Pak. J. Bot., 38(5): 1759-1767, 2006.

MORPHOBIOCHEMICAL ANALYSIS OF SALINITY STRESS RESPONSE OF WHEAT

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Abstract

The experiments were planned to investigate the effect of salt stress on morphobiochemical response of wheat cv. Uqab-2000. Seeds were given different priming treatments like hydro priming, chilling, kinetin, $CaCl_2.2H_2O$ and controlled (with no treatment). After priming treatments the seedlings were grown under normal and salt stress (NaCl 125 mM) for 8-days in petridishes at 25 ±1°C in an incubator. Various morphological parameters for seedlings and biochemical parameters (catalase activity and ascorbic acid contents) for roots were determined. The results revealed that generally all treatments except hydropriming increased the morphological growth of the seedlings, particularly chilling, kinetin, and calcium chloride showed a significant difference compared to control. An overall increasing trend in the catalase activity (units/ mg fresh weight) was observed except kinetin treatment in normal and chilling in saline conditions. The profound increase in activity of catalase was observed after chilling and hydro priming in normal and saline, respectively. However, all seed priming treatments decreased the ascorbic acid concentration under salinity, while reverse was true for normal conditions. Application of salt stress have overall substantial negative affect on all the visual growth and biochemical parameters, However seed priming treatments tend to alleviate the adverse effects of salinity.

Key words: Seed priming, wheat, salt tolerance, pretreatment, growth, kinetin, chilling.

Introduction

Salinity is one of the major and increasing problems in irrigated agriculture in Pakistan, particularly in wheat and rice grown areas. The areas affected by varying degrees of salinity are reported at around 6.3 million hectare within canal command areas, of which half is cultivable or cultivated to some extent (Rafique, 1990). Ghassemi *et al.* (1995) estimated about 14 % of irrigated land to be badly affected by salinity in Pakistan.

Wheat (*Triticum* spp.) and rice are the world's major cereal crops, with the annual production of wheat being over 627 million ton in 2004 (http://faostat.fao.org). Wheat is grown under irrigated and rain-fed conditions: both types of agriculture are threatened by salinization (Ghassemi *et al.*, 1995; Kazi & Leon, 2002). Data collected at CIMMYT suggest that 8–10%of the area planted to wheat in India, Pakistan, Iran, Egypt, Libya, and Mexico is affected by salinity (Kazi & Leon, 2002). Salinity affect plant growth at all stages of development however sensitivity varies from one growth stage to another (Shannon, 1998).

The adverse effects of high concentration of salts for plants are due to the osmotic retention of water and to specific ionic effects on the protoplasm. Water is osmotically held in salt solutions, so as the concentration of salt increased water becomes less and less accessible to the plant. Soil salinity is known to suppress the growth of most crop

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species (Maas & Hoffman, 1977). It either completely inhibits germination at higher levels or induces a state of dormancy at low levels. Salinity also reduces imbibition of water because of lowered osmotic potentials of the medium and causes changes in metabolic activity (Yupsanis *et al.*, 1994).

To get more yields under stressed environment, effect of salinity is being tried to be replenish by different research tools. Seed priming treatments are therefore in practice to reduce the effects of salinity with lesser input of capital and energy (Taylor *et al.,* 1998). Many seed invigoration treatments are being used to improve the rate and speed of germination (Lee & Kim, 2000). Pre-sowing treatments with different salts (Idris & Aslam, 1975; Cantliffe, 2003) and water (Twitchell, 1955) stimulate the germination process.

To counteract the toxicity of reactive oxygen species, a highly efficient antioxidative defense system, including both nonenzymic and enzymic constituents, is present in plant cells. Catalase a front line antioxidant is a tetrameric heme-containing enzyme, which by dismutation, degrade hydrogen peroxide to water and oxygen and occurs in all aerobic organisms. Ascrobic acid acts both as an abundant antioxidant free radical scavenger and a reductant in enzymatic reactions. A small pool of ascorbic acid located outside the plant cell wall in the apoplastic space is thought to have a major role in the detoxification of reactive oxygen species (Luwe *et al*., 1993). The analysis of physiological changes in plants associated with seed-priming may be useful for advancing the understanding of plant salt tolerance, it may suggest strategies by which plants acquire salt tolerance. The primary objective of the present study was to determine the effect of priming agents on seedling growth and the antioxidant level of roots of wheat seedlings under normal and saline conditions.

Materials and Methods

The growth and morphological characters like fresh and dry weights of seedlings, shoot and root dry weight, root and shoot length, their ratio and root number. The roots of seedlings were separated for catalase activity and ascorbic acid contents.

The seeds of wheat (*Triticum aestivum* L.) cultivar, namely Uqab 2000 were obtained from "The Nuclear Institute for Agriculture and Biology" (NIAB) Faisalabad, Pakistan. Different pretreatments of hydro priming (12 hour water soaking), chilling (-20^{o}C for 48 hours), hormonal priming (25 ppm kinetin 12 hours), halo priming (soaking in 100 mM $CaCl_2$ Solution) were given to seeds. After that, seeds were air dried for 12 hours at room temperature. All pretreated and untreated (control) seeds were allowed to germinate in darkness for 48 hours at 25 ± 1°C on wet filter papers in petridishes. All treated (primed) and untreated seedlings (non-primed) were divided into two groups (normal and saline), normal were treated with distilled water and saline with 125mM NaCl for next six days. The morphological character like fresh and dry weights of seedlings, shoots and roots, shoot and root lengths and root numbers were determined. Catalase activity (units / mg fresh weight) and ascorbic acid contents (µg/ g fresh weight) in roots of both control and salt stress 8 days old seedlings were determined. Catalase enzyme was extracted by homogenizing frozen roots (- 80 °C) with 50 mM phosphate buffer pH 7.0 and 1 mM dithiothreitol (Dixit *et al.,* 2001) and the activity of enzyme was determined in units/mg fresh weight of seedlings (Beers *et al.,* 1952). Ascorbic acid was determined in water extract of frozen ground roots of wheat seedlings (Kamfenkal *et al*., 1995).

The descriptive statistics including mean, and sample variance were applied to analyze and organize the resulting data. The significance of differences between means (for control and treated) for different parameters were measured using two factors factorial ANOVA assuming unequal variances at 0.01 and where applicable at 0.05 significance level. The means were compared by applying Duncan's Multiple Range test (Steel *et al.*, 1997).

Results

The morphological parameters of wheat seedlings including fresh and dry weight of seedlings, dry weight of root and shoot, root and shoot length and their ratio and their number under normal and saline conditions after various seed priming treatments are presented in Table. 1. Salinity decreased the seedling fresh weight, dry weight of shoots and roots, root number, length of shoot and root while seedling dry weight and root/shoot ratio were increased significantly under salinity. All seed priming treatments both in saline and normal conditions increased the length of roots and shoots in these conditions however kinetin treatment showed profound effect on length of root and shoot of wheat seedling under saline conditions (Table 2).

An overall increasing trend in the catalase activity (units/ mg fresh weight) was observed except kinetin treatment in normal and chilling in saline conditions (Fig.1). The profound increase in activity of catalase was observed after chilling and hydro priming in normal and saline, respectively. Generally all treatments increased the ascorbic acid concentrations significantly under normal conditions compared to control while reverse was true for seedlings grown under salinity (Fig.2).

Table 1. Morphological Parameters of wheat seedlings under normal and saline conditions.

Treatments	**Root length (cm)**		**Shoot length (cm)**		**Root Shoot ratio (length)**		**Root No.**	
	Normal	**Saline**	**Normal**	**Saline**	**Normal**	**Saline**	**Normal**	**Saline**
Control	3.611	2.16	13.16	2.41	0.274	0.89	5.75	3.66
Hydro priming	4.37	2.66	15.4	3.08	0.283	0.86	5.0	5.01
Chilling	6.27	3.0	16.77	2.87	0.373	1.04	6.11	5.5
Kinetin	5.1	4.5	15.7	9.83	0.324	0.46	5.44	6.33
Calcium chloride	6.5	4.0	16.3	8.0	0.398	0.50	6.11	5.3
	Seedling fresh wt. (mg)		Seedling dry wt. (mg)		Root dry wt. (mg)		Shoot dry wt. (mg)	
Control	125.7	82.64	19.99	26.66	4.315	4.05	8.8	5.05
Hydro priming	188.4	104.7	21.88	22.22	3.88	4.28	9.5	6.75
Chilling	211.1	128.0	22.77	30	5.44	2.7	10.6	3.1
Kinetin	202.4	164.0	14.44	20	8.1	5.1	7.4	4.6
Calcium chloride	202.5	155.2	19.5	11.9	5.1	4.7	10.6	7.2

Table 2. Treatment means of wheat seedlings

Treatments	Root length (cm)	Shoot length (cm)	Root Shoot ratio (length)	Root No.
Control	2.885a	7.785a	0.5850a	4.705a
Hydro priming	3.515a	9.240b	0.5717a	5.000ba
Chilling	4.635b	9.820b	0.7050b	5.805c
Kinetin	4.800b	12.76c	0.3900c	5.885c
Calcium chloride	5.100b	12.15c	0.4500c	5.720c
	Seedling fresh wt. (mg)	**Seedling dry wt. (mg)**	**Root dry wt. (mg)**	**Shoot dry wt. (mg)**
Control	104.2a	23.33a	4.183a	6.905a
Hydro priming	146.6b	22.05b	4.080a	8.080b
Chilling	169 cb	26.39a	4.070a	6.860a
Kinetin	183.2c	24.44c	6.600b	6.000c
Calcium chloride	178.9c	23.33d	4.895a	8.90d

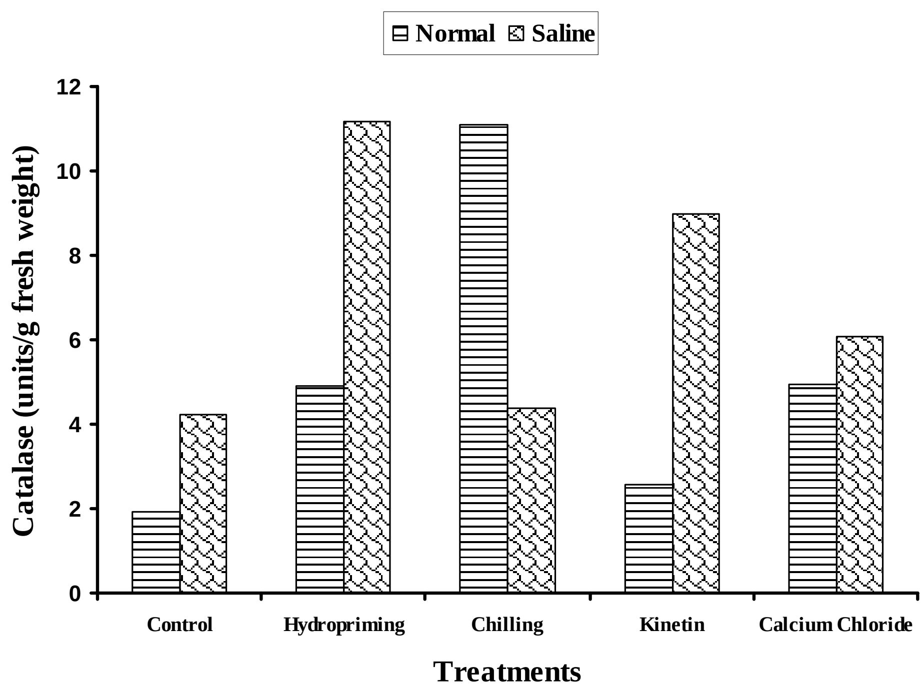

Fig. 1. Catalase activity of roots of wheat seedlings primed with different priming agents grown under normal and saline conditions.

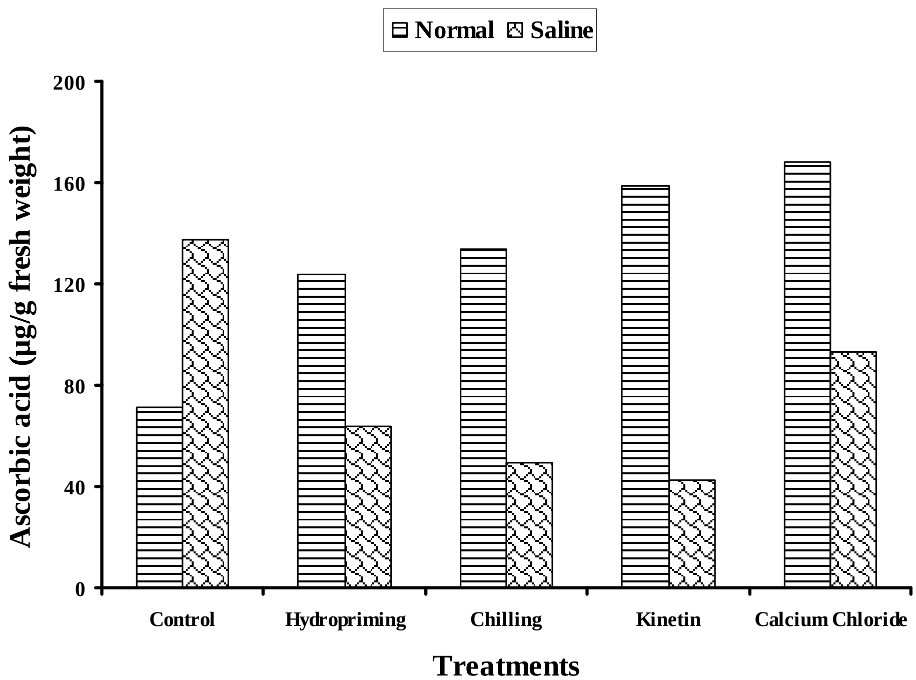

Fig. 2. Ascorbic acid of root of wheat seedlings primed with different priming agents grown under normal and saline conditions.

Discussion

Different presowing treatments with various priming agents improved seedling growth in both normal and saline conditions. Generally, priming improves the seedling growth, particularly under saline conditions in our studies and it was also conformed by earlier reports (Bradford, 1976; Sivritepe *et al.,* 2003). The pre-sowing treatments cause initiation of the early metabolic processes and the re-drying of seeds arrest, but do not reverse, the initial stages of germination so that on the availability of suitable conditions, the time taken to germinate is reduced (Bewley & Black, 1982).

Hydro-priming significantly increased the fresh weight of seedling, shoot length and shoot dry weight of cultivar Uqab 2000 compared with non-primed (control) seeds under both normal (non-saline) and saline conditions in our studies. There are numerous studies in the literature which exhibit the considerable effectiveness of hydro-priming on germination and later growth in different plant species under both saline and non-saline conditions, e.g., wheat (Idris and Aslam, 1975) *Acacia tortilis* and *A.coriacea* (Rehman *et al.,* 1998) and only under normal non-saline conditions e.g., maize (*Zea mays*), rice (*Oryza sativa*), chickpea (*Cicer arietinum*) (Harris *et al*., 1999) and white-flowered gourd (*Lagenaria siceraria*) (Jumsoon *et al*., 2000). Hydro-priming did not increase the fresh weight of seedling, root dry weight, root length and root number of cultivar Uqab 2000 compared with non-primed (control) seeds under both normal (non-saline) and saline conditions in this study.

Chilling increased all parameters of growth under both normal and saline conditions in the present study except root and shoot dry weight under salinity. The presowing chilling of seeds improved the growth in pearl millet cultivars IC-8206 and 18-BY under normal non-saline conditions (Ashraf *et al*., 2003). It is thus possible that during pre-chilling some factors/genes may have been stimulated in seeds of wheat whose effect lasted until the vegetative growth of the seedling.

Kinetin significantly increases the rate of germination on most salinity levels (Khan *et al.*, 1998). Even at highest salinities, application of kinetin has proved to be effective in improving plant growth (Zeinab & Sallam., 1996). In our studies kinetin priming was more effective in alleviating adverse effects of salinity on seedling growth. Similar positive effects of seed priming were reported earliar (Ahmed *et al.,* 1998; Ashraf & Rauf, 2001; Harris *et al.,* 1999; Hardegree & Vactor, 2000; *Henckel* & Strogonov, 1961; Khan, 1993) on different crops. The possible mechanism of kinetin treatment has been reported, it was explained that the association between the internal mineral elements concentrations was largely affected by kinetin treatment. It reduced Na^+, Ca^{+2}, and Cl^- accumulation and improved K^+ uptake under salinity stress. Increased K^+/Na^+ ratio helped the plants to avoid Na^+ toxicity and enhanced shoot growth. Kinetin also reduced membrane injury by dehydration and heat stressed and improved the water status of plants (Ashraf *et al.,* 2003) this may be the reason for which the seedlings grown in our studies give improved growth under kinetin treatments.

Presowing seed treatment with inorganic salts (halo priming) is an easy, low cost, and low risk technique, and it is being used effectively to overcome the salinity problem in agricultural lands. Halo priming has been shown to improve germination and plant establishment under saline conditions for different plants (Strogonov ,1964; Cayuela *et al*., 1996; Rehman *et al*., 1998; Ashraf & Rauf, 2001; Sivritepe *et al*., 2003). Ca^{2+} is known to have an antagonistic effect on the uptake of Na^+ in plants subjected to NaCl stress and, thus, mitigate the toxic effect of Na^+ on plant metabolism (Greenway & Munns, 1980; Ashraf, 2004). There are a number of reports that show that increasing the Ca^{2+} and K^+ concentration in seeds of different crops increases their germination in NaCl solutions, including wheat (Chaudhri & Wiebe, 1968). For halopriming, we used chloride salts of Ca^{2+} and there was an increasing trend for all growth parameters under salinity. Calcium protects plants from the adverse effects of NaCl salinity and improves the growth of plants under saline conditions (Cramer *et al*., 1990). The possible reason may be that salinity interacts with plant nutrients which become unavailable to seedlings however calcium chloride treatment made the nutrients available to the plants. In this study, calcium chloride treatment significantly increased root length under both normal and saline conditions. Ca^{2+} could have a protective effect in root tips, which is of fundamental importance for the maintenance of root elongation in NaCl-stressed seedlings (Franco *et al.,* 1999).

An overall increasing trend in the catalase activity (units/ mg fresh weight) was observed except kinetin treatment in normal and chilling in saline conditions. Kinetin tends to increase the catalase activity above normal under salinity, as kinetin try to normalize the adverse effects of salinity (Younis *et al.,* 2003). Chilling and hydro priming significantly increased the catalase activity (units/ mg fresh weight) in roots of wheat seedling in normal and saline conditions, respectively. Previously it has been reported that salinity stress increases catalase in wheat (Sairam *et al.,* 2002).

The adverse effects of salt stress on wheat seedling growth can be mitigated by presowing seed treatments particularly by chilling, kinetin and calcium chloride treatments. Alleviation of adverse effects of salt stress by seed soaking treatments was associated with enhanced antioxidant capacity of the seedling.

However, all seed priming treatments decreased the ascorbic acid concentration under salinity, while reverse was true for normal conditions. Ascorbic Acid was detected in leaf and although its absolute level did not change in response to salt stress, the ascorbate/dehydro ascorbate ratios decreased progressively with the severity of stress. However this antioxidative response does not seem to be sufficient to remove the harmful effects of high salinity (Hernandez *et al.,* 2001) and similar trend has been observed in this study, because the growth of the seedlings was much affected by salinity than under normal conditions, when two controls were compared. However seed priming treatments tend to enhance the level of antioxidants substantially and growth of the seedlings was also improved by seed priming treatments. In our experiments the level of antioxidants increased under salinity, so, it can be postulated that, ROS (Reactive oxygen species) are produced as a result of salinity, which act as a second messenger for the production of anti oxidants (catalase and ascorbic acid) to counter act the adverse effects of salinity.

It is evident that salt stress reduces plant growth as has been observed in the present study however presowing treatment have profound increase in all parameters of wheat seedlings under normal and saline conditions. Chilling and hydro priming significantly increased the catalase activity (units/ mg fresh weight) in roots of wheat seedling in normal and saline conditions, respectively. All seed priming treatments decreased the ascorbic acid concentration under salinity, while reverse was true for normal conditions.

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(Received for publication 18 September, 2006)