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Salicylic Acid Induced Physiological and Biochemical Changes in Wheat Under Drought Stress Conditions

Sami Ullah Khan[a], Asghari Bano[b], Jalal Ud Din[a]* and Suba Sadiq Tahir[c]

[a]Plant Physiology Program, CSI, NARC, Islamabad, Pakistan

[b]Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan

[c]Environmental Analytical Laboratory, NPSL, PCSIR, H-8, Islamabad

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Abstract. Experiment for finding the effect of pre-soaking of wheat seeds varieties, *viz* Wafaq-2001 and Punjab-96, in salicylic acid (SA) solution on the drought tolerance of wheat, revealed increase in the total biomass and grain yield per plant as well as in spikes per plant, 100 seed weight, proline, total soluble sugars, membrane stability index (MSI), superoxide dismutase (SOD) and ascorbate peroxidase (APOX) activity in both the tested varieties. The yield increase in drought tolerant variety Wafaq-2001 was more as compared to drought sensitive Punjab-96. Results signify the role of SA in regulating the drought response of wheat and that SA could be seed primed and used as a potential growth regulator under drought stress conditions.

Keywords: wheat, salicylic acid, drought resistance

Introduction

Wheat (*Triticum aestivum* L.) is the most important food crop of the world including Pakistan and ranks first among all the cereals. In Pakistan, it occupies around 8.6 million hectares with annual production of 22.0 million tones (Economic Survey, 2007). Wheat yields of the country are much lower as compared to many other countries of the world due to abiotic stresses particularly drought, salinity and high temperature (Sial *et al*., 2005; Khan, 2003; Reynolds *et al*., 2001).

To overcome the consumption pressure of ever increasing population, efforts are concentrating on improving wheat-yield by developing new varieties with desirable genetic make up. Although selection and breeding is the ultimate way to produce stress tolerant crop plants, exogenous application of osmoprotectants, growth promoters and antioxidant compounds to plants has been considered a short-term solution for alleviating the adverse effects of different stresses on plants during the last decade (Arfan *et al*., 2007; Raza *et al.*, 2006; Iqbal and Ashraf, 2005).

Various physiological and biochemical effects of salicylic acid on plant systems have been documented in response to environmental stresses (Raskin, 1992). These include effects on membrane permeability, SOD activity, chlorophyll, relative water contents etc. (Agarwal *et al*., 2005). It is also an important molecule for modulating plant responses to stress (Senaratna *et al*., 2000). Any compound can be applied exogenously either as a pre-sowing seed treatment, as a foliar spray or through the rooting medium (Ashraf and Foolad, 2007; Iqbal and Ashraf, 2005) but pre-sowing seed treatment is easy, time saving and economical for the farmers to mitigate the adverse effects of drought stress.

The hypothesis of present research study was to analyze the effects of salicylic acid seed pre-treatment in ameliorating the adverse effects of drought stress in wheat.

Materials and Methods

Seeds of two wheat varieties *viz* Wafaq-2001 and Punjab-96, obtained from wheat programme, National Agriculture Research Centre (NARC) Islamabad, Pakistan, were treated in aerated aqueous solution of salicylic acid (10^{-4}M) for 12 h and control (no SA) in black painted flasks. A separate set of plants was maintained which served as well watered. After hormone treatment, seeds were washed with distilled water and sown in pots filled with soil.

Drought stress was imposed at three developmental stages *viz* tillering (48 DAS), preanthesis (80 DAS) and mid-milky stage (128 DAS) by withholding irrigation for about 5-7 days till the signs of temporary wilting/leaf rolling started. At this satge, samples of flag leaf were collected and analyzed for proline, soluble sugar, superoxide dismutase (SOD), ascorbate peroxidase (APOX) activity and membrane stability index (MSI). After sampling, pots were regularly irrigated. Proline content of flag leaf was determined by the method of Bates *et al*. (1973). Total soluble sugars were measured as described by Pattanaik and Mohapta (1988).

*Author for correspondence; E-mail: jalaludin10@yahoo.co.uk

For SOD activity measurement, leaves (0.5 g) were homo genized in 50 mM sodium phosphate buffer containing 1% polyvinyl pyrolidone (PVP). The homogenate was centrifuged for 15 min at 4 °C and the supernatant was used for assay of the activities of APOX and SOD. The activity of SOD was assayed by monitoring its ability to inhibit the photochemical reduction of NBT (Beauchamp and Fridovich, 1971). One unit of SOD was defined as the amount of enzyme necessary to inhibit the reduction of absorbance reading to 50% in comparison with tubes lacking enzyme.

APOX was assayed by recording the decrease in optical density due to ascorbic acid at 290 nm (Nakano and Asada, 1981). Reaction mixture (3 ml) contained, 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM EDTA, 0.1 mM H_2O_2 and 0.1 ml enzyme. The reaction was started with the addition of 0.1 mM hydrogen peroxide. Decrease in absorbance for a period of 30 sec was measured at 290 nm. Activity was expressed by calculating the decrease in ascorbic acid content by comparing with a standard curve drawn with known concentrations of ascorbic acid.

The MSI was determined according to Sairam *et al.* (2002). Leaf samples (0.1 g each) were cut into discs of uniform size and placed in 10 ml of double- distilled water in two sets. One set was kept at 40 °C for 30 min and its conductivity was (C_1) recorded using conductivity meter. The second set was kept in a boiling water bath (100 °C) for 15 min and its conductivity was also recorded (C_2). The MSI was calculated as:

$$MSI = [1-(C_1/C_2)] \times 100$$

At maturity, agronomic data were recorded for biomass, spikes, grain yield per plant and 100 seed weight. Data were analyzed using a completely randomized design three factor factorial split plot arrangement, where, factor A=hormone, factor B=varieties and factor C=growth stages. Factor C is split plot on factor A and B. The treatment means were compared by LSD test at 0.01 and 0.05 probability levels (Steel and Torrie, 1984).

Results and Discussion

The analysis of data revealed significant differences between the well watered, salicylic acid pretreated seeds and the control. However, there were non-significant differences among the varieties for biomass accumulation (Fig. 1). In both wheat varieties, significant ($p<0.001$) increase in biomass was observed as compared to the control. SA treatment enhanced biomass production by 38.84% as compared to the control.

Further significant differences were found among the treatments for grain yield per plant (Fig. 2), 100 seed weight (Fig. 3) and proline content (Fig. 4). The highest grain yield per plant was recorded in pretreated Wafaq-2001; the percent increase in grain yield alone by SA seed pretreatment was 26.17% as compared to control. Similarly, there was 29.24% increase in yield of pretreated seeds in case of (HXV). In Punjab-96 this increase was 21.45%.

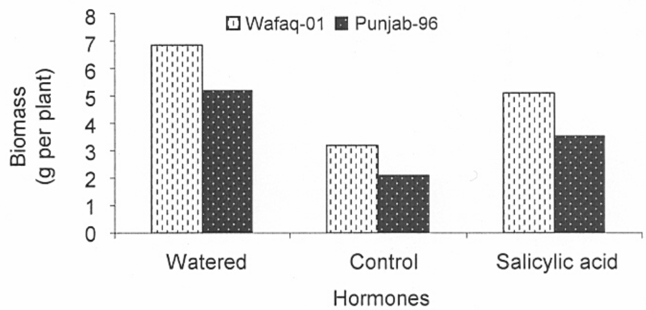

Fig. 1. Effect of salicylic acid seed pretreatment on the biomass of wheat under watered and water stressed (control and salicylic acid) conditions.

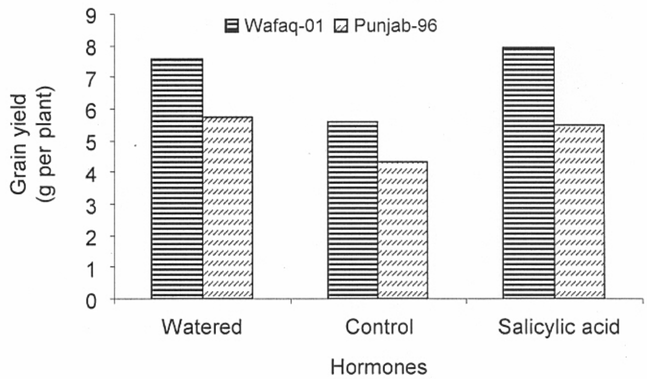

Fig. 2. Effect of salicylic acid seed pretreatment on the grain yield of wheat under watered and water stressed (control and salicylic acid) conditions.

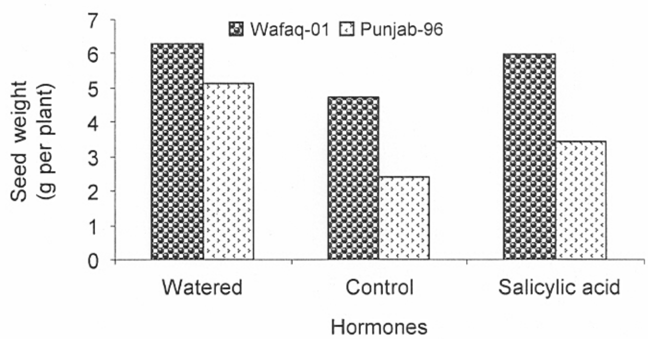

Fig. 3. Effect of salicylic acid seed pretreatment on 100-seed weight of wheat under watered and water stressed (control and salicylic acid) conditions.

The highest 100 seed weight (23.9%) increase was observed in pretreated Wafaq-2001. However, in case of HxV, this increase was 20.8% in case of Wafaq-2001 and 29.61% in case of Punjab-96.

Wafaq-2001 accumulated highest proline content, whereas, proline content was non-significant under HxV interaction. There was 40.4% increase in proline as compared to the control.

The data showed significant differences among the treatments for total soluble sugars accumulation (Fig. 5), membrane stability index, superoxide dismutase and ascorbate peroxidase activities and also between the two varieties, by SA seed pretreatment. Wafaq-2001 accumulated the highest total soluble sugar as well as displayed high membrane solubility index (Fig. 6), high superoxide dismutase activity (Fig. 7) and high ascorbate peroxide activity (Fig. 8).

In Waqaf-2001, there was 29.23% increase in total soluble sugar accumulation as compared to the control; however, in case of HxV, total soluble sugars increased 36.7% in Wafaq-2001 and 19.68% in Punjab-96.

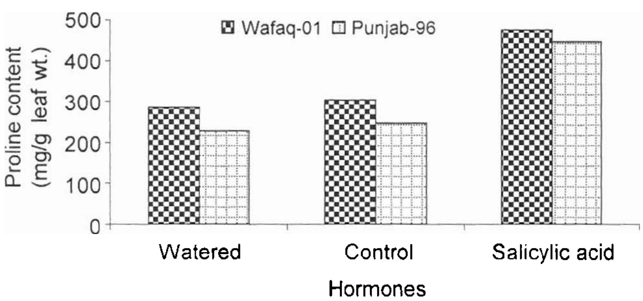

Fig. 4. Effect of salicylic acid seed pretreatment on the proline concentration in wheat leaves of wheat under watered and water stressed (control and salicylic acid) conditions.

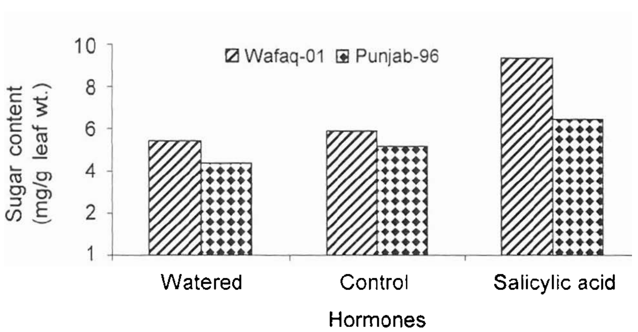

Fig. 5. Effect of salicylic acid seed pretreatment on the soluble sugar content in wheat leaves under watered and water stressed (control and salicylic acid) conditions.

There was 20.07% improvement in membrane stability index, highest being at mid milky growth stage followed by preanthesis and tillering.

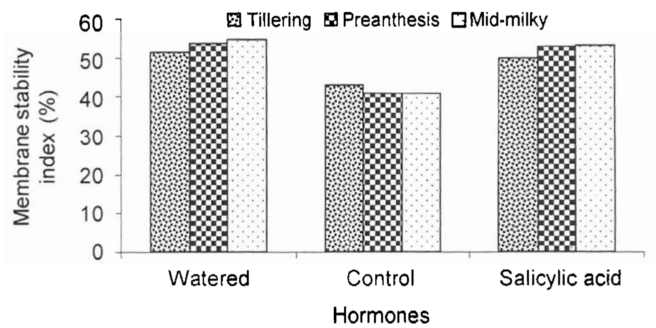

Fig. 6. Effect of salicylic acid seed pretreatment on the membrane stability index of wheat under watered and water stressed (control and salicylic acid) conditions.

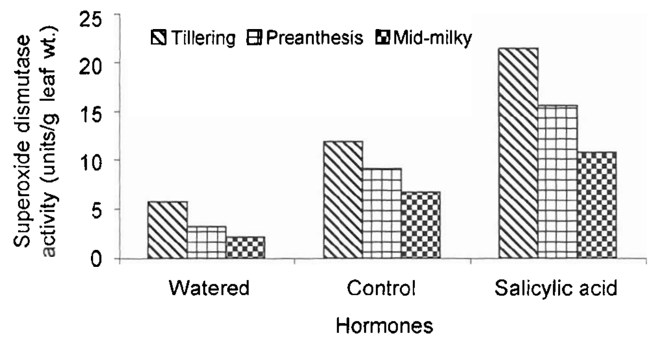

Fig. 7. Effect of salicylic acid seed pretreatment on the superoxide dismutase activity in wheat at different growth stages under watered and water stressed (control and salicylic acid) conditions.

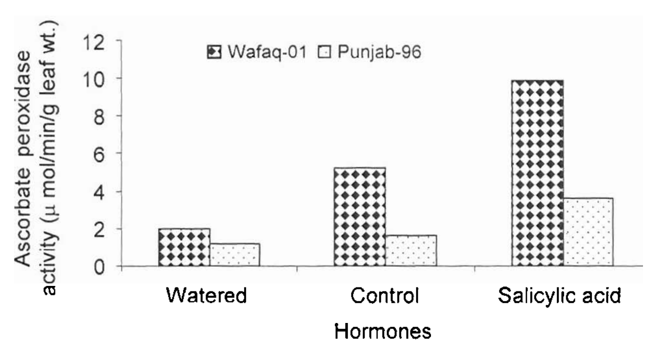

Fig. 8. Effect of salicylic acid seed pretreatment on the ascorbate peroxidase activity of wheat under watered and water stressed (control and salicylic acid) conditions.

There was 40.5% and 49.3% increase in SOD and APOX activities, respectively, by SA seed pre-treatment alone. In case of HxV, the increase was 40.2% and 46.6%, respectively, in Wafaq-2001 and 43.8% and 55.5%, respectively, in Punjab-96 by SA seed pretreatment.

SA seed pretreatment significantly affected plant growth properties i.e., biomass per plant, grain yield per plant,100 seed weight, proline, total soluble sugars, activity of SOD and APOX enzymes. Drought stress caused a significant reduction in the growth of two wheat varieties. However, application of salicylic acid seed pretreatment counteracted the adverse effects of low water availability on the growth of the varieties.

From the results it can be concluded that beneficial effect of SA application depends on type of cultivar. Bezrukova *et al*. (2004) also reported improvement in growth of wheat by SA application. In the present study increase in grain yield and 100-grain weight of Wafaq-2001 was mainly due to increase in grain size and number with SA application. This is in agreement with findings of Grieve *et al*. (1992), who inferred that the beneficial effects of SA on grain may have been due to translocation of more photo-assimilates to grains during grain filling, thereby increasing the grain yield per plant. Zhou *et al*. (1999) also reported 9% increase in grain weight of maize plants seed-soaked with SA. The second possible mechanism of SA induced yield enhancement might be an increase in the number of spikelets and number of grains, because SA has the capacity to both directly and indirectly regulate the yield (Agarwal *et al*., 2005).

SA treatment increased proline content more in Wafaq-2001 than Punjab-96. Proline indirectly causes increase in metabolic activation by providing osmoregulation for the plants under stress (El-Tayeb, 2005; Shakirova *et al*., 2003; Pesserakli and Huber, 1987) and also found increase in proline content with SA treatment under salinity and drought.

SOD activity increased significantly, by SA seed pretreatment (Fig. 4). The highest increase in SOD activity was observed in Wafaq-2001 by SA seed pretreatment as compared to control. SA seed pre-treatment increased the activity of ascorbate peroxidase. The highest increase in ascorbate peroxidase was observed in Wafaq-2001 followed by Punjab-96 (Fig. 5). This might be due to drought tolerant character of Wafaq-2001. Salicylic acid-induced-increase in SOD, CAT and GR activities has also been reported by Agarwal *et al*. (2005), Clark *et al*. (2002) and Molina *et al*. (2002). The higher MSI in drought tolerant Wafaq-2001 suggests a better protection from the oxidative damage. The better protection in Wafaq-2001 seems to result from the high CAT and APOX activity. These results are in agreement with the results of Bor *et al*. (2003) and Sairam *et al*. (2002) who found a correlation between increased anti-oxidant enzymes activities and decreased lipid peroxidation in salt drought tolerant wheat and wild beet, *Beta maritema*, respectively, under salt stress.

Conclusion

From the results it was inferred that SA seed treatment caused an increase in biomass and grain yield in both the two wheat varieties. The increase in yield was more pronounced in the tolerant variety Wafaq-2001 as compared to drought sensitive Punjab-96. The SA pre-soaking increased total biomass, proline, total soluble sugars, membrane stability index, activity of superoxide dismutase and ascorbate peroxidase in both the tested varieties. Results signify the role of SA in regulating the drought response of wheat and suggest that SA could be seed primed and used as a potential growth regulator under drought stress conditions.

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