Pak. J. Bot., 40(1): 361-367, 2008.

INFLUENCE OF SALICYLIC ACID SEED PRIMING ON GROWTH AND SOME BIOCHEMICAL ATTRIBUTES IN WHEAT GROWN UNDER SALINE CONDITIONS

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Abstract

A sand culture experiment was conducted to study the effect of salicylic acid seed priming on growth and some biochemical attributes in wheat (*Triticum aestivum* L.) grown under saline conditions. Wheat seeds of cv. Inqlab and S-24 were soaked in water and 100 mg L^{-1} salicylic acid solution for 24 hours and sown in sand salinized with 0, 50 or 100 m*M* NaCl. Pots were irrigated with ¼ strength Hoagland's nutrient solution. Fourteen days old seedlings were harvested and growth parameters (shoot and root length, and shoot and root dry weights) were recorded and chlorophyll *a* and *b* contents and soluble sugars (reducing and non-reducing) in the leaves were estimated. Salt stress significantly reduced all growth parameters. However, salicylic acid treatment alleviated the adverse effect of salinity on growth. Salinity decreased the chlorophyll *a* and *b* content and chlorophyll a/b ratio in both the lines, but reduction in chlorophyll a/b ratio was lower in salt tolerant wheat line S-24, which could be a useful marker for selection of salt tolerant wheat. Salinity (NaCl) stress considerably increased the accumulation of reducing, non-reducing and total soluble sugars in the leaves of 14 days old wheat seedlings of both cultivars. Salt tolerant line S-24 accumulated higher sugar content which could also be a useful marker for salt tolerance in wheat.

Introduction

Salinity in the growth medium causes significant reduction in plant growth parameters like leaf area, leaf length, root and shoot dry weight (Hamada & Al-Hakimi, 2001; Ashrafuzzamana *et al*., 2002), decrease carotenoid and induce reduction in chlorophyll and photosynthetic activity. Similarly, saline conditions decrease the activity of ribulose 1, 5-bisphosphate carboxylase (Rubisco) which results in reduction in formation of carbohydrates (El-Shihaby *et al*., 2002).

One of the approaches to cope with the problem is to find out some bio-chemicals that might mitigate the effect of salinity on plants and enable them to grow normally and produce reasonable yields. Salicylic acid and related compounds have been reported to induce significant effects in various biological aspects in plants (Gill *et al*., 2001; Wang & Li, 2006). These compounds inhibit certain processes and enhance others (Raskin, 1992). Different levels of acetylsalicylic acid function as anti-transpirant and inhibit the opening of stomata (Larque-Saavedra, 1979). Salicylic acid also reverses the closure of stomata caused by abscisic acid (Rai *et al*., 1986). Exogenous application of salicylic acid improves the yield of crops (Singh & Kaur, 1980; Arfan *et al*., 2007). Salicylic acid treatment retards ethylene synthesis; stimulates photosynthetic machinery and increase the content of chlorophyll (Leslie & Romani, 1988). Recently, it has been recognized that salicylic acid is required in the signal transduction for inducing systemic acquired resistance against pathogenic infections (Metraux *et al*., 1990; Gaffney *et al*., 1993; Vernooij *et al*., 1994).

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The objective of this study was to investigate whether salicylic acid could be used to mitigate the adverse effects of salinity on wheat seedlings and to identify the biochemical criteria conferring the tolerance to salinity stress in wheat.

Materials and Methods

A sand culture experiment was conducted in plastic pots in net house at NIAB, Faisalabad, Pakistan. Homogenous lots of healthy seeds of two wheat varieties, Inqlab and S-24 were sterilized with Sodium hypchloride solution (5%) for five minutes, washed thoroughly with distilled water before use. Before sowing seeds were soaked in Salicylic acid solution (100 mg L^{-1}) and distilled water for 24 hours. The silica sand thoroughly washed with distilled water and dried sand was filled in plastic pots (15 cm inner diam., and 15 cm deep) maintaining the weight of sand at 2 kg per pot.

Three levels of salinity (0, 50 and 100 m*M* NaCl) were developed in pots filled with sand before sowing. The seeds of two wheat varieties were soaked for 24 h in 0 and 100 mg L^{-1} Salicylic acid. Ten seeds per pot containing ¼ strength Hoagland nutrient solution (Hoagland and Arnon, 1950) and sand were sown. All the treatments were arranged in a completely randomized block design. The wheat seedlings were allowed to grow for 14 days. The roots and shoots of seedlings were collected and rinsed with distilled water. The seedlings were immediately dried in tissue paper. The fresh leaves were used for the estimation of chlorophyll, reducing, non-reducing and total soluble sugars. The dry weights of roots and shoots were recorded after drying in an oven at 70±2^{o}C for 72 h.

Chlorophyll was determined according to the method of Arnon (1949) and Davies (1976). Total soluble, reducing and non-reducing sugars were extracted in 0.2 M phosphate buffer with pH 7 and analyzed according to the method of Razi *et al*., (1985).

All the collected data were subjected to analysis of variance and LSD worked out for comparison of means (Steel *et al*., 1997).

Results

Salinity affected adversely the growth parameters such as shoot and root length, dry weight of shoot and root. In wheat cultivar Inqlab shoot length was severely reduced at 100 m*M* NaCl salinity (Table 1). Wheat cultivar S-24 performed better than Inqlab at the highest salinity level. Wheat cultivar Inqlab showed 49% reduction in shoot length at the highest salinity level which was 33.33% in S-24. Application of salicylic acid stimulated growth in both the cultivars under control and salinity stress. Cultivar S-24 showed more increase in shoot length than that of Inqlab.

The growth of root was also adversely affected by salinity in both the wheat cultivars (Table 1). In cultivar Inqlab, root length reduction was more prominent than that of S-24, under 50 and 100 m*M* NaCl. The salicylic acid treatment increased root length in both wheat cultivars. Salinity adversely affected the shoot and root dry weight of the two cultivar used in this study (Table 1). However, application of salicylic acid enhanced the shoot and root dry weight.

Root dry weight was also adversely affected by salinity (Table 1). Under control dry weight of root was 0.134 and 0.130 g $plant^{-1}$ in Inqlab and S-24, respectively. The dry weight of root was reduced by 18.7, 44.0% in Inqlab and 12.3 and 30.8% in S-24 under 50 and 100 m*M* NaCl stress, respectively. Salicylic acid treatment increased root dry weight by 11.9, 18.4 and 33.7% in Inqlab and 7.7, 7.9 and 12.1% in S-24 under control and 50, 100 m*M* NaCl stress, respectively.

Salinity had an adverse effect on chlorophyll in wheat seedlings (Table 2). Both the salinity levels adversely affected Chlorophyll contents in both the wheat varieties however, reduction was more pronounced in Inqlab. Application of salicylic acid improved the chlorophyll 'a' content of wheat seedlings of both the cultivars under normal and saline conditions. Wheat variety S-24 performed better than that of Inqlab.

Like chlorophyll 'a' and 'b' content of wheat seedlings was also adversely affected by salinity (Table 2), the reduction in chlorophyll 'b' was more than that in chlorophyll 'a'. Severe reduction in chlorophyll 'b' was found at 100 m*M* NaCl in both the wheat cultivars. Salicylic acid application increased the content of chlorophyll 'b' in wheat seedlings of the two cultivars under study. Wheat line S-24 maintained higher chlorophyll b than Inqlab.

An interesting feature of chlorophyll 'a' and 'b' under stress is the shift of chlorophyll a/b ratio and results indicated that the chlorophyll a/b ratio increased with increase in salinity in both the cultivars. The increase in chlorophyll a/b ratio was more in Inqlab than S-24. Application of salicylic acid reduced the chlorophyll a/b ratio under normal as well as saline conditions in both the wheat varieties except in case of Inqlab at 100 m*M* NaCl where it significantly increased (Table 2).

Salinity affected significantly the non-reducing sugars in wheat seedlings (Table 3). Non-reducing sugars increased due salinity in both the wheat cultivars however, S-24 was successful in maintaining higher concentrations of non-reducing sugars than that of Inqlab (Table 3). Application of salicylic acid further increased the concentration of non-reducing sugars in seedlings of both the varieties under normal and salt stressed conditions.

Reducing sugars also influenced by salinity in both the wheat cultivars (Table 3). Reducing sugars significantly enhanced by in both the wheat cultivars, however, cultivar S-24 maintained high reducing sugars than Inqlab. Salicylic acid treatment further increased the concentration of reducing sugars in the both the wheat cultivars included in this study.

Salinity significantly affected the concentration of total soluble sugars in seedlings of the two cultivars (Table 3). Total sugars increased with increase in salinity in both the wheat cultivars and application of Salicylic acid was effective in further increasing the total sugars. Wheat cultivar S-24 maintained higher concentration of sugars than Inqlab.

Discussions

The results of the present study show that salinity reduced the growth and disturbed the plant metabolism. Seed priming with salicylic acid was beneficial in alleviating the adverse effects of salinity by enhancing growth, chlorophyll contents and sugars necessary for osmotic adjustment under adverse environmental conditions. Hamada & Al-Hakimi (2001) observed that soaking of wheat seed in salicylic acid before sowing was effective in reducing the stress effect of salinity on growth. Gutierrez *et al.*, (1998) reported increase in the growth of shoots and roots of soybean plants in response to salicylic acid treatment. Khodary (2004) observed that salicylic acid treated maize plants exhibited an increase in tolerance to salinity reflected in growth parameters like length, fresh and dry weight of shoot and root.

Table 1. Effect of Salicylic acid on growth characteristics of wheat seedlings of two varieties under NaCl stress.

Treatment		Shoot length cm		Root length cm		Shoot dry weight g $plant^{-1}$		Root dry weight g $plant^{-1}$		Shoot/root ratio	
SA mg L^{-1}	NaCl m*M*	Inq.	S-24	Inq.	S-24	Inq.	S-24	Inq.	S-24	Inq.	S-24
	0	29.6	25.5	34.3	31.9	0.264	0.260	0.134	0.130	1.970	2.00
0	50	22.4	21.5	25.5	27.9	0.204	0.236	0.109	0.114	1.872	2.070
	100	15.1	17.0	17.2	19.7	0.105	0.130	0.065	0.09	1.615	1.444
	0	32.2	29.6	42.1	38.9	0.300	0.283	0.150	0.140	2.000	2.021
100	50	23.8	25.7	30.8	33.0	0.229	0.253	0.129	0.123	1.775	2.056
	100	17.4	22.6	20.2	24.6	0.125	0.179	0.100	0.112	1.250	1.598
LSD ($p \geq 0.05$)		**4.62**		**5.04**		**0.053**		**0.009**		**0.052**	

SA = Salicylic acid; Inq. = Inqlab; LSD = Least significant difference

Table 2. Effect of Salicylic acid on chlorophyll content of wheat seedlings of two varieties under NaCl stress.

Treatment		Chlorophyll 'a' mg g^{-1} F.Wt.		Chlorophyll b mg g^{-1} F.Wt.		a/b ratio	
SA mg L^{-1}	NaCl m*M*	Inq.	S-24	Inq.	S-24	Inq.	S-24
	0	0.874	0.885	0.539	0.564	1.621	1.569
0	50	0.748	0.780	0.424	0.480	1.764	1.625
	100	0.549	0.656	0.278	0.345	1.974	1.901
	0	0.889	0.895	0.575	0.655	1.546	1.366
100	50	0.765	0.798	0.560	0.640	1.366	1.247
	100	0.650	0.712	0.202	0.414	3.218	1.719
LSD ($p \geq 0.05$)		**0.119**		**0.075**		**ND**	

SA = Salicylic acid; Inq. = Inqlab; LSD = Least significant difference; ND = Not determined

Table 3. Effect of Salicylic acid on non-reducing sugars, reducing sugars and total soluble sugars content of wheat seedlings of two varieties under NaCl stress.

Treatment		Non reducing sugar mg g^{-1} F.Wt.		Reducing sugars mg g^{-1} F.Wt.		Total soluble sugars mg g^{-1} F.Wt	
SA mg L^{-1}	NaCl m*M*	Inq.	S-24	Inq.	S-24	Inq.	S-24
	0	1.626	1.618	0.453	0.448	2.079	2.066
0	50	1.697	1.799	0.552	0.741	2.249	2.540
	100	1.706	2.018	0.653	0.914	2.359	2.932
	0	1.675	1.705	0.616	0.775	2.291	2.480
100	50	2.014	2.570	0.745	0.880	2.759	3.450
	100	2.409	2.755	0.934	0.982	3.343	3.737
LSD ($p \geq 0.05$)		**0.465**		**0.131**		**0.315**	

SA = Salicylic acid; Inq. = Inqlab; LSD = Least significant difference

Growth reduction due to salinity is well documented (Khan *et al.*, 1990; Saqib *et al*., 1999; Ashraf & Harris, 2004; Ashraf *et al.,* 2005) and is mainly attributed to water deficit due to lowered water potential in the root zone, nutritional imbalance and specific ion toxicity arising from higher concentration of Na^+ and Cl^- (Khan & Ashraf, 1988). A secondary effect of high concentration of Na^+ and Cl^- in the rooting medium is the suppression of uptake of essential nutrients such as K^+, Ca^{2+} and NO_3^- etc., (Ashraf, 2004; Gorham & Wyn Jones, 1993). Different physiological traits such as selectivity for potassium, exclusion of Na^+ and Cl^- ions, osmotic adjustments by accumulation of organic solutes have all been related to salt tolerance of crop plants (Khan *et al.,* 1995; Ashraf *et al.,* 2002). There is also the possibility that NaCl treated plants utilize energy for the osmotic adjustment process (Ashraf *et al.,* 2006) at the expense of growth.

Regarding salicylic acid, people are not the only ones to benefit from Salicylic acid, the active ingredient is aspirin. Research has shown that spraying this naturally occurring compound on to some plants triggers natural defenses that keep harmful fungi, bacteria and viruses at bay. Plant scientists first encountered the phenomenon called systemic acquired resistance (SAR) in 1930s. Plants make Salicylic acid, particularly after encountering a pathogen, and use it as a key regulator or SAR expression of defense genes. But only recently have companies begun marketing Salicylic acid and other similar compounds as a way to activate SAR in crops.

Systemic acquired resistance (SAR) is the basic mechanism by which plants protect themselves. Treatment of wheat plants with Salicylic acid increased the level of cell division within the apical meristem of seedling roots which caused an increase in plant growth (Sakhabutdinova *et al*., 2003). Phytohormones are known to play a key role in plant growth regulation. It was found that salicylic acid treatment caused accumulation of both Abscisic acid and indole acetic acid in wheat seedling. However, the Salicylic acid treatment did not influence cytokinin content.

It is supposed that the protective and growth promoting effects of Salicylic acid are due to phenomenon described above. The Salicylic acid treatment reduces the damaging action of salinity and water deficit on seedling growth and accelerates the restoration of growth processes. Treatment with Salicylic acid diminished the alteration of phytohormones level in wheat seedlings under salinity and water deficit. The Salicylic acid treatment prevented the decrease in Indole acetic acid and cytokinin content completely which reduces stress reduced inhibition of plant growth. The protective Salicylic acid action includes the development of antistress programs and acceleration of normalization of growth processes after removal of stress factors.

From the results presented here it might be concluded that NaCl salinity significantly reduced the growth of wheat seedlings (Shoot was affected more than root as is evident from root/shoot ratio) and chlorophyll content and increased the accumulation of soluble sugars in leaves. Wheat variety S-24 showed better performance than that of Inqlab in all parameters studied in this investigation. The above mentioned two criteria could be useful markers for selecting salt tolerant variety of wheat for breeding program for salt affected areas. Salicylic acid treatment of salt-stressed wheat plants stimulated their salt tolerance in both wheat varieties *via* accelerating their photosynthesis performance and carbohydrate metabolism so it can be utilized for enhancing the salt tolerance potential of wheat varieties and obtaining the economical wheat yield if cultivated on salt-affected soils.

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(Received publication 24 February 2007)