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Indole-3-Acetic Acid Induces Biochemical and Physiological Changes in Wheat under Drought Stress Conditions

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Impact of foliar application of indole-3-acetic acid (IAA) was studied to induce drought tolerance in commercially important wheat cultivars. Four hexaploid wheat (*Triticum aestivum* L.) cultivars viz. Aari, Baras, Sahar and Aas were grown under controlled conditions. Plants were subjected to drought stress 14 d after germination. After 21 d, 80 ppm solution of IAA was used for foliar application. Sugar and proline contents increased due to drought stress in all four wheat cultivars. Exogenous application of IAA had a varying effect on sugar and proline contents. Protein content, abiotic tolerance index (ATI) and stress tolerance index (STI) showed variation among different varieties under drought stress conditions and after IAA foliar application. Under drought conditions, relative water content (RWC) and fresh weight of wheat seedlings decreased significantly, which were recovered in the tolerant variety Aas through the foliar application of IAA. Exogenous application of IAA showed variable effects on antioxidant system. The activity of peroxide dismutase (POD) remained unaffected in the four cultivars, while superoxide dismutase (SOD) was promoted in all cultivars except Baras. After foliar application of IAA, drought-stressed plants of Aas variety showed 10.3% improvement in fresh weight compared with the untreated plants. These results lead us to conclude that IAA showed a positive cultivar specific effect in wheat drought tolerance. IAA not only helps plants to accumulate proteins, but it also restores plant fresh weight by increasing relative water content. These results suggest exogenous application of IAA to recover wheat plants from drought stress conditions.

Key Words: drought stress, foliar application, fresh weight, IAA, peroxide dismutase, superoxide dismutase, wheat

Abbreviations: ABA – abscisic acid, ATI – abiotic tolerance index, BSA – bovine serum albumen, CAT – catalase, FP – free proline content, FW – fresh weight, GAA – gibberellic acid, H_2O_2 – hydrogen peroxide, IAA – indole-3-acetic acid, O_2^- – superoxide, OH^- – hydroxyl radicals, PGRs – plant growth regulators, POD – peroxide dismutase, ROS – reactive oxygen species, rpm – rotation per minute, RWC – relative water content, SOD – superoxide dismutase, STI – stress tolerance index, TSP – total soluble protein content

INTRODUCTION

Wheat is the leading crop of the temperate climates of the world and is a unique world food grain. Wheat is considered the main staple food crop for more than one-third of the world's population (Shirazi et al. 2010) and is the staple food in Pakistan. Wheat plants are exposed to various environmental stresses during the course of their life cycle. Among these are drought, temperature, salinity and cold stress. Wheat production in the Mediterranean region is restricted mainly by the accessibility of water resources. Water stress decreases the photosynthetic rate and accelerates leaf senescence (Martinez-Tome et al. 2001).

Drought is a major environmental cue impairing many physiological and metabolic processes in plants, which may lead to suppressed plant growth and development, reducing crop productivity, and eventually resulting in plant death. Across plant species, drought imposes various physiological and biochemical limitations and adverse effects (Chaves and Oliveira 2004; Wang et al. 2003). For example, drought stress elevates the generation of reactive oxygen species (ROS) when plants are exposed to moisture stress (Foyer and Noctor 2005). Accumulation of ROS, including superoxide (O_2^-), hydroxyl radicals (OH^-) and hydrogen peroxide (H_2O_2), is the earliest plant response to drought stress (Apel and Hirt 2004; Ashraf 2009; Ashraf and Akram 2009; Mittler 2002). Accumulation of ROS may lead to many deleterious effects, e.g., protein degradation, lipid peroxidation and pigment bleaching. To protect cells from such deleterious effects, plants increase activities of key antioxidant enzymes in the cytosol, including superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), which are believed to counteract the effects of ROS (Foyer et al. 1997; Foyer and Noctor 2000). In some cases, *de novo* synthesis of such antioxidant enzymes is a way to overcome the negative effects of drought stress (Alscher et al. 1997). Drought stress also induces the accumulation of compatible solutes such as glycerol, sugars, glycinebetaine and proline, a phenomenon that also varies among plant genotypes. Proline, for example, is one of the most common osmolytes accumulating in plants in response to a variety of environmental stresses (Bates et al. 1973; Nikolaeva et al. 2010; Ibrahim et al. 2014). Accumulation of free proline is likely a part of the general adaptation of plants to drought stress, as it promotes water retention in plants, thereby alleviating the negative effects of water deficit (Ashraf and Foolad 2007; Szabados and Savoure 2010). Thus, the concentration of free proline in plants has been suggested as a metabolic measure of drought tolerance (Farooq and Bano 2006). Furthermore, in many plant species, there is a strong positive correlation between increased concentration of intracellular proline and the ability of plants to survive under high salt or water deficit conditions (Chaves and Oliveira 2004).

Increased concentration of intracellular proline and consequently enhanced plant drought tolerance can also be achieved by exogenous application of plant growth regulators (PGRs). IAA plays a major role in regulating plant growth. For example, it controls vascular tissue development, cell elongation, and apical dominance (Wang et al. 2003). Plants respond to drought through various biochemical changes, including changes of the endogenous hormone levels, especially that of abscisic acid (ABA). Plants exposed to drought stress can recruit ABA as an endogenous signal to initiate adaptive responses. However, the variation of IAA and GA contents under drought stress is contradictory. It was reported that drought resulted in a decrease of IAA content in the leaves of wheat (Xie et al. 2003). However, other reports have shown that adaptation to drought in plants was accompanied with an increase in IAA content. Xie et al. (2003) reported that drought stress significantly decreased IAA and GA concentration in wheat leaves compared with the control treatment. It may be due to decreased IAA and GA synthesis or increase in the destruction of IAA and GA by increasing the activity of oxidase enzyme (Davenport et al. 1980). Auxins are used commercially for enhancing crop production; they also regulate plant growth and development, and result in rapid growth of plant parts such as shoot tissues, young leaves and developing seeds. However, auxin application increases pod numbers, seed weight or seed yield in pea, but this is based on varietal sensitivity and correct time of application (Amal et al. 2009). IAA was useful for the increase of growth and yield.

In view of the above-mentioned studies, we hypothesized that IAA would alleviate the harmful effects of drought stress in wheat plants by altering the activities of some key metabolites. In the present study, we examined the effectiveness of exogenous application of IAA in mitigating the adverse effects of drought in four widely grown wheat cultivars of Pakistan.

MATERIALS AND METHODS

Seed Collection and Sterilization

Wheat seeds of Aari 2011, Aas 2011, Baras 2009 and Sahar 2006 varieties were collected from the National Agriculture Research Center, Islamabad in 2013–2014 and brought to Quaid-i-Azam University, Islamabad for further experimentation. The seeds were soaked in 75% ethanol for 3 min for sterilization and washed thrice, thoroughly with distilled water. Wheat seeds were sown in pots containing sterilized sandy soil for 4 wk in a growth chamber adjusted at 25 °C, 80% relative humidity and 14 h photoperiod. Light was provided by cool-white fluorescent lamps. Eight seeds were sown in each 6-inch diameter pot and thinned after 1 wk to four seedlings per pot. Moisture level was maintained by adding distilled water. Plants of each variety were grown in 30 pots and these were divided into 2 groups with 10 and 20 pots per group. Two weeks after sowing, plants of the 1st group were left without any treatments to serve as healthy control. Drought stress was done by withholding water on the 2nd group. Three weeks thereafter, 10 pots from the 2nd group were selected for the exogenous application of 80 ppm IAA solution through hand sprayer. After 4 wk of sowing, the plants were uprooted and preserved at −80 °C for further analysis of biochemical parameters.

Biochemical Parameters

Biochemical parameters such as proline, sugar, protein and antioxidant enzymes were studied to evaluate the possible effects of drought and IAA treatment. Proline content of the leaves was determined by using the method of Bates et al. (1973). Estimation of sugar content in the leaves was determined following the method of Dubois et al. (1956). Protein content of the leaves harvested at the seedling stage was determined following the method of Lowry et al. (1951) using Bovine Serum Albumen (BSA) as standard.

For extraction of antioxidant enzymes, 0.5 g of leaves was ground in 5 mL of 50 mM phosphate buffer placed in an ice bath. The homogenate was centrifuged at 13000 rotations per minute (rpm) for 20 min at 4 °C. The supernatant was used for assays of the activities of enzymes. Superoxide dismutase activity was determined following the method of Beauchamp and Frodovich (1971). Peroxidase activity was estimated by the method of Vetter et al. (1958). Relative water content (RWC) of entire leaves was measured after 7 d of induction of water stress, by using the method of Weatherley (1950).

Fresh Weight of Seedlings

To determine the fresh weight of seedlings, 30-d-old seedlings (root and shoot tissue) were taken out from the pots, washed with running tap water, blotted dry with blotting paper and weighed.

Statistical Data Analysis

Data were subjected to analysis with Statistic version 8.1 Software using analysis of variance (ANOVA) under completely randomized design (CRD). Means were compared by Least Significant Difference (LSD) test at 0.05 probability level.

RESULTS AND DISCUSSION

Free Proline Content (FP)

Results showed significant variation in free proline content within a variety as well as among varieties under different treatments. Maximum proline content was found in Aas followed by Sahar, Aari and Baras under controlled conditions (Fig. 1). Free proline content increased significantly in Aas (88%), Sahar (42%) and Baras (110%) under drought treatment. IAA treatment gave good result under drought stress and decreased proline content in Sahar (62%) and Aas (41%) while these were increased in Aari by 44% (Fig. 1).

Proline, as an amino acid, accumulates in large quantities in response to abiotic stresses in plants (Ozturk and Demir 2002; Hsu et al. 2003; Kavi-Kishor et al. 2005). This increasing trend in proline content indicates that stressed plants got relief by increasing proline content which is responsible for osmotic adjustment, stabilizing sub-cellular structures (e.g., membranes and proteins), scavenging free radicals and buffering cellular redox potential of plant cells to alleviate stress conditions (Shereen et al. 2007). Generally proline content is higher in the tolerant variety compared with the susceptible variety. Proline content accumulated more in Sahar and Aas than in Baras and Aari, which indicate that Sahar and Aas are more resistant against abiotic stress. Similar findings have already been reported in rice (Shereen et al. 2007), alfalfa (Petrusa and Winicov 1997), maize (Cha-um and Kirdmanee 2009), pigeon pea (Waheed et al. 2006) and potato (Rahnama and Ebrahimzadeh 2004).

Exogenous application of IAA had a significant effect on proline content under drought stress. Proline content in tolerant varieties (Sahar and Aas) decreased because IAA treatment alleviated water limiting effect. This decrease shows that IAA application helped plants to restore their normal condition (2010).

Sugar Content

A significant variation in sugar content was observed among all wheat genotypes and maximum sugar content was observed in Sahar (Fig. 2). Results revealed that foliar application of IAA significantly increased the sugar content in Sahar (19%) and Aas (16%) when sprayed at the vegetative stage compared with both stressed and control varieties (Fig. 2). Sugars are vital for plant growth and metabolism, both as a source of energy and structural components. Sugar content affects many biochemical processes such as photosynthesis, glycolysis, nitrogen metabolism, cell cycle and defense mechanism (Zhu 2002). In four tested varieties, soluble sugar accumulation increased and this increase indicates the activation of the plant's defense mechanism in response to environmental stress (Kaya et al. 2013). Increase in sugar content was an adaptive mechanism in plants to minimize the deleterious effects of abiotic stress. Under drought conditions, sugar content increased due to increased activity of enzymes such as α amylase which converts stored starch into soluble sugar to adjust osmotic pressure in plants (Bolarían et al. 1995; Lippmann 1991).

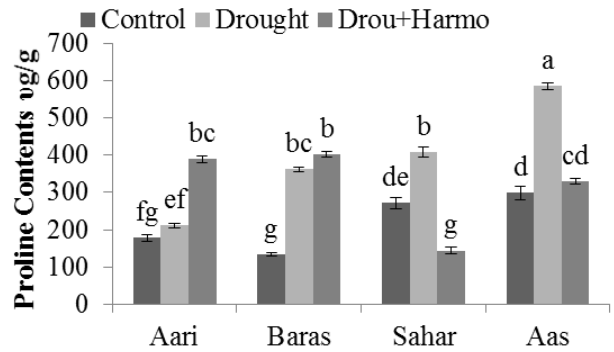

Fig. 1. Variation in proline content under different treatments. Means with a common letter are not significantly different at p<0.05 level while vertical bars show standard deviation of means.

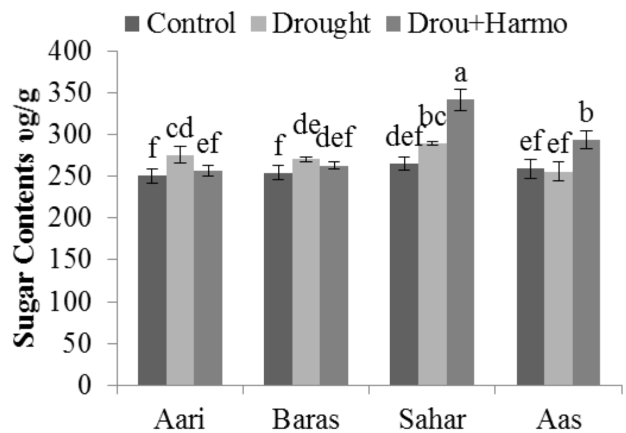

Fig. 2. Variation in sugar content under different treatments. Means with a common letter are not significantly different at p<0.05 level while vertical bars show standard deviation of means.

Increased sugar content declares Sahar to be more tolerant compared with other susceptive varieties. Foliar application of IAA showed an increasing trend in sugar content which indicates its role under adverse environmental condition. An increase in the concentration of soluble sugar content of IAA treatment was due to the increase in the rate of photosynthesis (Candan and Tarhan 2003).

Total Soluble Protein Content (TSP)

Total soluble protein varied significantly in selected varieties and maximum TSP content was observed in Baras, followed by Sahar, Aas and Aari under controlled conditions (Fig. 3). Drought stress decreased the protein content of susceptible varieties Aari and Baras by 26% and 49%, respectively. These values were increased in Aas variety by 19%. This decrease in the protein content of Aari and Baras could be the reason for their susceptible nature. After IAA treatment, TSP content was increased in Aari and Baras by 40% and 43%, respectively, while significant decreases of 51% and 28% were observed in Sahar and Aas, respectively (Fig. 3). The increase in TSP level is a natural occurring

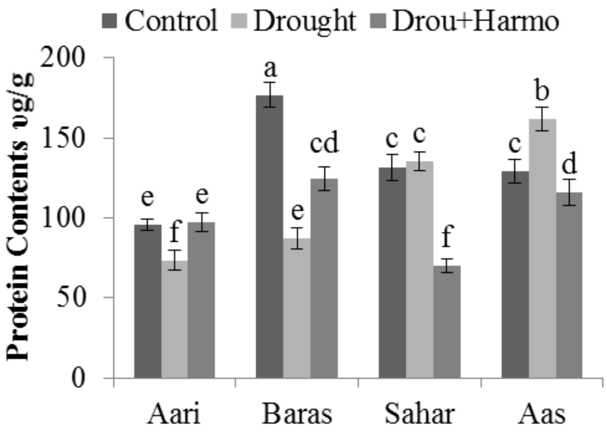

Fig. 3. Variation in protein content under different treatments. Means with a common letter are not significantly different at p<0.05 level while vertical bars show standard deviation of means.

phenomenon under drought stress to increase plant adaptation to water deficit conditions. In the present study, exogenous application of IAA provided relief to tolerant varieties by starting other processes such as photosynthesis while in susceptible varieties, protein accumulated in response to drought stress (Mittler 2002).

Superoxide Dismutase (SOD) and Peroxide Dismutase (POD) Activity

SOD activity varied significantly within the variety as well as among the varieties under different treatments. Maximum SOD activity was observed in Aari and Baras while the minimum was observed in Sahar and Aas. IAA treatment positively affected and raised SOD activity in Sahar, Aas and Aari by 33%, 15.26% and 38%, respectively (Fig. 4). POD activity in selected varieties changed insignificantly under controlled conditions. By imposition of drought, increasing trend in POD activity was observed in Baras (90%), Aas (77%) and Sahar (82%) while in Aari, POD activity was decreased by 22% (Fig. 5). Highest POD activity has been found in Baras under drought stress. Exogenous application of IAA showed a 42% decrease in POD activity in the cultivar Sahar whereas in the other three cultivars, insignificant variation was seen (Fig. 5). All these results show increased SOD as well as POD activity under environmental stress conditions. Under unfavorable environmental conditions, ROS are generated which damage plants (Arfan et al. 2007). In response to oxidative stress, the plant's defense mechanism is activated, e.g., SOD and POD activities increase to scavenge properly and protect plants against ROS damage (Chen and Li 2001). Under drought stress, foliar application of IAA gave positive results. Increase in SOD activity indicates that unfavorable environmental stress alters ion homeostasis in wheat cultivars, resulting in ion toxicity and osmotic stress that generate ROS in plants to cause oxidative stress (Sairam and Saxena 2000). Exogenous application of phytohormones is one of the best options to alleviate oxidative stress by increasing the activity of SOD in plants.

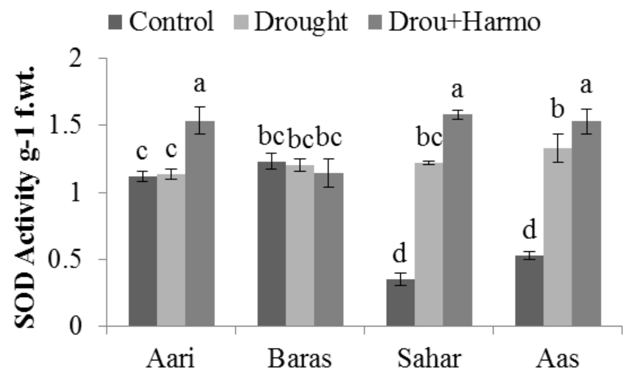

Fig. 4. Variation in superoxide dismutase (SOD) activity under different treatments. Means with a common letter are not significantly different at p<0.05 level while vertical bars show standard deviation of means.

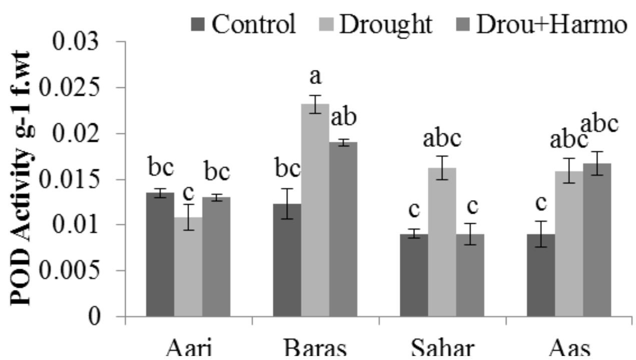

Fig. 5. Variation in peroxide dismutase (POD) activity under different treatments. Means with a common letter are not significantly different at p<0.05 level while vertical bars show standard deviation of means.

Fresh Weight and Relative Water Content (RWC)

Drought stress decreased fresh weight of all varieties under study (Fig. 6). After foliar application of IAA, drought-stressed plants of Aas variety showed 10.3% improvement in fresh weight compared with the untreated plants. Analysis of variance for relative water content in selected varieties showed non-significant variation under control condition. The drought treatment caused significant decrease in RWC in selected varieties (Fig. 7). IAA application significantly increased RWC in Aas by 2% and in Baras by 1.7%, while Sahar and Aari showed minor changes (Fig. 7). In the current study, RWC and fresh weight (FW) were reduced drastically under moisture stress conditions. This result may be due to the loss of more water through transpiration, compared with absorption of water through the roots. The current study showed that foliar application of IAA gave positive relationship to absorption of water under abiotic stress because under adverse conditions, IAA concentration is decreased (Xie et al. 2003). Abiotic stress inhibits the synthesis of growth-promoting phytohormone and accelerates the degradation of IAA (Davenport et al. 1980). The best option to overcome IAA deficiency is exogenous treatment.

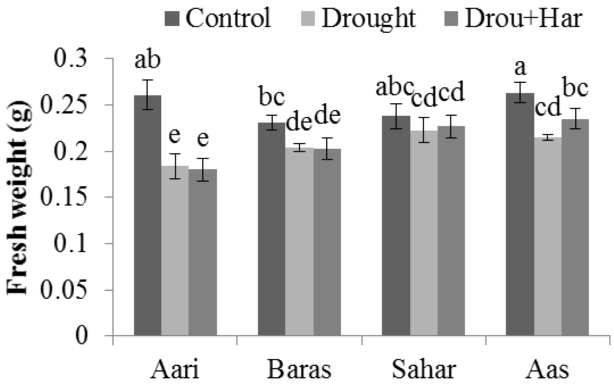

Fig. 6. Variation in fresh weight under different treatments. Means with a common letter are not significantly different at p<0.05 level while vertical bars show standard deviation of means.

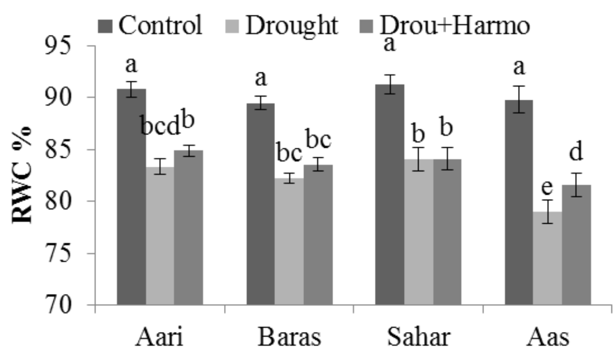

Fig. 7. Variation in relative water content (RWC) under different treatments. Means with a common letter are not significantly different at p<0.05 level while vertical bars show standard deviation of means.

CONCLUSION

The results of this study demonstrated that proline is a good indicator of stress condition and that IAA application helped plants to restore their normal condition. The most promising effect of IAA application resulted in increased sugar content which indicates the activation of the plant's defense mechanism. IAA application decreased protein content in the tolerant varieties due to the possible start of other important processes. IAA application also improved fresh weight and relative water content of drought-stressed plants. These biochemical and physiological parameters showed that the varieties Sahar and Aas were tolerant while Aari and Baras were susceptible varieties. Exogenous IAA application improved tolerance in both susceptible and tolerant wheat varieties and it can be used to defy drought stress in wheat.

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