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Effect of Seed Priming on the Activity of Antioxidant Enzymes and Changes of Biochemicals of Rice under Timely and Late Sown Conditions

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In the present investigation rice genotype HUR 105 was selected to see the effect of seed priming on the activity of antioxidant enzymes and changes of biochemicalsof rice under timely and late sown conditions. Priming of seeds has been done with distilled water (hydro), $Mg(NO_3)_2$ salt (halo), kinetin and salicylic acid (hormonal) and activities of SOD and nitrate reductase, ascorbate peroxidise proline and protein contents were studied at different study periods with respect to two sowing conditions, representing timely and late sowing of rice crop. Priming of seeds was found to improve antioxidant enzymes activity and other biochemical parameters over non-primed (control) sets in timely as well as in late sown conditions. The work therefore presents the late sown stress ameliorating role of seed priming in rice genotype.

Introduction

Rice is a most important food crop, nearly one-half of the world population, including all East and Southeast-Asia completely dependent upon rice. The world population is increasing continuously day by day while Agricultural land is decreasing due to rapid urbanisation. Providing food to growing population is became a challenge in future for agricultural scientist. The sowing time is one of the most important yield deciding factor of rice. Early sowing of rice crop got more time for vegetative growth and development that lead to high yield and delay in sowing date causes significant reduction in growth and yield (Abou Khalifa 1996; Sharief *et al.*, 2000; Abou Khalifa 2005).Seed holds a key position in plant science because it is the main source of food supply. Further a hand full of seed is always easy to handle rather than 100 plants growing in the field. Hence seed research always attracts plant scientists. During germination the seed of any crop suffers from many kind of stresses may be biotic or abiotic because the soil itself is a harbour of many kind of stresses. Germination status decides the fate of the growing crop and finally the yield. Seed priming is a technique that improves seed germination, germination speed, seedling vigor, root length, seedling dry weight, photosynthetic efficiency and many

other plant growth traits. Other than this it also improve biochemical status of plant by improving α- amylase activity and soluble sugar contents during seed germination, SOD, nitrate reductase activity (Anaytullah and Bose 2007; Sananda and Bose, 2012; Kumar *et al.,* 2016). Seed priming enhanced seed germination and seedling vigor under adverse conditions in diverse species, such as soybean, maize, spinach, mustard, pepper and wheat and rice etc. (Bose *et al*.2007, Iqbal and Ashraf 2007, Farooq *et al.,* 2008, Krishnottar *et al.,* 2009, Korkmaz and Korkmaz, 2009, Zhuo *et al.,* 2009, Chen *et al.,* 2011, Srivastava and Bose2012). Pre-soaking and priming improved seedling establishment in flooded soil, enhanced the capacity to scavenge reactive oxygen species in seeds by increasing CAT and SOD activities, and enhanced carbohydrate mobilization. Seed priming treatment exhibit increased germination rate, greater germination uniformity, and greater total germination percentage (Sananda and Bose, 2012).

Materials and Methods

Rice variety HUR 105 was selected for experimental purpose. Healthy and bold seeds were surface sterilized by keeping them in 0.01% $HgCl_2$ (Mercuric chloride) solution for 5 minutes and then washed with distilled water for 4-5 times. Three types of priming treatments were used for this experiment included hydropriming(T_1) (using distilled water), halopriming and hormonal priming using 5 mM/ppm concentration of $Mg(NO_3)_2$ (T_2), Kinetin (T_3) and 0.75mMconcentration of salicylic acid(T_4) respectively for 18 h. A set containing non primed control seeds (T_0) was used during experimentation. After 18 h these seeds were air dried under fan to bring back to its initial weight further the seeds were kept in paper bags and used within one month. All parameters were taken at 20, 40, 60 and 80 DAT (Days after transplanting). Experiments were conducted in Factorial Randomized Block Design (RBD) during 2015 and 2016 at Agricultural Research Farm, Banaras Hindu University, Varanasi.

Estimation of superoxide dismutase (SOD) activity (U g^{-1}FWmin^{-1})

Superoxide dismutase activity was assayed by the method of Dhindsa *et al.,* (1981).

Preparation of enzyme extract

Enzyme extract for SOD was prepared by first freezing the weighed amount of leaf samples (1.0g) in ice basket to prevent proteolytic activity. The sample was grounded with 10mL of extraction buffer (0.1M phosphate buffer, pH 7.5 containing (0.5mM EDTA). The grinded sample was centrifuged in cooling centrifuge machine at 10000*g* for 10 minutes, after centrifugation supernatant was collected and this supernatant was used as enzyme source.

Assay of SOD activity

3mL of the reaction mixture containing 0.1mL of 1.5M sodium carbonate, 0.2mL of 200mM methionine, 0.1mL of 2.25mM NBT, 0.1mL of 3mM EDTA, 1.5mL of 100mM potassium phosphate buffer, 1mL of distilled water and 0.1mL of enzyme extract were taken in test tubes in duplicate from each enzyme sample. Two tubes without enzyme extract were taken as control. The reaction was started by adding 0.1mL riboflavin (60µM) and placing the tubes below a light source of two 15W florescent lamps for 15 minutes. Reaction was stopped by switching off the light and covering the tubes by black cloth. Tubes without enzyme extract developed maximum colour. A non-irradiated complete mixture that did not develop colour served as blank. Absorbance was recorded at 560nm in spectrophotometer.

$$\text{Enzyme Unit (EU)} = \frac{\text{Enzyme}^{(-)}{}_{\text{light}} - (\text{Enzyme}^{(+)}{}_{\text{light}} - \text{Enzyme}^{(+)}{}_{\text{dark}})}{\text{Enzyme}^{(-)}{}_{\text{light}} / 2}$$

Where,

(-) = Without enzyme
(+) = With enzyme

The EU was expressed on per g fresh weight basis.

Determination of nitrate reductase activity (NRA)

In vivo activities of enzyme nitrate reductase of green leaves of rice were determined by the method of Srivastava (1974).

Estimation of proline Content (mg g^{-1}DW)

Proline content was determined by the method of Bates *et al.*, (1973).

Procedure

Leaf sample (0.5g) was homogenized in 5mL of sulphosalicylic acid (3%). It was centrifuged at 6000g for 10 minutes and supernatant was saved. Residue was again extracted twice with 5mL of 3 per cent aqueous sulphosalicylic acid. All the supernatant fractions were pooled and final volume was made to 15mL. 2mL of this extract was taken in the test tube and 2mL ninhydrin reagent and 2mL glacial acetic acid were added. The reaction mixture was put in boiling water bath for 30 minutes. After cooling the reaction mixture was added with 5mL toluene. Then solution mixture was shaken vigorously and toluene fraction was separated by separating funnel. The absorbance of toluene fraction was read at 520nm with the help of spectrophotometer against toluene blank. Concentration of proline in the plant samples was estimated by referring to a standard curve of proline.

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Estimation of ascorbate peroxidase (APX) activity (U g^{-1}FW min^{-1})

Enzyme extraction

100mg of leaves was homogenised in phosphate buffer in a pre chilled pestle and mortar. Homogenate was strained through two folds of muslin cloth and centrifuged at 16000 rpm for 20 min at 4^0C and the supernatant was used as enzyme extract.

Enzyme assay

Ascorbate peroxidase activity was assayed according to the method as proposed by Nakano and Asada (1981). It was based on the decrease in absorbance at 290nm as ascorbate was oxidized with time. The reaction mixture for the peroxidase contained 0.1mL of 100mM potassium phosphate buffer (pH 7.5), 1.5mM H_2O_2, 0.40mL of 3mM ascorbic acid, 0.1mL of 3mM EDTA and 1mL of enzyme extract in a total volume of 3mL. In blank, ascorbic acid was not added. The reaction was started by adding the enzyme and H_2O_2 simultaneously and the decrease in absorbance was recorded 30 seconds after this addition at 290nm. The ascorbate peroxidase activity was estimated by referring to the standard curve.

Estimation of protein

Protein content of leaf samples was measured by using the method of Lowry *et al.*, (1951).

Results and Discussion

Table 1 represents data regarding Superoxide dismutase (SOD) analysed at 20,40,60 and 80 DAT. Maximum SOD activity was noted at 40 DAT, after that reduction in SOD activity was noted. Primed sets showed higher SOD

activity over non primed sets and maximum SOD activity was found in kinetin primed set T_3. The increment in SOD activity was also noted under late sown condition. Same result was obtained in both consecutive years.

Data (Table 2.) regarding nitrate reductase activity(NR) of rice showed that NR activity was found to increase till 40 DAT, after that activity of NR decreases. Result showed that in late sown condition NR activity was found to decrease compared to timely sown condition. However primed sets showed higher NR activity over non primed control set and maximum NR activity was noted in set T_2.. The trend was found similar for both the studied years.

Table 3 represents data regarding proline content in rice leaves, obtained from primed and non primed sets The proline content was found to increase with age of plant and maximum proline content was noted in T_4 set. It was also found that primed sets showed higher proline content over non primed control set under both sowing conditions. Same result was observed in both the consecutive years. Table 4depicted the effect of seed priming on ascorbate peroxidase activity of leaves, under timely and late sown conditions at 20, 40, 60 and 80 DAT. Result showed that ascorbate peroxidase activity was found to increase with increasing plant age under both sowing conditions. Primed sets showed higher ascorbate peroxidase activity in comparison to non primed control set. The maximum ascorbate peroxidase activity was noted in T_4 set. It was also noted that activity of ascorbate peroxidise was found lesser in late sown condition. Similar result was obtained in both the consecutive years.

Table.1 Effect of hydro, halo and hormonal seed priming on superoxide dismutase activity (SOD) of rice leaf (Var. HUR 105)

Year	Sowing Time	Treatments***	SOD activity (unit×10^2 g-^{1}FW min^{-1})			
			20 DAT	**40 DAT**	**60 DAT**	**80 DAT**
2015	**I***	T_0	24.08±0.27^{j}	40.45±0.50^{e}	53.18±0.59^{g}	77.25±0.81^{f}
		T_1	25.71±0.36^{h,i}	42.48±0.58^{c,d}	56.13±0.31^{f}	79.83±0.66^{e}
		T_2	27.77±0.33^{f}	45.18±0.63^{a,b}	59.32±0.63^{e}	80.70±0.18^{d,e}
		T_3	29.48±0.58^{a,b,c,d}	45.55±0.52^{a,b}	61.53±0.54^{a,b,c}	82.81±0.38^{b,c}
		T_4	28.42±0.45^{e,f}	44.91±0.38^{e}	60.77±0.40^{b,c,d}	80.76±0.31^{d,e}
	II**	T_0	24.54±0.10^{j}	40.95±0.41^{e}	53.62±0.52^{g}	77.58±0.67^{f}
		T_1	26.42±0.29^{g,h}	42.98±0.41^{c}	56.67±0.11^{f}	80.69±0.12^{d,e}
		T_2	28.32±0.26^{e,f}	45.55±0.54^{a,b}	59.78±0.62^{d,e}	81.09±0.37^{d,e}
		T_3	29.85±0.32^{a,b,c}	45.85±0.37^{a,b}	61.99±0.64^{a,b}	83.35±0.28^{a,b}
		T_4	29.05±0.35^{b,c,d,e}	45.18±0.43^{a,b}	61.19±0.28^{b,c}	81.30±0.33^{d}
2016	**I***	T_0	24.59±0.12^{j}	40.92±0.28^{e}	53.65±0.55^{g}	77.82±0.61^{f}
		T_1	26.36±0.30^{g,h}	42.92±0.43^{c}	56.45±0.36^{f}	80.51±0.15^{d,e}
		T_2	28.47±0.35^{e,f}	45.65±0.57^{a,b}	59.69±0.61^{d,e}	81.36±0.34^{d}
		T_3	29.95±0.36^{a,b}	46.01±0.37^{a,b}	61.98±0.40^{a,b}	83.24±0.25^{a,b}
		T_4	28.94±0.30^{c,d,e}	45.37±0.31^{a,b}	61.22±0.34^{b,c}	81.33±0.39^{d}
	II**	T_0	24.93±0.34^{i,j}	41.22±0.39^{d,e}	54.05±0.36^{g}	77.83±0.38^{f}
		T_1	26.71±0.04^{g}	43.26±0.24^{c}	56.95±0.31^{f}	80.82±0.42^{d,e}
		T_2	28.65±0.06^{d,e,f}	46.10±0.66^{a,b}	60.37±0.28^{c,d,e}	81.72±0.03^{c,d}
		T_3	30.19±0.28^{a}	46.42±0.33^{a}	62.68±0.14^{a}	84.26±0.71^{a}
		T_4	29.25±0.21^{a,b,c,d}	45.37±0.30^{a,b}	61.58±0.08^{a,b,c}	81.60±0.10^{c,d}

Data presented are means from three replicates with standard errors. Within each treatment, different letters indicate significant differences by Duncan's multiple range test at P<0.05

***Timely sown**

****Late sown**

***** T_0: Non primed control; T_1: Hydro primed; T_2: $Mg(NO_3)_2$; T_3: Kinetin and T_4: Salicylic acid

Table.2 Effect of hydro, halo and hormonal seed priming on nitrate reductase activity (NR) of rice leaf (Var. HUR 105)

Year	Sowing Time	Treatments***	NR activity (n mol NO_2^- h^{-1} g^{-1} FW)			
			20 DAT	40 DAT	60 DAT	80 DAT
2015	I*	T_0	360.83±3.05^{j,k,i}	1020.00±1.26^{h}	982.57±3.07^{f}	743.60±0.67^{h}
		T_1	374.33±1.99^{g,h,i}	1174.57±1.04^{c}	1082.40±2.34^{c}	817.50±1.57^{c,d,e}
		T_2	417.37±2.00^{a}	1288.57±4.25^{a}	1175.70±2.66^{a}	915.60±1.79^{a}
		T_3	385.47±2.31^{e,f}	1162.30±3.55^{d,e,f}	1076.00±3.18^{c,d,e}	822.43±1.55^{c}
		T_4	414.87±1.17^{a}	1172.13±2.32^{c,d}	1078.83±1.^{72c,d}	823.50±2.57^{c}
	II**	T_0	356.33±2.78^{k,l}	1009.47±2.32^{i,j}	976.77±2.58^{f,g}	738.70±1.78^{h,i}
		T_1	369.53±2.07^{h,I,j}	1163.23±3.94^{d,e,f}	1078.43±1.65^{c,d}	808.03±2.10^{f,g}
		T_2	408.77±1.42^{a,b}	1285.30±3.76^{a,b}	1170.30±3.29^{a,b}	909.50±2.31^{a}
		T_3	381.50±2.17^{f,h}	1157.87±3.95^{e,f,g}	1071.53±2.78^{d,e}	816.30±2.93^{c,d,e,f}
		T_4	403.77±1.15^{b,c}	1168.53±1.88^{c,d}	1075.57±2.09^{c,d,e}	820.73±2.89^{c,d}
2016	I*	T_0	356.37±2.19^{j,k}	1011.70±3.32^{d}	979.77±3.00^{g}	739.23±1.17^{i}
		T_1	370.70±1.19^{g,h,i}	1167.90±2.92^{b,c}	1078.77±2.30^{c,d,e}	809.83±2.95^{f,g,h}
		T_2	407.57±1.97^{a,b}	1284.63±2.32^{b,c}	1169.93±2.91^{a,b}	906.53±2.62^{b}
		T_3	378.47±3.99^{f,g}	1158.97±5.14^{b,c}	1072.13±3.41^{d,e,f}	816.50±1.05^{e,f}
		T_4	406.80±3.00^{a,b,c}	1167.83±2.28^{b}	1075.13±0.86^{d,e,f}	823.10±2.96^{d,e}
	II**	T_0	361.87±2.00^{i,j}	1018.57±1.50^{d}	981.17±3.77^{g}	743.47±1.44^{i}
		T_1	375.77±2.16^{g,h}	1168.50±3.57^{b,c}	1083.57±1.55^{c}	816.17±1.16^{e,f,g}
		T_2	416.40±0.85^{a}	1289.43±4.12^{a}	1174.87±3.32^{a}	917.80±1.92^{a}
		T_3	387.13±2.94^{e,f}	1165.10±5.18^{b,c}	1076.53±2.34^{c,d,e,f}	821.73±1.91^{d,e}
		T_4	411.90±0.26^{a}	1173.13±1.69^{b,c}	1080.23±1.85^{c,d}	828.33±1.65^{d}

Note: Detail of the conditions has given in table 1

Table.3 Effect of hydro, halo and hormonal seed priming on proline content of rice leaf (Var. HUR 105)

Year	Sowing Time	Treatments***	Proline content (µg g^{-1} DW)			
			20 DAT	**40 DAT**	**60 DAT**	**80 DAT**
2015	I*	T_0	2.12±0.22^{d,e,f}	9.98±0.30^{f,g}	18.33±0.29^{g,h}	20.18±0.17^{h,i}
		T_1	2.18±0.21^{d,e,f}	10.45±0.00^{e,f}	18.84±0.28^{f,g}	20.90±0.35^{g,h}
		T_2	2.81±0.03^{a,b,c,d}	12.88±0.18^{b,c,d}	19.90±0.13^{c,d,e}	23.96±0.32^{b,c}
		T_3	2.74±0.06^{a,b,c,d}	12.99±0.18^{b,c,d}	21.20±0.36^{b}	23.08±0.34^{d,e,f}
		T_4	2.92±0.11^{a,b,c}	14.92±0.28^{a}	22.96±0.25^{a}	25.82±0.37^{a}
	II**	T_0	1.69±0.34^{f}	9.62±0.03^{g}	17.71±0.43^{h}	19.82±0.38^{i}
		T_1	1.91±0.20^{e,f}	10.22±0.24^{e,f,g}	18.31±0.13^{g,h}	20.12±0.29^{h,i}
		T_2	2.43±0.03^{b,c,d,e}	12.37±0.13^{d}	19.75±0.25^{d,e}	23.55±0.01^{b,c,d,e}
		T_3	2.28±0.03^{c,d,e,f}	12.45±0.06^{c,d}	20.65±0.40^{b,c}	22.41±0.07^{f}
		T_4	2.91±0.42^{a,b,c}	14.42±0.07^{a}	22.32±0.03^{a}	25.42±0.03^{a}
2016	I*	T_0	2.24±0.11^{c,d,e,f}	10.12±0.16^{f,g}	18.28±0.03^{g,h}	20.47±0.08^{g,h,i}
		T_1	2.73±0.09^{a,b,c,d}	10.75±0.05^{e}	19.21±0.29^{e,f}	21.09±0.28^{g}
		T_2	2.78±0.24^{a,b,c,d}	13.11±0.29^{b,c}	20.02±0.29^{c,d}	24.15±0.26^{b}
		T_3	2.88±0.29^{a,b,c}	13.22±0.33^{b}	21.41±0.03^{b}	23.14±0.32^{c,d,e,f}
		T_4	3.36±0.24^{a}	15.02±0.34^{a}	23.12±0.35^{a}	25.99±0.37^{a}
	II**	T_0	2.09±0.28^{d,e,f}	9.79±0.26^{f,g}	18.09±0.34^{g,h}	20.04±0.40^{i}
		T_1	2.45±0.09^{b,c,d,e}	10.45±0.35^{e,f}	18.72±0.07^{f,g}	20.29±0.42^{g,h,i}
		T_2	2.73±0.08^{a,b,c,d}	12.63±0.08^{b,c,d}	19.95±0.36^{c,d,e}	23.80±0.03^{b,c,d}
		T_3	2.69±0.03^{a,b,c,d}	12.98±0.18^{b,c,d}	20.85±0.27^{b}	22.76±0.05^{e,f}
		T_4	3.05±0.25^{a,b}	14.72±0.09^{a}	22.72±0.06^{a}	25.84±0.05^{a}

Note: Detail of the conditions has given in table 1.

Table.4 Effect of hydro, halo and hormonal seed priming on ascorbate peroxidase activity of rice leaf (Var. HUR 105)

Year	Sowing Time	Treatments***	Ascorbate peroxidase activity (U g^{-1}FW min^{-1})			
			20 DAT	**40 DAT**	**60 DAT**	**80 DAT**
2015	**I***	T_0	1.58±0.04^{i}	2.59±0.02^{i}	2.69±0.02^{k,l}	2.84±0.02^{k,l}
		T_1	1.70±0.03^{g}	2.73±0.03^{g}	2.83±0.02^{i}	2.98±0.02^{j}
		T_2	2.54±0.03^{d}	3.32±0.03^{c,d,e}	3.52±0.02^{d,e}	3.86±0.01^{b,c,d,e}
		T_3	2.53±0.03^{d}	3.28±0.05^{e}	3.50±0.02^{d,e}	3.78±0.00^{e,f,g}
		T_4	2.69±0.04^{a,b}	3.37±0.04^{b,c,d}	3.56±0.01^{c,d}	3.89±0.05^{b,c}
	II**	T_0	1.46±0.02^{j}	2.49±0.01^{j}	2.65±0.02^{l}	2.77±0.01^{l}
		T_1	1.62±0.04^{h,i}	2.64±0.02^{h,i}	2.73±0.03^{j,k}	2.81±0.03^{k,l}
		T_2	2.43±0.02^{e}	3.17±0.04^{f}	3.36±0.05^{f}	3.67±0.04^{h}
		T_3	2.40±0.03^{e}	3.11±0.02^{f}	3.28±0.02^{g}	3.62±0.02^{h}
		T_4	2.60±0.03^{c,d}	3.28±0.03^{d,e}	3.48±0.02^{e}	3.80±0.03^{d,e,f}
2016	**I***	T_0	1.68±0.02^{g,h}	2.69±0.03^{g,h}	2.76±0.01^{i,j,k}	2.92±0.02^{j,k}
		T_1	1.79±0.02^{f}	2.78±0.03^{g}	2.93±0.01^{h}	3.19±0.03^{i}
		T_2	2.66±0.02^{b,c}	3.41±0.02^{a,b}	3.64±0.02^{a,b}	3.95±0.02^{a,b}
		T_3	2.58±0.02^{c,d}	3.37±0.02^{b,c}	3.61±0.03^{b,c}	3.82±0.01^{c,d,e,f}
		T_4	2.77±0.02^{a}	3.48±0.03^{a}	3.70±0.02^{a}	4.04±0.11^{a}
	II**	T_0	1.59±0.02^{i}	2.59±0.02^{i}	2.75±0.02^{i,j,k}	2.84±0.01^{k,l}
		T_1	1.71±0.03^{f,g}	2.76±0.01^{g}	2.80±0.03^{i,j}	2.90±0.03^{j,k}
		T_2	2.51±0.02^{e}	3.31±0.03^{c,d,e}	3.49±0.05^{d,e}	3.76±0.03^{f,g,h}
		T_3	2.56±0.00^{d}	3.27±0.03^{e}	3.38±0.02^{f}	3.72±0.04^{g,h}
		T_4	2.68±0.04^{b}	3.41±0.04^{a,b}	3.60±0.03^{b,c}	3.88±0.03^{b,c,d}

Note: Detail of the conditions has given in table 1.

Table.5 Effect of hydro, halo and hormonal seed priming on protein content of rice leaf (Var. HUR 105)

Year	Sowing Time	Treatments***	Protein content (mg g^{-1} FW)			
			20 DAT	**40 DAT**	**60 DAT**	**80 DAT**
2015	**I***	T_0	4.35±0.20^{h,i}	9.85±0.26^{def}	9.30±0.29^{f,g,h}	7.00±0.17^{c,d}
		T_1	4.75±0.20^{f,g}	10.35±0.20^{c,d,e}	9.75±0.20^{d,e,f,g,h}	7.45±0.14^{b,c}
		T_2	5.23±0.13^{c,d,e}	11.40±0.35^{a,b}	10.79±0.51^{a,b,c,d}	8.60±0.23^{a}
		T_3	5.85±0.20^{a}	11.95±0.38^{a}	11.53±0.36^{a}	8.60±0.17^{a}
		T_4	4.93±0.13^{e,f}	10.25±0.20^{d,e,f}	9.88±0.22^{c,d,e,f,g,h}	7.15±0.09^{c,d}
	II**	T_0	4.38±0.07^{h,i}	9.80±0.12^{d,e,f}	9.25±0.09^{g,h}	7.05±0.14^{c,d}
		T_1	4.70±0.00^{f,g,h}	10.20±0.17^{d,e,f}	9.63±0.04^{e,f,g,h}	7.50±0.12^{b,c}
		T_2	5.33±0.10^{b,c,d}	11.70±0.12^{a,b}	10.45±0.08^{a,b,c,d,e}	8.25±0.26^{a}
		T_3	5.90±0.06^{a}	11.85±0.09^{a}	11.20±0.06^{a,b}	8.55±0.32^{a}
		T_4	4.95±0.03^{e,f}	10.50±0.23^{c,d}	9.88±0.18^{c,d,e,f,g}	7.50±0.23^{b,c}
2016	**I***	T_0	3.80±0.06^{k}	9.60±0.29^{e,f}	9.08±0.33^{g,h}	6.90±0.12^{c,d}
		T_1	4.18±0.10^{i,j}	9.95±0.32^{d,e,f}	7.20±1.04^{i}	7.25±0.09^{c,d}
		T_2	4.93±0.13^{e,f}	11.05±0.32^{b,c}	10.48±0.51^{a,b,c,d,e}	8.30±0.29^{a}
		T_3	5.50±0.12^{b,c}	11.50±0.46^{a,b}	11.25±0.38^{a,b}	8.35±0.14^{a}
		T_4	4.50±0.12^{g,h,i}	9.95±0.20^{d,e,f}	9.75±0.03^{d,e,f,g,h}	7.15±0.09^{c,d}
	II**	T_0	3.98±0.07^{j,k}	9.50±0.00^{f}	9.00±0.12^{h}	6.65±0.03^{d}
		T_1	4.50±0.06^{g,h,i}	9.95±0.20^{d,e,f}	9.45±0.03^{f,g,h}	7.25±0.09^{c,d}
		T_2	5.05±0.09^{d,e,f}	11.50±0.12^{a,b}	10.20±0.12^{b,c,d,e,f}	8.00±0.23^{a,b}
		T_3	5.62±0.05^{a,b}	11.70±0.06^{a,b}	10.90±0.00^{a,b,c}	8.55±0.20^{a}
		T_4	4.80±0.00^{f,g}	10.30±0.23^{d,e,f}	9.75±0.15^{d,e,f,g,h}	7.20±0.23^{c,d}

Note: Detail of the conditions has given in table 1.

Table 5 represents protein content of rice leaves, obtained from primed and non primed sets at 20, 40, 60 and 80 DAT. Data showed that primed sets have more protein content over non primed control sets under both sowing conditions and maximum protein content was noted in T_3. It was also noted that protein content was found to increase in late sown condition. Same result was observed in both the consecutive years.

Antioxidant enzymes and metabolites increase under various environmental stress, with their comparatively higher activity in stress-tolerant cultivars, suggesting that higher antioxidant activity imparts tolerance (Polle 1997, Sairam and Saxena 2000). SOD is an enzyme that constitutes the first line of defence against ROS and it has already been established that seed priming with nitrate salts of Mg and K has shown an increment in SOD activity in wheat (Siddique *et al.,* 2015). It may be due to the fact that, nitrate acts as a signalling molecule which triggers a number of changes in metabolic pathway (Bose and Pandey 2003, Magalhes *et al.,* 2002). In the present study, late sowing of rice influence with various types of abiotic stresses cause accumulation of H_2O_2. It has been suggested that the accumulation of H_2O_2 levels caused by various environmental stresses would result in the combined activity of catalase and ascorbate peroxidase in order to protect plant cells. (Sofo *et al.,* 2015).Bose et al.(2016) reported that rice seed priming with nitrate salts significantly improved SOD and NR activity in leaves under deferent sowing conditions. Pre sowing and soaking treatment in *Brassica juncea* seed with magnesium salts significantly induce growth, nitrate reductase activity, total protein content and yield (Bose and Mishra, 1999).

From the above study, we concluded the significant role of seed priming in rice seed which could mitigate the deleterious affect of abiotic stress by increasing the antioxidant activity and biochemical contents under late sown and timely sown conditions.

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