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IMPROVEMENT OF ANTIOXIDANT DEFENSE SYSTEMS IN WHEAT (*Triticum aestivum* L.) SEEDLINGS BY HYDROPRIMING

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Abstract: In the present study, effect of hydropriming on seedling growth and antioxidant response was investigated in two wheat cultivars PBW 175 (drought tolerant) and PBW 621 (drought sensitive). In general, hydropriming promoted seedling growth of wheat by increasing the activities of antioxidant enzymes viz. superoxide dismutase (SOD), peroxidase (POX) and ascorbate peroxidase (APX), contents of non-enzymatic antioxidants viz. ascorbate and total phenols and lowering of H_2O_2 content. Superoxide dismutase activity increased to greater extent in hydroprimed PBW 175 seedlings as compared to PBW 621. Peroxidase activity was significantly higher in PBW 175 seedlings when compared with PBW 621. Correlation between PPO activity and total phenolic content indicated that increase of total phenolic content in hydroprimed PBW 175 seedlings could be due to *denovo* synthesis of these compounds. Hydropriming did not affect proline and malondialdehyde contents of wheat seedlings. Therefore, hydropriming improved seedling growth of wheat by promoting antioxidant response.
Keywords: Antioxidant response, hydropriming, wheat.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the main cereal crop in India with an area of about 29.8 million hectares (FAOSTAT and IGC reports, 2012). The production of wheat in the country has increased significantly from 75.81 million tonnes in 2006-07 to an all time record high of 94.88 million tonnes in 2011-12. However this increase is not sufficient to meet the demands of continually growing population. Secondly, over the years percentage area under irrigation has witnessed a downward trend and proportionately the wheat acreage under diesel operated tube wells has increased in the North West plains (FAOSTAT and IGC reports, 2012). Moreover, yields will have to grow without depleting the natural resource base on which agriculture depends.

Major environmental constraints limiting the growth and productivity of wheat are extreme temperatures, salinity and drought stress. These climatic stresses induce the generation of reactive oxygen species (ROS) such as superoxide radicals ($^{\cdot}O_2^-$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\cdot}OH^-$) etc thereby creating a state of oxidative stress (Khan and Singh,

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2008). Furthermore, these stresses may lead to the disruption of cellular structures as well as disorganization of membranes. Plants have evolved efficient ROS scavenging mechanisms to achieve control over the ROS toxicity and use them as signalling molecules. Various enzymes involved in scavenging ROS are superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), catalase (CAT) and peroxidase (POX) whereas non-enzymatic antioxidants include glutathione, ascorbic acid, α-tocopherol, phenols, proline and carotenoids (Malik *et al.,* 2010).

Plants also respond to abiotic stresses by accumulation of compatible solutes, such as proline and glycine-betaine in the cells which results in the improvement of environmental stress tolerance (Ashraf and Foolad, 2007). Proline is an important molecule in redox signalling and an effective quencher of ROS generated due to various abiotic stresses (Mafakheri *et al.,* 2010). Pre-treatment of wheat and barley seeds with phenolic acids showed higher levels of proline and betaine and accumulation of these osmolytes were correlated with greater tolerance of these seedlings against saline stress (Deef 2007). Malondialdehyde accumulation takes place in plants due to membrane peroxidation. It is an effecting means of assessing oxidation stress induced membrane damage.

Therefore the prime importance is to find out the means by which antioxidant response could be augmented in order to counteract the damaging effects of upcoming abiotic stresses. Seed priming is a seed pre-sowing imbibition treatment that is used to improve germination percentage and uniformity of emergence in various field crops. Seed priming techniques strongly influence metabolic, biochemical and enzymatic status of seed, thereby helping in germination and seed establishment (Farooq *et al.,* 2010). Out of these techniques, hydropriming is an economic method with advantageous effects on many crops *viz.* maize, rice, chickpea and soybean (Ashraf and Foolad, 2007). Indeed, there are several reports demonstrating the positive effects of hydropriming on seedling growth of alfalfa and wheat (Amooaghaie, 2011 and Ghobadi *et al.,* 2012). Hydropriming induced seedling growth is correlated with higher water uptake by primed seeds (Ghobadi *et al.,* 2012). It also decreased malondialdehyde content of *Pinus* seeds (Guo *et al.,* 2012). Water-primed canola seeds showed improved seedling growth under saline and drought stressed conditions (Aboutalebian *et al.,* 2012).

Although several studies had been conducted in the past involving the exogenous use of various eco-friendly chemicals such as phenolic acids, ascorbic acid and hydrogen peroxide in different agricultural crops to counteract the damaging effects of reactive oxygen species

(Mohammed and Tarpley, 2011). However, to the best of our knowledge, there is not even a single report showing hydropriming induced changes in antioxidant response of contrasting wheat cultivars grown under normal conditions.

MATERIALS AND METHODS

Plant material and growth conditions: The present investigation was carried out in two wheat cultivars PBW 175 and PBW 621. PBW 175 is recommended for cultivation under rainfed conditions whereas PBW 621 is recommended for cultivation under irrigated conditions. Seeds were pre-treated with water (hydroprimed) for 12 hours before germination at room temperature in the dark. After 12 hours, the seeds were dried by placing them in an incubator for 2 hours at 25±1ºC. Pre-treated seeds were germinated in plastic cups containing untreated and well irrigated soil. For each treatment, three cups with 6 seeds in each were used. Plastic cups containing seeds were then placed in an incubator at 25±1ºC in the dark. Data for the seedling growth was taken at 6th day of seedling growth. Fresh samples were placed in an oven at 60°C for 6-8 hrs and dry weights were taken. Drying of these samples continued till constant weight was obtained.

Activities of antioxidant enzymes and contents of non-enzymatic antioxidants were determined in roots and shoots at 4th and 6th days after germination (DAG).

Extraction and assay of enzymes: All enzymes were extracted at 4ºC. Each sample was taken from a representative sample of at least three seedlings. All activities were determined at 30ºC.

Extraction and assay of GR, POX and SOD: Root and shoot (100 mg each) tissue was extracted with 1.5 ml of ice cold 100 mM sodium phosphate buffer (pH 7.5) containing 1 mM EDTA, 1% PVP and 10 mM β-mercaptoethanol. Homogenate was centrifuged at 10000xg at 4°C for 15 min and clear supernatant was used for enzyme assays according to the methods earlier standarized in the lab (Devi *et al* 2012).

Extraction and estimation catalase and ascorbate peroxidase: Catalase (EC 1.11.1.6) activity was extracted and estimated by the procedure described by Chance and Maehly (1955). Ascorbate peroxidase (EC 1.11.1.1) was assayed according to the method of Nakano and Asada (1987). The reaction mixture consisted of 30 mM sodium phosphate buffer (pH 7.0), 0.13 mM ascorbic acid, enzyme extract and 13 mM H_2O_2 solution. The enzyme activity was measured as decrease of absorbance at 290 nm. Extinction coefficient of monodehydro ascorbic acid is 2.8 mM^{-1} cm^{-1}.

Extraction and estimation of polyphenol oxidase: Polyphenol oxidase (EC 1.14.18.1) was

extracted and assayed according to the method of Zauberman *et al* (1991). One unit of enzyme activity has been defined as an increase of 0.01 in absorbance $min^{-1}mg^{-1}$ of protein.

2.6 Extraction and estimation of H_2O_2 and malondialdehyde: The shoot (300 mg) and root (400 mg) was crushed with 2.0 ml of ice cold 10 mM potassium phosphate buffer (pH 7.0). Homogenate was centrifuged at 10000xg for 20 minutes. Then supernatant was collected and used for estimating H_2O_2 content (Sinha, 1971).

Malondialdehyde content was extracted and estimated by the standard procedure of of Heath and Packer (1968). It was calculated using an extinction coefficient of 155 $mM^{-1}cm^{-1}$ and expressed as nmole g^{-1} FW.

Extraction and estimation of reduced ascorbic acid, proline, total phenols and protein content: For extraction of ascorbate content, the shoot (300 mg) and root tissues (400 mg) were homogenized in 2.0 ml of 5% ice cold metaphosphoric acid and centrifuged at 10,000xg for 10 min. Supernatant was taken for the estimation (Law *et al* 1983). Proline was extracted by crushing 300 mg tissue in 4 ml of 3% aqueous sulfosalicylic acid and the homogenate was filtered through Whatman filter paper and filtrate was used for proline estimation (Bates *et al* 1973). Extraction and estimation of total phenols was done by the method of Swain and Hills (1959). Protein was estimated by the method of Lowry *et al* (1951) using Folin-phenol reagent.

Statistical analysis: Growth data has been presented as mean $\pm$ SD of three samples of 18 seedlings each. It was statistically analysed by applying one-way analysis of variance (ANOVA) followed by post hoc analysis, the LSD (Least significant difference) test. Data for other biochemical parameters was analysed by applying two-way ANOVA with interaction. LSD-A, LSD-B and LSD-AB represent the values for water priming, days and interaction between these two factors.

RESULTS AND DISCUSSION

Wheat is the second most important staple food in India after rice and provides 50 % of calories and proteins to the people. Although wheat is relatively tolerant to abiotic stresses, it is frequently grown in environments in which water deficit stress is of common occurrence. Globally, almost 50% of the wheat cultivated in the developing world (50 million ha) is sown under rainfed systems that receive less than 600mm per annum (CIMMYT 2005). Moreover seed priming seems to be a promising technique to raise successful crop in semiarid tropics. Therefore, effect of hydropriming on antioxidative defense system of two wheat cultivars differing in drought tolerance was investigated.

In comparison to non-treated PBW 175 seedlings, hydropriming increased root and shoot lengths by 3 and 2% respectively however the increase was about 6 and 13% respectively in PBW 621 seedlings (Table 1). Earlier Kaur *et al* (2002) also found increased root and shoot lengths in hydroprimed chickpea seedlings compared to non-primed seedlings. Hydropriming also increased fresh weights of roots in both cultivars of wheat. Although water pre-treatment had no effect on fresh and dry biomass of endosperms in PBW 175 whereas it resulted in decreased biomass of endosperms in PBW 621 (Table 1). Therefore, it seems that hydropriming promoted seedling growth in drought tolerant cultivar PBW 175 by increasing water absorption whereas in case of PBW 621, enhanced growth could be attributed to biomass partitioning. Ghobadi *et al.* (2012) also reported improved seedling growth after hydropriming and it was correlated with higher water uptake by primed seeds as in case of PBW 175. Indeed, one of the proteomic studies revealed that hydropriming improved seedling growth by inducing some embryonic proteins in maize seedlings (Gong *et al.,* 2013). Reactive oxygen species can act as secondary messengers in stress signal transduction pathways but excess ROS have detrimental effects on normal metabolism through oxidative damage to various biomolecules. Antioxidative defense system plays a vital role in protecting the plant cells from oxidative damage. To analyse the effect of hydropriming on antioxidant response, activities of superoxide dismutase, peroxidase, catalase, ascorbate peroxidase, glutathione reductase and polyphenol oxidase were determined. In general, hydropriming led to a significant increase in SOD activity of wheat seedlings (Table 2). It increased by more than 75% in roots and 90% in shoots of PBW 175 seedlings however the increase was about 29 and 56% respectively in the roots and shoots of PBW 621 seedlings at 4th DAG (Table 2). Amooaghaie *et al.* (2011) also found increased SOD activity in the hydroprimed alfalfa seedlings as compared to non-primed ones. It was also upregulated by hydropriming in bitter gourd and cucumber seedlings (Wang *et al.,* 2003 and Huang *et al.,* 2006). The dismutation of $^{\cdot}O_2^-$ is accompanied by the production of H_2O_2, increased levels of which is lowered by the activities of CAT, POX and enzymes of ascorbate-glutathione cycle.

Hydrogen peroxide is detoxified by catalase. In general, shoots possess higher CAT activity as compared to roots of wheat seedlings (Table 2). In comparison to non-primed seedlings, CAT activity either increased or remained unaffected in hydroprimed wheat seedlings with the exception of shoots in PBW 175 at 6th DAG and roots of PBW 621 at 4th DAG where it declined significantly (Table 2). Hydropriming also upregulated CAT activity in cucumber and rye seedlings (Huang *et al.,* 2006 and Ansari and Sharif-Zadeh, 2012).

Peroxidase activity was higher in PBW 175 seedlings as compared to PBW 621 (Table 2). In comparison to non-treated seedlings, water priming caused significant upregulation of POX activity in roots and shoots of wheat seedlings. Shoots of PBW 175 showed upregulation of POX activity by more than 1.7 fold at both stages of growth whereas it increased by 1.5 fold in the shoots of PBW 621 at 6th DAG (Table 2). Amooaghaie *et al.* (2011) also observed enhanced POX activity in water primed alfalfa plants grown under normal conditions. Increased activities of SOD, CAT and POX pointed towards better scavenging of H_2O_2 as shown by Rouhi *et al.* (2012) in osmoprimed seeds of Berseem clover.

In comparison to non-treated seedlings, water-primed seedlings of both the cultivars showed an increase in APX activity at 4th DAG but remained unaffected at 6th DAG (Table 3). Induction of APX activity helps in detoxification of H_2O_2 and thus maintaining its lower level in growing seedlings. Ascorbate peroxidase activity was also upregulated by hydropriming in cucumber and rye seedlings (Huang *et al.,* 2006 and Ansari and Sharif-Zadeh, 2012). Hydropriming promoted seedling growth as well as antioxidant response by enhancing SOD, CAT, POX and APX activities therefore it can be said that improvement in germination characteristics of hydroprimed seeds could be the results of enhancement in the antioxidant profile of treated seeds (Ansari and Zadeh, 2012). In general, GR activity was significantly higher in PBW 621 seedlings as compared to PBW 175 (Table 3). Although, hydropriming caused a significant decrease in GR activity of both cultivars of wheat at 6th DAG but it increased in the shoots of PBW 175 at 4th DAG. However, water primed seedlings of two wheat cultivars did not vary significantly with respect to GR activity.

Hydropriming led to significant increase of polyphenol oxidase activity in seedlings of both wheat cultivars (Table 3). However, its activity was significantly higher in PBW 621 seedlings as compared to PBW 175 under control conditions. Roots of hydroprimed PBW 621 seedlings showed upregulation of PPO activity by more than 2.4 fold whereas it increased by more than 1.2 fold in PBW 175 (Table 3). Although, PPO activity remained unaffected in the shoots of water-primed PBW 175 seedlings but increased in PBW 621 shoots. Moosavi *et al.* (2009) also reported that seed hydropriming highly increased PPO activities in Amaranth genotypes.

Although water priming led to slight increase in malondialdehyde content in the roots of PBW 621 seedlings at 4th DAG but remained unaffected at 6th DAG. Hydropriming did not affect MDA content of PBW 175 seedlings but it declined significantly in the shoots of PBW 621 (Table 4). Malondialdehyde content decreased in osmoprimed sweet pepper and water

primed pinus seedlings (Guo *et al.,* 2012 and Siri *et al.,* 2013). In general, H_2O_2 contents were more in the roots and shoots of PBW 175 seedlings as compared to PBW 621 (Table 4). At 6th DAG, hydropriming decreased H_2O_2 content by 31 and 38% respectively in the roots and shoots of PBW 175 whereas it declined by 36 and 14% respectively in PBW 621 (Table 4). At lower concentrations, H_2O_2 might be acting as a signalling molecule, thus activating the antioxidant defense response in the seedlings which could be correlated with the upregulated activities of CAT, APX and POX.

Phenolic compounds constitute a part of antioxidant system. In general, total phenolic contents were more in the roots and shoots of PBW 175 seedlings as compared to PBW 621 (Table 5). At 6th day of seedling growth, hydropriming increased total phenolic content of roots and shoots of PBW 175 by 98 and 29% respectively whereas it declined significantly in PBW 621. It decreased by about 24 and 31% respectively in the roots and shoots of PBW 621 at 4th DAG (Table 5). Correlation between total phenols and PPO activity showed that this increased phenolic content in PBW 175 may be due to increased activity of enzymes involved in *de novo* synthesis of these compounds.

In comparison to 4th DAG, ascorbic acid content was less in roots and shoots at 6th DAG (Table 5). Ascorbate is an antioxidant that acts as a redox buffer and protects plasmalemma from oxidation (Pignocchi and Foyer, 2003). Present results depict that water priming increased the ascorbate content in the roots of wheat seedlings at 4th DAG thus, assuring the activation of ascorbate-glutathione pathway. Ascorbate content also increased in sweet pepper seedlings after osmopriming (Siri *et al.,* 2013). The accumulation of ascorbate and total phenols in hydroprimed wheat seedlings improved the defense mechanism of plants and thereby hydropriming could help in protection of the plant cells from reactive oxygen species. Although hydropriming led to slight decrease in proline content of PBW 175 seedlings at 4th DAG but at 6th DAG, it remained unaffected in both cultivar of wheat. However, it increased significantly in the shoots of water primed PBW 621 seedlings. Proline content also increased in water-primed alfalfa plants grown under salt stress and control conditions (Amooaghaie *et al.,* 2011).

Hydropriming augmented seedling growth and antioxidant response of wheat by enhancing enzymatic (SOD, CAT, POX and APX) and non-enzymatic antioxidants (ascorbate and total phenols) and lowering H_2O_2 content. Further, with the progress of seedling growth water priming did not affect MDA content of wheat seedlings thus indicating towards membrane integrity. Therefore it appears that hydropriming improved germination characteristics of

wheat seedlings by enhancing the antioxidant response.

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Table 1: Effect of hydropriming on seedling growth of wheat at 6th DAG

Tissue	Parameters	PBW 175			PBW 621		
		Control	Hydroprimed	LSD (5%)	Control	Hydroprimed	LSD (5%)
Roots	Length (cm)	13.1±0.9	13.5±1.1	NS	15.7±0.3	16.6 ± 0.5	NS
	Fresh weight (mg)	58.2±2.1	68.8±4.4[a]	7.8	84.2±8.1	99.1 ± 3.4 [a]	14.0
	Dry weight (mg)	4.6±0.4	4.7±1.2	NS	5.6 ± 0.6	6.4 ± 0.8	NS
	Moisture Content (%)	92.1±1.6	93.2±0.8	NS	93.3±0.4	93.5 ± 1.7	NS
Shoots	Length (cm)	13.2±0.6	13.5±0.7	NS	12.7±0.3	14.4 ± 0.3[a]	0.68
	Fresh weight (mg)	102.3±8.1	112.8±3.2	NS	120.8±11.7	126.1 ± 10.0	NS
	Dry weight (mg)	8.6±0.7	8.7±1.1	NS	9.2 ± 0.4	9.9 ± 0.8	NS
	Moisture Content (%)	91.2±1.8	92.2±1.4	NS	92.4 ± 0.2	92.2 ± 0.8	NS
Endosperms	Fresh weight (mg)	49.3±2.6	48.2±2.6	NS	33.5 ± 3.4	28.2 ± 4.0	NS
	Dry weight (mg)	12.3±1.4	12.1±0.5	NS	6.8 ± 0.4	5.5 ± 0.3[a]	0.80
	Moisture Content (%)	75.1±0.4	74.9±0.3	NS	79.1 ± 0.8	80.1± 0.2	NS

Values are mean±SD of 18 seedlings.
Least significant difference (LSD) at 5% probability level
[a]significant differences from respective controls

Table 2: Effect of hydropriming on activities of SOD, CAT and POX in wheat seedlings.

SOD (units/ min/ mg protein)							
Tissue	DAG	PBW 175		LSD (5%)	PBW 621		LSD (5%)
		Control	Hydroprimed		Control	Hydroprimed	A= 0.93
Roots	4	2.8±1.7	5.2±1.4[a]		3.8 ± 0.7	4.9 ± 0.2[a]	B=NS AB=1.32

	6	11.6±0.4	20.5±0.8[a]	A=1.5 B=1.5 AB=2.2	22.7 ± 0.9	21.6 ± 0.8[a]	
Shoots	4	2.6±2.7	5.8±0.1	A=NS B=0.95 AB=NS	3.9 ± 0.6	6.1 ± 0.1[a]	A= 1.2 B= 1.2 AB=NS
	6	3.1±1.2	6.7±0.3[b]		13.2 ± 0.8	14.4 ± 1.5[a,b]	

CAT (µmoles of H_2O_2 decomposed/ min/ mg protein)

Tissue	DAG	PBW 175			PBW 621		
		Control	Hydroprimed	LSD (5%)	Control	Hydroprimed	LSD (5%)
Roots	4	3.2±0.4	3.6±0.7	A=0.6 B=NS AB=NS	4.1 ± 0.7	3.6 ± 0.5	A=0.82 B= NS AB=NS
	6	2.0±0.2	2.6±0.6[a]		3.0 ± 0.8	2.0 ± 0.4[a]	
Shoots	4	3.8±1.2	3.6±0.9	A=1.6 B=1.6 AB=NS	9.5 ±1.2	11.5 ±1.4	A=NS B= NS AB=NS
	6	7.4±1.6[b]	4.4±1.2[a]		8.9 ±1.6	9.6 ± 1.0	

POX (change in absorbance/ min/ mg protein)

Tissue	DAG	PBW 175			PBW 621		
		Control	Hydroprimed	LSD (5%)	Control	Hydroprimed	LSD (5%)
Roots	4	0.97±0.8	1.2±0.4	A=1.03 B=NS AB=NS	0.1 ± 0.01	-[a]	A=0.06 B=0.06 AB=0.09
	6	2.6±0.4	2.4±1.2		0.2 ± 0.004	0.6 ± 0.09[a,b]	
Shoots	4	0.47±0.5	1.9±0.3[a]	A=0.7 B=0.69 AB=0.98	0.3 ± 0.09	0.2 ± 0.04	A=0.8 B=0.8 AB=1.08
	6	10.2±0.6[b]	17.5±0.7[a,b]		9.4 ± 0.32	14.6 ± 1.1[a,b]	

Values are mean±SD of data obtained from triplicate samples.
Least significant difference (LSD) at 5% probability level- A-Hydropriming, B-Days, AB-Interaction between hydropriming and days
[a]significant differences from respective controls
[b] significant differences from initial day (4th day)

Table 3: Effect of hydropriming on activities of APX, GR and PPO in wheat seedlings

APX (nmoles of monodehydroascorbate formed/min/mg protein)

Tissue	DAG	PBW 175			PBW 621		
		Control	Hydroprimed	LSD (5%)	Control	Hydroprimed	LSD (5%)

Roots	4	788.7±48.8	1110.2±92.7 [a]	A=72.5	880.2±49.2	1297.3 ± 95.8[a]	A= 72.8
	6	473.2±28.4 [b]	399.1±10.4[b]	B=72.5 AB=102.5	346.5±15.1 [b]	293.9 ± 12.1[b]	B= 72.8 AB=103.03
Shoots	4	381.4±78.2	480.7±65.4	A=NS B=NS	165.8 ± 65.0	477.2 ± 88.6[a]	A= 73.4 B= 73.4
	6	428.8±68.2	307.9±54.8	AB=126.5	241.3 ± 7.6[b]	192.6 ± 7.3[b]	AB=103.8

GR (nmoles of NADP formed/ min/ mg protein)

Tissue	DAG	PBW 175			PBW 621		
		Control	Hydroprimed	LSD (5%)	Control	Hydroprimed	LSD (5%)
Roots	4	7.5±0.6	7.2±1.3	A=1.09 B=1.09 AB=1.54	10.8 ± 0.9	11.2 ± 1.1	A= 5.7 B=5.7 AB=8.18
	6	31.1±0.7	27.5±0.3[a,b]		56.4 ± 7.1[b]	37.0 ± 5.3[a,b]	
Shoots	4	9.7±1.2	12.5±0.6 [a]	A=1.04 B=1.04 AB=2.03	16.6 ± 1.4	20.4 ± 1.0	A= 4.5 B= NS AB=6.5
	6	38±1.2	36.3±0.4[a,b]		77.0 ± 5.3	71.0 ± 4.0[a]	

PPO units /min/g FW (units/min/ mg protein)

Tissue	DAG	PBW 175			PBW 621		
		Control	Hydroprimed	LSD (5%)	Control	Hydroprimed	LSD (5%)
Roots	4	5.7 ±0.7	10.5 ±0.5[a]	A=1.1 B=1.1 AB=1.5	15.9±1.8	38.4±3.4[a]	A=2.4 B=2.4 AB=3.4
	6	4.1±0.6	4.8 ±1.3		5.6±0.7[b]	14.5±0.8[a,b]	
Shoots	4	3.6±0.7	5.3±0.4	A=NS B=0.6 AB=NS	8.85±0.5	12.51±0.9[a]	A=1.2 B=1.2 AB=NS
	6	4.1±0.2	4.8±0.5		8.4±1.9	10.2±0.5[a,b]	

Values are mean±SD of data obtained from triplicate samples.
Least significant difference (LSD) at 5% probability level- A-Hydropriming, B-Days, AB-Interaction between hydropriming and days
[a]significant differences from respective controls
[b] significant differences from initial day (4th day)

Table 4: Effect of hydropriming on MDA and H_2O_2 contents in wheat seedlings

MDA (nmoles/FW)

Tissue	DAG	PBW 175			PBW 621		
		Control	Hydroprimed	LSD (5%)	Control	Hydroprimed	LSD (5%)
Roots	4	4.8±0.3	5.0±0.84	A=1.1 B=NS AB=NS	8.6 ± 0.3	10.1 ± 0.8[a]	A= 0.74 B=NS AB =
	6	10.3±1.2	11.3±0.9		5.4 ± 0.5	5.9 ± 0.9	

							1.05
Shoots	4	5.7±0.6	6.0±1.1	A=1.15 B=NS AB=NS	8.1 ± 0.6	6.5 ± 0.6 [a]	A=0.69
	6	11.6±0.9	12.3±0.8		7.5 ± 0.3	5.3 ± 0.5[a,b]	B=0.69 AB=NS
H_2O_2 (µmoles/FW)							
Tissue	DAG	PBW 175			PBW 621		
		Control	Hydroprimed	LSD (5%)	Control	Hydroprimed	LSD (5%)
Roots	4	310±16.4	321±39.06	A=29.3 B=NS AB=41.4	53.7 ±2.9	74.9 ± 3.4[a]	A= 8.13 B=NS AB=11.5
	6	220.9±10.1	151.5±6.7[a]		61.0 ±5.8	38.5 ± 10.4[a]	
Shoots	4	938.7±9.9	688±24.9[a]	A=19.3 B=19.3 AB=27.3	100.6 ±7.0	105.3 ± 9.9	A= 17.3 B=NS AB= NS
	6	449±9.3[b]	278±6.2[a,b]		143.7±18.2	122.7 ± 16.2[a]	

Values are mean±SD of data obtained from triplicate samples.
Least significant difference (LSD) at 5% probability level- A-Hydropriming, B-Days, AB-Interaction between hydropriming and days
[a]significant differences from respective controls
[b] significant differences from initial day (4th day)

Table 5: Effect of hydropriming on contents of total phenols, ascorbic acid and proline in wheat seedlings.

Total phenols (µg / g FW)							
Tissue	DAG	PBW 175			PBW 621		
		Control	Hydroprimed	LSD (5%)	Control	Hydroprimed	LSD (5%)
Roots	4	218±46.2	537.8±32.9[a]	A=65.03 B=65.3 AB=NS	168.8 ±5.5	128.1 ± 2.0[a]	A =6.4 B=6.4 AB=9.15
	6	386±66.5[b]	771±43.6[a,b]		182.5 ±6.3[b]	171.0 ± 4.2[a,b]	
Shoots	4	678.4±54.8	516±82.1[a]	A=79.09 B=NS AB=111.8	287.5±22.0	197.5 ± 25.2[a]	A= 24.2 B=NS AB=34.2
	6	815.9±18.3	1055.9±63.4[a]		194.3±11.5	240.0 ± 8.4[a]	
Ascorbic acid (nmoles / g FW)							
Tissue	DAG	PBW 175			PBW621		
		Control	Hydroprimed	LSD (5%)	Control	Hydroprimed	LSD (5%)
Roots	4	20.16±6.6	42.2±6.6[a]	A=7.96 B=7.95 AB=11.2	30.34 ± 7.7	54.2 ± 2.6[a]	A=6.05 B=6.05 AB=8.6
	6	19.91±3.3	24.2±6.7[a,b]		15.7 ± 3.8[b]	13.2 ± 2.1[b]	
Shoots	4	53.6±5.4	56.6±5.4	A=NS B=NS AB=NS	67.7 ± 1.4	61.7 ± 1.9[a]	A=2.3 B=2.3 AB=3.2
	6	41.8±18.2	45.9±12.6		45.4 ± 1.8[b]	46.5 ± 1.8[b]	
Proline (nmoles / g FW)							

Tissue	DAG	PBW 175			PBW 621		
		Control	Hydroprimed	LSD (5%)	Control	Hydroprimed	LSD (5%)
Roots	4	102.5 ±5.7	104 ±5.1	A=5.78	52.8 ± 8.9	49.5 ± 5.6	A= 7.4
	6	81.2 ±3.2	73.4 ±1.3[a]	B=NS AB=NS	25.7 ± 2.5	26.7 ± 3.1	B= NS AB = NS
Shoots	4	98.9 ±3.9	104 ±14.8	A=10.3	43.7 ± 6.7	41.1 ± 2.0	A = 5.8
	6	87.5 ±1.4	80.2 ±1.1	B=NS AB=NS	25.1 ± 2.5	30.9 ± 5.0[a]	B=NS AB=NS

Values are mean±SD of data obtained from triplicate samples.
Least significant difference (LSD) at 5% probability level- A-Hydropriming, B-Days, AB-Interaction between hydropriming and days
[a]significant differences from respective controls
[b]significant differences from initial day (4th day)