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EFFECTS OF SALICYLIC AND GIBBERELLIC ACIDS ON WHEAT (*TRITICUM AESTIVUM* L.) UNDER SALINITY STRESS

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Abstract

Salinity decreased seed germination, the length, fresh and dry weight of the root and shoot, chlorophyll and carotenoid contents of wheat. The proline content was increased by salinity. The application of salicylic- and gibberellic acids, was found to alleviate the adverse effects of salinity stress on the above parameters.

Introduction

Soil salinity is one of the major problems affecting crop productivity in the world. The problem is rapidly increasing on a global scale and currently affects more than 10% of the arable land which drastically decreased the average yields of major crops greater than 50% (Wang *et al.* 2009). Therefore, understanding the mechanisms of plant tolerance to high salinity stress to combat this problem is necessary.

The injurious effects of salinity are associated with water deficit, ionic imbalance, stomatal behaviour, photosynthetic efficiency, proline accumulation and oxidative damage (Ashraf and Harris 2004, Munns *et al.* 2006). On the other hand salicylic acid (SA) is recognized as an endogenous regulator of plant metabolism, mainly involved in biotic and abiotic stress (Lian *et al.* 2000, Aydin and Nalbantoglu 2011). Gibberellic acid (GA_3) regulate growth and development (Schwechheimer 2008).

The present study was conducted to asses whether the exogenous application of SA and GA_3 could ameliorate the adverse effects of salinity on wheat.

Materials and Methods

In the first group of experiments, healthy seeds of uniform size were sterilized with sodium hypochlorite solution (5%) for five minutes and washed three times with sterilized distilled water. Twenty five seeds were maintained in Petri dishes (10 cm diameter) provided with two layers of filter paper saturated with 5 ml of distilled water (control) and treatments. Four replicates were prepared for each treatment. Seeds were left to germinate at 25±1ºC in light/dark regime of 16/8 h and were considered to be germinated after the radicle emerged from the caryopses. The final germination percentage was recorded after a period of one week. After germination, coleoptile and embryonic root length and fresh weight of one-week-old seedlings were measured. Dry weights were recorded after 48 h of oven-drying at 65ºC.

For the second group of experiments, seeds were sown in pots (20 × 30 cm^2, 25 seeds for each) filled with perlite soaked with Hoagland nutrient solution (Hoagland and Arnon 1950) and seedlings were grown under controlled conditions (light/dark regime of 16/8 h at 25 ± 1ºC , relative humudity of 60 - 70% and light intensity 350 µE m^2/s). Seedlings were irrigated with 200 ml distilled water every other day, with full strength Hoagland nutrient solution and treatments once a week for four weeks. After 24 h from the final application seedlings were harvested. Plant samples were analyzed for growth measurement, leaf pigment content and proline amount.

The treatments of the experiments were as follows: C_0: Control (non saline condition), C_1: 100 mM NaCl, C_2: 200 mM NaCl, $1A_1$: 100 mM NaCl + 200 ppm SA, $2A_1$: 200 mM NaCl+ 200 ppm SA, $1A_2$: 100 mM NaCl + 400 ppm SA, $2A_2$: 200 mM NaCl + 400 ppm SA, $1B_1$: 100 mM NaCl + 10 ppm GA_3, $2B_1$: 200 mM NaCl + 10 ppm GA_3, $1B_2$: 100 mM NaCl + 20 ppm GA_3 and $2B_2$: 200 mM NaCl + 20 ppm GA_3.

Vigour index was determined after Abdul-Baki and Anderson (1973) as % germination × radicule length.

Chlorophyll was extracted from leaves with 80% acetone and absorbance of supernatants were measured spectrophotometrically. Total chlorophyll and carotenoids were determined at 652 and 450 nm, respectively (Lichtenthaler 1987).

Modified method of Bates *et al.* (1973) was used to dermine the proline content. Leaf samples were homogenized in 3% (w/v) sulfosalicylic acid solution and then centrifuged. The supernatant was taken into a test tube to which glacial acetic acid and acid ninhydrin solution were added. Tubes were incubated in a boiling water bath for one hour and then allowed to cool to room temperature. After adding cold toluene, the mixture was vortexed and allowed to stand for separation of toluene and aqueous phase. The absorbance of toluene phase was measured at 520 nm with a spectrophotometer. The concentration of proline was calculated from a proline standard curve and expressed as μmol/g FW.

The data presented were mean values from two independent experiments, each with four replicates. Experimental data were analyzed with the SPSS statistical computer package (SPSS for WINDOWS, Standart Version 16.0) with Tukey test at $p < 0.05$ level. Standard errors (±) were calculated.

Results and Discussion

Germination of wheat seeds was inhibited by 100 mM NaCl (6%) and by 200 mM NaCl (10%). However, SA and GA_3 treatments increased germination rate of the plants under salinity condition. Alleviation by 3.19% SA in 100 mM NaCl and 4.44% in 200 mM NaCl, by 3.19% GA_3 in 100 mM NaCl and 6.67% in 200 mM NaCl (Table 1). Under saline conditions, seed germination and seedlings growth have been improved by application of GA_3 (Sakhabutdinova *et al*. 2003, Khan *et al.* 2004) and SA (Farida *et al.* 1992, Rajan *et al*. 2000). The reduction in vigour index in all test groups compared to the control was determined.

Radicle elongation decreased in all treatments compared with that of control. Although plumule elongation decreased with 100 mM NaCl and 200 mM NaCl, SA and GA_3 treatments ameliorate this adverse effects. The highest elongation was $1B_1$ treatment in plumule by 17.59 cm (Table 1).

Similarly, fresh-dry weights of radicle and plumule were also reduced by salinity, and the effect was alleviated by PGRs (Table 2). The greatest alleviation was achieved with $1A_1$ (53.16%) and $2A_2$ (72.46%) in radicle fresh weight, with $1B_1$ (23.88%) and $2B_1$ (61.70%) in plumule fresh weight; with $1A_1$ (75%) and $2A_2$ (142.85%) in radicle dry weight, with $1B_1$ (44.44%) and $2B_1$ (140%) in plumule dry weight (Table 2).

Salinity caused a dramatic decrease especially in 200 mM NaCl in root and shoot lengths and fresh-dry weights in 4 weeks old seedlings, but PGRs treatments ameliorated this adverse effect. The most effective treatments were $1A_2$ in 100 mM NaCl and $2B_1$ in 200 mM NaCl in root and shoot lengths, dry and fresh weights for alleviating salinity effects (Tables 3, 4).

Hamayun *et al.* (2010) observed the role of exogenous gibberellic acid in salinity alleviation of soybean. GA_3 application significantly promoted plant length and plant fresh-dry weights under salt stress. Shoot fresh-dry weights and root fresh-dry weights of strawberry plants were

also lower at salt stress as compared with non saline conditions. However, exogenous SA applications alleviate the inhibitory effect on these parameters as compared with the control under salt stress (Karlıdag *et al*. 2009, Afzal *et al*. 2006, Azooz 2009).

Table 1. Effects of PGRs on the percentage of germination in seeds, radicle and plumule length on wheat under salinity stress.

Treatments	Germination (%)	Vigour index	Radicle length (cm)	Plumule length (cm)
C_0	$96^{a,b}$	1097.28	11.43 ± 3.27^{a}	11.32 ± 1.14^{a}
C_1	94^{a}	0916.50	09.75 ± 1.81^{a}	10.56 ± 0.74^{a}
$1A_1$	$97^{a,b}$	1092.22	11.26 ± 1.85^{a}	12.49 ± 0.72^{a}
$1A_2$	$97^{a,b}$	0982.61	10.13 ± 2.07^{a}	11.45 ± 1.63^{a}
$1B_1$	$97^{a,b}$	0955.45	09.85 ± 1.79^{a}	17.59 ± 1.26^{b}
$1B_2$	$96^{a,b}$	0944.64	09.84 ± 2.52^{a}	12.73 ± 0.79^{a}
C_2	90^{c}	0518.40	05.76 ± 1.03^{a}	04.52 ± 0.68^{c}
$2A_1$	94^{a}	0825.32	08.78 ± 2.59^{a}	09.83 ± 1.32^{a}
$2A_2$	94^{a}	0815.92	08.68 ± 0.85^{a}	10.00 ± 0.79^{a}
$2B_1$	$96^{a,b}$	0802.56	08.36 ± 2.21^{a}	11.21 ± 1.24^{a}
$2B_2$	$95^{a,b}$	0790.40	08.32 ± 2.66^{a}	10.69 ± 1.03^{a}

n = 100, ± SE. Values followed by the same letter within a column are not significantly different.

Table 2. Effects of PGRs on radicle and plumule fresh-dry weight on wheat under salinity stress.

Treatments	Radicula fresh weight (g)	Plumula fresh weight (g)	Radicle dry weight (g)	Plumule dry weight (g)
C_0	0.128 ± 0.030^{a}	0.069 ± 0.020^{a}	0.037 ± 0.005^{a}	0.010 ± 0.001^{a}
C_1	0.079 ± 0.015^{a}	0.067 ± 0.009^{a}	0.016 ± 0.004^{b}	0.009 ± 0.001^{a}
$1A_1$	0.121 ± 0.023^{a}	0.083 ± 0.011^{a}	0.028 ± 0.004^{ab}	0.012 ± 0.001^{a}
$1A_2$	0.113 ± 0.020^{a}	0.069 ± 0.014^{a}	0.031 ± 0.005^{a}	0.011 ± 0.002^{a}
$1B_1$	0.100 ± 0.028^{a}	0.083 ± 0.016^{a}	0.020 ± 0.005^{b}	0.013 ± 0.002^{a}
$1B_2$	0.095 ± 0.022^{a}	0.079 ± 0.015^{a}	0.020 ± 0.005^{b}	0.012 ± 0.002^{a}
C_2	0.069 ± 0.013^{b}	0.047 ± 0.014^{b}	0.020 ± 0.004^{b}	0.005 ± 0.001^{b}
$2A_1$	0.104 ± 0.024^{a}	0.072 ± 0.013^{a}	0.031 ± 0.005^{ab}	0.009 ± 0.001^{a}
$2A_2$	0.119 ± 0.042^{a}	0.071 ± 0.019^{a}	0.034 ± 0.004^{a}	0.010 ± 0.002^{a}
$2B_1$	0.117 ± 0.021^{a}	0.076 ± 0.013^{a}	0.033 ± 0.005^{a}	0.012 ± 0.002^{a}
$2B_2$	0.099 ± 0.017^{a}	0.071 ± 0.014^{a}	0.025 ± 0.004^{ab}	0.011 ± 0.002^{a}

n = 100, ± SE. Values followed by the same letter within a column are not significantly different.

Total chlorophyll was reduced by 46.73% in 100 mM NaCl and 18.28% in 200 mM NaCl compared to control. Also, carotenoid contents were decreased by 73.68% in 100 mM and 5.41% in 200 mM. The greatest alleviation was achieved with $1B_1$ (30.24%) and $2B_1$ (57.61%) in total chlorophyll and with $1B_1$ (91.70%) and $2B_1$ (3.52 %) in carotenoid (Table 5).

Similarly, leaf chlorophyll and carotenoid content were significantly reduced in salt treated plants (El-Tayeb 2005, Shah 2007). The observed chlorophyll depletion may be considered to be a result of the inhibition of chlorophyll biosynthesis (Khan 2003). However, treatment of the salt-

stressed plants with SA (Khan *et al.* 2003) and GA_3 (Shah 2007) were found to restore normal chlorophyll levels. These data supported tthe present findings.

Table 3. Effects of PGRs on root length, fresh and dry weight on wheat under salinity stress.

	Root		
Treatments	Length (cm)	Fresh weight (g)	Dry weight (g)
C_0	6.55 ± 1.12^{a}	0.090 ± 0.001^{a}	0.018 ± 0.001^{a}
C_1	3.83 ± 1.61^{a}	0.050 ± 0.001^{b}	0.013 ± 0.001^{b}
$1A_1$	4.00 ± 1.89^{a}	0.060 ± 0.001^{ab}	0.0150 ± 0.001^{b}
$1A_2$	4.95 ± 0.95^{a}	0.060 ± 0.001^{ab}	0.016 ± 0.001^{a}
$1B_1$	4.00 ± 1.53^{a}	0.060 ± 0.001^{ab}	0.015 ± 0.001^{b}
$1B_2$	2.93 ± 0.88^{a}	0.050 ± 0.002^{b}	0.009 ± 0.001^{c}
C_2	3.65 ± 1.34^{a}	0.050 ± 0.001^{b}	0.012 ± 0.001^{bc}
$2A_1$	4.63 ± 1.40^{a}	0.090 ± 0.001^{a}	0.016 ± 0.001^{a}
$2A_2$	4.08 ± 1.11^{a}	0.050 ± 0.001^{b}	0.012 ± 0.001^{bc}
$2B_1$	5.48 ± 2.21^{a}	0.080 ± 0.002^{a}	0.013 ± 0.001^{b}
$2B_2$	5.13 ± 2.13^{a}	0.040 ± 0.001^{b}	0.011 ± 0.001^{bc}

n = 100, ± SE. Values followed by the same letter within a column are not significantly different.

Table 4. Effects of PGRs on shoot length, fresh and dry weight on wheat under salinity stress.

	Shoot		
Treatments	Length (cm)	Fresh weight (g)	Dry weight (g)
C_0	19.16 ± 1.40^{a}	0.080 ± 0.020^{a}	0.012 ± 0.001^{a}
C_1	18.76 ± 1.36^{a}	0.070 ± 0.020^{a}	0.010 ± 0.001^{a}
$1A_1$	20.93 ± 1.10^{a}	0.080 ± 0.020^{a}	0.011 ± 0.001^{a}
$1A_2$	21.33 ± 1.90^{a}	0.080 ± 0.010^{a}	0.012 ± 0.001^{a}
$1B_1$	18.58 ± 1.27^{a}	0.070 ± 0.010^{a}	0.010 ± 0.001^{a}
$1B_2$	18.53 ± 1.47^{a}	0.060 ± 0.020^{a}	0.007 ± 0.001^{b}
C_2	15.15 ± 0.70^{b}	0.060 ± 0.030^{a}	0.006 ± 0.001^{b}
$2A_1$	21.18 ± 0.93^{a}	0.070 ± 0.010^{a}	0.009 ± 0.001^{ab}
$2A_2$	15.60 ± 0.94^{b}	0.060 ± 0.010^{a}	0.006 ± 0.001^{b}
$2B_1$	21.53 ± 1.20^{a}	0.090 ± 0.020^{a}	0.010 ± 0.001^{a}
$2B_2$	20.85 ± 1.50^{a}	0.060 ± 0.020^{a}	0.009 ± 0.001^{ab}

n = 100, ± SE. Values followed by the same letter within a column are not significantly different.

The proline contents increased following the treatment with $1B_1$ in 100 mM NaCl and $2B_1$ in 200 mM NaCl (Table 5). Similarly, salt stress (NaCl) caused a marked increase in proline amino acid (Chakrabarti and Mukherji 2003, Mohammed 2007). Exogenous GA_3 applications decreased proline contents in *Vigna radiata* under saline conditions (Mohammed 2007) which are in agreement with the present results.

Table 5. Effects of PGRs on total chlorophyll, carotenoid and proline amounts on wheat under salinity stress.

Treatments	Total chlorophyll (mg/g)	Carotenoid (mg/g)	Proline [μmol/g FW]
C_0	0.854 ± 0.062^a	1.947 ± 0.084^a	4.051 ± 0.029^a
C_1	0.582 ± 0.012^b	1.121 ± 0.016^b	6.603 ± 0.086^b
$1A_1$	0.6791 ± 0.016^b	1.922 ± 0.011^a	6.074 ± 0.014^c
$1A_2$	0.650 ± 0.026^b	1.847 ± 0.054^a	6.584 ± 0.080^b
$1B_1$	0.758 ± 0.045^a	2.149 ± 0.047^c	6.384 ± 0.013^b
$1B_2$	0.552 ± 0.032^b	1.707 ± 0.019^d	6.993 ± 0.100^d
C_2	0.722 ± 0.023^a	1.847 ± 0.009^a	6.108 ± 0.015^c
$2A_1$	0.686 ± 0.034^b	1.833 ± 0.001^a	4.431 ± 0.063^e
$2A_2$	0.853 ± 0.031^a	1.497 ± 0.016^e	4.030 ± 0.011^a
$2B_1$	1.138 ± 0.030^c	1.912 ± 0.015^a	3.213 ± 0.014^f
$2B_2$	0.847 ± 0.078^a	1.905 ± 0.037^a	3.868 ± 0.034^a

n = 4, ± SE. Values followed by the same letter within a column are not significantly different.

Results of the present study indicate SA and GA_3 ameliorate the adverse effects of salt stress on wheat plants and restore normal growth and development. It can be concluded that increasing salinity was associated with decreases in SA and GA_3 in the plant tissues. Changes in hormone levels in plant tissue were thought to be an initial process controlling growth reduction due to salinity. Therefore, NaCl-induced reduction in the plant growth and development can be mitigated by exogenous application of plant growth regulators as SA and GA_3.

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