatments increased it.

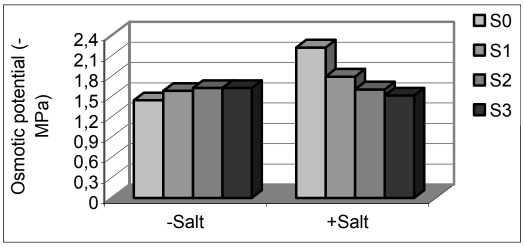

Figure 3. Effects of SA on osmotic potential (-MPa) under salinity and non salinity conditions

Conclusion

The effects of SA is higher on emergenge percentage, K^+/Na^+ ratio and osmotic potential in salinity condition compared to non salinity condition. The highest effects on plant growth and some physiological characters in wheat was obtained level of SA with 10^{-2} mol/L. In conclusion, data presented indicate that the presowing seed treatment of SA in regulating stress response of wheat, and suggest that SA could be used as a potential growth regulator to improve plant growth under salinity conditions.

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