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PACLOBUTRAZOL PREVENTS YIELD LOSSES IN MUNGBEAN (*Vigna radiata* L.) BY ESCALATING ANTIOXIDANT DEFENSE MECHANISMS UNDER FLASH FLOODING STRESS

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Abstract: *Mungbean (Vigna radiata L.) is a flooding sensitive protein yielding leguminous crop. Present investigations were carried out with two mungbean genotypes, viz., HUM-16 (flooding tolerant) and HUM-12 (flooding susceptible), to understand the mitigative role of paclobutrazol under flash flooding stress. Eight seeds were sown in plastic pots containing 7 kg pulverized soil. Paclobutrazol treatments were given as pre-seed soaking treatment as well as foliar spray (at pre-flowering stage) @ 10ppm and 25ppm along with its suitable combinations. Flash flooding stress was imposed at pre-flowering stage for 7 days subsequently excess water was drained out. Plant samples were collected and immediately dipped in liquid nitrogen container wrapped with aluminum foil. After 24 hours, the samples were withdrawn from liquid nitrogen and stored in deep freezer (-80°C) till further analysis. Observations were recorded viz., protein content, hydrogen peroxide (H_2O_2), catalase (CAT), polyphenol oxidase (PPO) and superoxide dismutase (SOD) including yield parameters i.e., number of pods plant^{-1} and seed yield plant^{-1}. Paclobutrazol both pre-soaking and foliar spray significantly enhanced aforementioned parameters in both tolerant and susceptible genotypes in response to flooding stress, though, the values were quite higher in flooding tolerant genotype (HUM-16) than the susceptible (HUM-12) one. However, the level of H_2O_2 was fairly higher in susceptible genotype. Our findings suggest that paclobutrazol treatments in tolerant mungbean genotype provides a greater tolerance potential to minimize the yield losses through enhancing protein content, CAT, PPO and SOD enzyme activities for scavenging H_2O_2 as compared to susceptible genotype under flash flooding stress.*

Key words*: Mungbean, flooding stress, paclobutrazol, protein, antioxidant enzymes, seed yield.*

Introduction: Pulses are the basic ingredient in the diets of a vast majority of the Indian population, as they provide a perfect mix of vegetarian protein component of high biological value when supplemented with cereals. Mungbean (*Vigna radiata* L.) is a staple legume in many diets around the world. The seeds are highly nutritious with protein 23–25%, carbohydrate, minerals and vitamins. Mungbean is a cheap source of dietary protein for the poor, with high levels of folate and iron compared with many other legumes [1]. The production of mungbean is limiting due to the abiotic stress constraints. Flooding stress is one of them which affect the normal physio-biochemical functions under oxidative stress and inhibiting crop performance and yield [2-3].

Flooding stress has been established as a vital problem for the agricultural practices that significantly affects the plants development and productivity [4-5]. Despite the fact that oxygen is important for life on earth, its reduction by any means could result in the production of ROS perturbing several cellular metabolic processes of plants [6-7]. Oxygen is required in plants for energy production through respiration, and low oxygen conditions (hypoxia) leading to radical and noticeable changes in metabolism to provide alternative sources of ATP [8]. This hypoxic conditions can occur through changes in the environmental surrounding of the plant (e.g., flooding), through physical barriers imposed by plant anatomy or during developmental processes with high energy demands [9-10, 8]. Hypoxic condition slowly leads to deficiency of oxygen

primarily and secondly, it leads to anoxia (complete O_2 deficiency) in the rhizosphere that inhibits aerobic respiration and leads to lesser energy production. Due to unavailability of oxygen, the intermediate electron carriers in electron transport chain become reduced. The highly reduced intracellular environment and low energy supply favors the generation of reactive oxygen species i.e., O_2^-, H_2O_2, OH• etc. [11]. However, under such conditions plants adopt various antioxidant defense mechanisms i.e., APX, CAT, PPO, POX and SOD, to balance their production and quantity [11-12]. There are many reports on the flash flooding tolerance in various crops like wheat, maize, tomato, but such study on mungbean is limited. Development of waterlogging tolerant varieties will help to reduce damage caused by unseasonal heavy rains during the growing season and increase the versatility of this otherwise hardy crop. Comparatively meager work is on record regarding the role of paclobutrazol (PBZ) on flooding tolerance in mungbean. Therefore, investigations were done with paclobutrazol which is supposed to be an effective plant growth regulator in mitigating the harmful effects of different abiotic stresses, hence, identified and thought worthwhile for understanding its effects on flash flooding stress.

Materials and Methods

Plant Material, Treatments and Flooding Stress Exposure: Investigations were performed in *kharif* (rainy season) during the year 2012-13 at the Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi with two screened mungbean (*Vigna radiata* L.) genotypes viz., HUM-16 (flooding tolerant) and HUM-12 (flooding susceptible). Eight seeds were sown in each plastic pot (diameter 15×15×15 cm) having 7 kg pulverized soil. After germination, five healthy uniform growth plants were maintained for further growth. Paclobutrazol treatments were given as pre-soaking seed as well as foliar application before 24 hours of flooding exposure i.e., pre-flowering stage. Flash flooding stress was produced up to one week by replacing the small plastic pots into large sized plastic pots (diameter 30×30×30 cm) and the level of water was maintained 5 cm above the soil surface of pot. After seven days, excess water was removed and plant samples were collected and instantly dipped in liquid nitrogen container wrapped with aluminum foil for 24 hours thereafter samples were stored at –80°C for further analysis. Following observations were recorded:

Protein Content: Protein content was determined by the method of [13]. 200mg plant sample was homogenized with 10 mL ethanol (80% v/v). The homogenate was centrifuged at 4000 rpm for 20 min. Supernatant was kept aside and the residue was hydrolyzed with 5 mL of 1N NaOH for overnight and centrifuged it again at 4000 rpm for 20 min. Supernatant was collected and volume was made up to 10 mL. Thereafter, 0.5 mL supernatant + 5 mL alkaline copper solution were mixed together and left for 10 min, subsequently 0.5 mL folin reagent was added and then incubated at room temperature for 1 hour. The absorbance of blue colour was recorded at 730 nm against blank by using UV-Vis double beam spectrophotometer (ELICO SL-196). The amount of protein was calculated using standard curve of bovine serum albumin (BSA).

Determination of Hydrogen Peroxide: Hydrogen peroxide contents were determined as per the protocol [14]. Plant sample (100mg) was homogenized in 10 mL of cold acetone (90%, v/v). Then homogenate was filtered and thereafter 4 mL of titanium reagent was added followed by 5 mL of concentrated ammonium solution to precipitate the peroxide titanium complex. The contents were centrifuged in a refrigerated centrifuge for 5 min at 10000 rpm, the supernatant was discarded and precipitate was dissolved in 10 mL of 2M H_2SO_4. It was re-centrifuged to remove the undissolved materials and the absorbance was recorded at 415 nm against the blank. Concentration of H_2O_2 was determined using a standard curve plotted with known concentration of H_2O_2.

Antioxidant Enzyme Assays: Catalase (EC 1.11.1.6) enzyme activity was assayed according to the protocol given [15]. 100mg plant sample was homogenized in 5 mL of 0.1M phosphate buffer in a chilled medium and centrifuged at 10,000 rpm for 20 min at 4 °C. After that reaction mixture was prepared and mixed rapidly by taking 2.6 mL 0.1M phosphate buffer (pH 6.4), 0.1mL enzyme extract and 0.1mL 1% H_2O_2.. Changes in absorbance at 240 nm (δ A_{240}) at an interval of 15 sec for 2 min were noted. The enzyme activity was estimated using extinction coefficient 43.6 for H_2O_2 decomposition and expressed according to the formula i.e., specific activity: EU mg^{-1} protein = δ $A_{240/min}$ × 1000 / 43.6 × mg protein mL^{-1} reaction mixture

Polyphenol oxidase (EC 1.14.18.1) activity was assayed as per the protocol given [16].

Exactly 100mg plant material was homogenized in 5 mL 0.1M phosphate buffer and then centrifuged at 10,000 rpm for 20 min at 4 °C. Thereafter, reaction mixture was prepared by adding 4.5 mL of phosphate buffer (pH 6.4), 0.2 mL pyragallol and 0.2 mL enzyme extract, after incubation at 25°C for 5 min 0.5 mL 5% H_2SO_4 was added to terminate the reaction. Absorbance was measured at 420 nm with the help of UV-Vis double beam spectrophotometer (ELICO SL-196). Enzyme units were calculated as: PPO units mg^{-1} protein = change in absorbance mg^{-1} protein.

Superoxide dismutase (EC 1.15.1.1) activity was assayed by the method [17]. 100mg plant sample was homogenized with 1.0 mL of extraction buffer (0.1M phosphate buffer, pH 7.5 containing 0.5 mM EDTA) and centrifuged in cooling centrifuge machine at 10,000 rpm for 10 min, after centrifugation supernatant was collected and used as enzyme source. Reaction mixture was prepared by adding 0.1 mL of 1.5M sodium carbonate, 0.2 mL of 200 mM methionine, 0.1 mL of 2.25 mM NBT, 0.1 mL of 3 mM EDTA, 1.5 mL of 100 mM potassium phosphate buffer, 1 mL of distilled water and 0.1 mL of enzyme extract in test tubes in duplicate. Two tubes without enzyme extract were taken as control. The reaction was started by adding 0.1 mL riboflavin (60 µM) 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 were developed maximum colour. A non irradiated complete mixture that did not develop colour served as blank. Absorbance was recorded at 560 nm by using UV-Vis double beam spectrophotometer (ELICO SL-196). Enzyme units were calculated as follows:

$$\text{Enzyme Units (EU)} = \frac{\text{Enzyme}^{*}_{(\text{light})} - (\text{Enzyme}^{\#}_{(\text{light})} - \text{Enzyme}^{*}_{(\text{dark})})}{\text{Enzyme}^{*}_{(\text{light})} / 2}$$

*Where, * without enzyme and # with enzyme*

Yield Contributing Observations: Number of pods $plant^{-1}$ and seed yield $plant^{-1}$ were taken in each treatment by random selection of three plants and an average number of pods $plant^{-1}$ and seed yield $plant^{-1}$ were determined.

Statistical Analysis: Experimental data were recorded with average mean values for three replicates of each treatment and data were subjected to ANOVA for completely randomized design (CRD). Critical differences were taken at 5% level of significance. Standard error mean was calculated as described [18].

Results

Protein Content: Significant differences in the protein contents were observed between PBZ treated and control (without PBZ) plants of both HUM-16 (tolerant) and HUM-12 (susceptible) genotypes in normal (non-flooded) and flooded plants. In response to flash flooding, the amount of protein (mg g^{-1} fresh weight) was higher with PBZ treatment T_5 (10ppm ST + 10ppm FS) i.e., 14.37 and 11.75, while it was minimum with control T_0 i.e., 9.53 and 8.00 in both tolerant and susceptible genotypes, respectively (Table 1). The per cent reduction on protein content was greater in susceptible genotype than the tolerant with respect to flooding stress over their respective normal plants. Rest of the PBZ treatments were also responded significantly on protein content under flash flooding stress.

Table 1 Effect of paclobutrazol on protein content (mg g^{-1} fresh weight) in mungbean (*Vigna radiata* L.) genotypes under flash flooding stress at pre-flowering stage

TREATMENTS	GENOTYPES					
	HUM-16			HUM-12		
	Normal	Flooding stress	Mean	Normal	Flooding stress	Mean
T_0 (0.0 ppm PBZ)	11.46	9.53 (-16.8)	10.50	10.45	8.00 (-23.4)	9.22
T_1 (10 ppm ST PBZ)	13.13	11.82 (-10.0)	12.48	11.80	9.73 (-17.5)	10.77
T_2 (25 ppm ST PBZ)	13.03	11.71 (-10.2)	12.37	11.69	9.57 (-18.2)	10.63
T_3 (10 ppm FS PBZ)	11.66	10.11 (-13.3)	10.88	10.91	8.67 (-20.6)	9.79
T_4 (25 ppm FS PBZ)	11.93	10.33 (-13.4)	11.13	10.83	8.67 (-20.0)	9.75
T_5 (10 ppm ST + 10 ppm FS PBZ)	15.60	14.37 (-7.8)	14.99	13.16	11.75 (-10.7)	12.45
T_6 (10 ppm ST + 25 ppm FS PBZ)	13.78	12.51 (-9.2)	13.14	11.96	10.10 (-15.6)	11.03
T_7 (25 ppm ST + 10 ppm FS PBZ)	14.97	13.63 (-9.0)	14.30	12.27	10.49 (-14.5)	11.38
T_8 (25 ppm ST + 25 ppm FS PBZ)	12.44	11.17 (-10.2)	11.80	11.49	9.75 (-15.1)	10.62
Mean	**13.11**	**11.69**		**11.62**	**9.64**	
Factors	**G**	**C**	**T**	**G × C**	**G × T**	**C × T**	**G × C × T**
SEm±	0.13	0.13	0.27	0.18	0.38	0.38	0.54
CD at 5 %	0.36	0.36	0.77	0.51	1.08	1.08	1.53

Where, ST= Seed treatment, FS= Foliar spray, PBZ= Paclobutrazol, G= Genotype, C= Condition (Normal and Flooding Stress), T= Treatment

Values in parentheses indicate per cent decrease under flash flooding stress over normal

Hydrogen Peroxide: Flash flooding stress significantly enhanced the hydrogen peroxide (H_2O_2) contents in both the genotypes. As compare to control (T_0) plants, genotype HUM-16 had lesser amount of H_2O_2 with PBZ treatment T_5 (10ppm ST + 10ppm FS) i.e., 158.61 µM g^{-1} fresh weight, while in HUM-12 it was 190.42 µM g^{-1} fresh weight under flooding condition (Table 2). All the PBZ treatments were reduced H_2O_2 production in both the genotypes in normal (non-flooded) as well as under flooding stress. Significant effects of PBZ treatments were also observed in the susceptible genotype, while it was not as much significant as in the tolerant genotype.

Table 2 Effect of paclobutrazol on hydrogen peroxide content (µM g^{-1} fresh weight) in mungbean (*Vigna radiata* L.) genotypes under flash flooding stress at pre-flowering stage

TREATMENTS		GENOTYPES					
		HUM-16			HUM-12		
		Normal	Flooding stress	Mean	Normal	Flooding stress	Mean
T_0 (0.0 ppm PBZ)		215.66	281.83 (+30.7)	248.75	255.73	373.80 (+46.2)	314.77
T_1 (10 ppm ST PBZ)		186.65	224.97 (+20.5)	205.81	192.11	250.30 (+30.3)	221.21
T_2 (25 ppm ST PBZ)		187.05	226.53 (+21.1)	206.79	197.56	260.67 (+31.9)	229.12
T_3 (10 ppm FS PBZ)		214.70	272.33 (+26.8)	243.52	256.47	352.57 (+37.5)	304.52
T_4 (25 ppm FS PBZ)		218.45	278.87 (+27.7)	248.66	266.14	368.60 (+38.5)	317.37
T_5 (10 ppm ST + 10 ppm FS PBZ)		143.76	158.61 (+10.3)	151.19	160.96	190.42 (+18.3)	175.69
T_6 (10 ppm ST + 25 ppm FS PBZ)		153.73	178.77 (+16.3)	166.25	177.74	228.53 (+28.6)	203.14
T_7 (25 ppm ST + 10 ppm FS PBZ)		145.23	163.43 (+12.5)	154.33	172.23	214.80 (+24.7)	193.52
T_8 (25 ppm ST + 25 ppm FS PBZ)		154.83	184.67 (+19.3)	169.75	183.58	238.00 (+29.6)	210.79
Mean		**180.01**	**218.89**		**206.95**	**275.30**	
Factors	**G**	**C**	**T**	**G × C**	**G × T**	**C × T**	**G × C × T**
SEm±	1.03	1.03	2.19	1.46	3.10	3.10	4.38
CD at 5 %	2.91	2.91	6.17	4.11	8.73	8.73	12.34

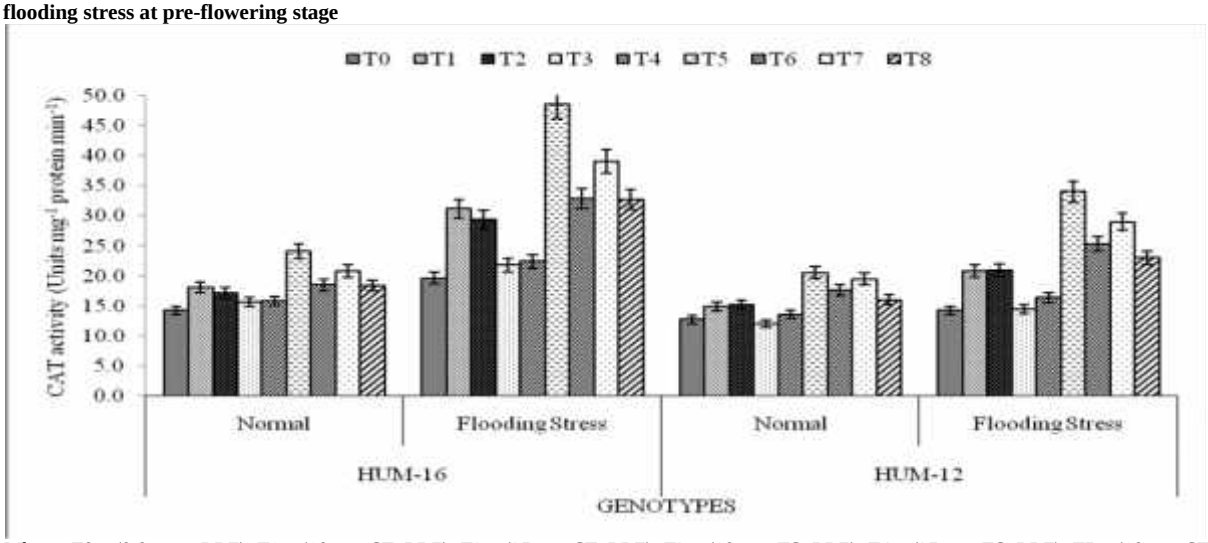
Where, ST= Seed treatment, FS= Foliar spray, PBZ= Paclobutrazol, G= Genotype, C= Condition (Normal and Flooding Stress), T= Treatment

Values in parentheses indicate per cent increase under flash flooding stress over normal

Antioxidant Enzyme Activities: In response to paclobutrazol (PBZ) treatments, flash flooding stress significantly increased catalase (CAT), polyphenol oxidase (PPO) and superoxide dismutase (SOD) activities in both tolerant and susceptible mungbean genotypes, however, activities of these enzymes were quite higher in tolerant (HUM-16) genotype than the susceptible (HUM-12) one.

Catalase Activity: The peak CAT activity was recorded with PBZ treatment T_5 (48.53 and 34.00 units mg^{-1} protein min^{-1}) and lesser with control T_0 (19.58 and 14.23 units mg^{-1} protein min^{-1}) under flash flooding stress (Fig. 1). As far as genotypes are concerned, tolerant genotype revealed elevated CAT activity as compared to susceptible genotype treated with different PBZ concentrations, as compared to the control (without PBZ) plants in normal and flooded plants. Tolerant genotype revealed about 2 folds more CAT activity with PBZ treatment T_5 in response to flash flooding over their normal (non-flooded) plants.

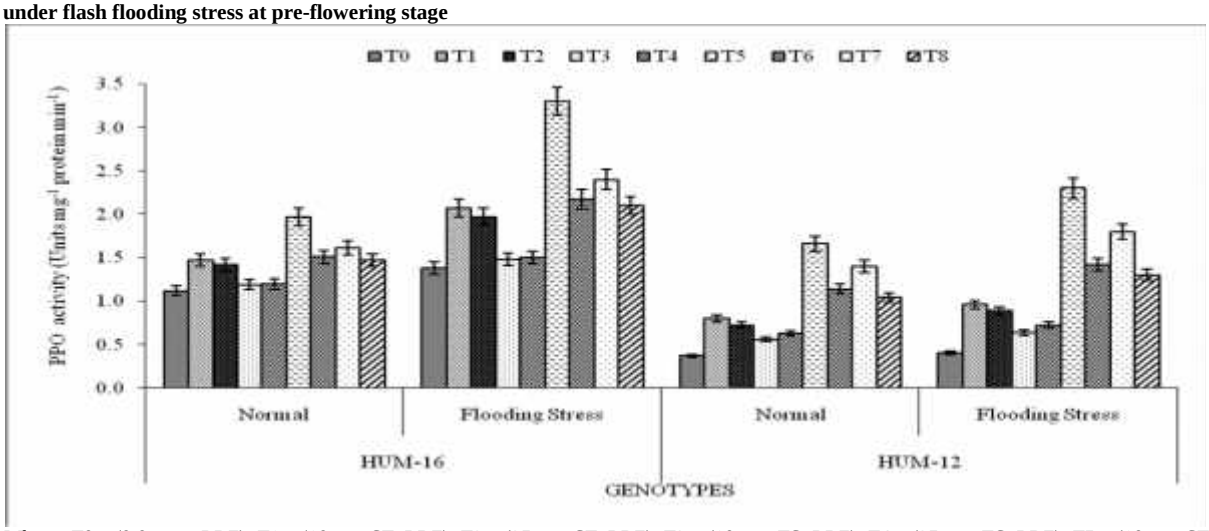
Fig. 1 Effect of paclobutrazol on catalase activity (Units mg^{-1} protein min^{-1}) in mungbean (*Vigna radiata* L.) genotypes under flash flooding stress at pre-flowering stage



Where, T0= (0.0 ppm, PBZ), T1= (10ppm ST, PBZ), T2= (25ppm ST, PBZ), T3= (10ppm FS, PBZ), T4= (25ppm FS, PBZ), T5= (10ppm ST + 10ppm FS, PBZ), T6= (10ppm ST + 25ppm FS, PBZ), T7= (25ppm ST + 10ppm FS, PBZ), T8= (25ppm ST + 25ppm FS, PBZ), ST= Seed treatment, FS= Foliar spray, PBZ= Paclobutrazol

Polyphenol Oxidase Activity: Polyphenol oxidase (PPO) activity significantly enhanced by flash flooding stress and PBZ treatments in both tolerant and susceptible mungbean genotypes. Under flash flooding stress, PBZ treatment T_5 exposed maximum i.e., 3.30 and 2.50 (units mg^{-1} protein min^{-1}) and minimum with control T_0 i.e., 1.38 and 0.40 (units mg^{-1} protein min^{-1}) PPO activities in both tolerant (HUM-16) and susceptible (HUM-12) genotypes, respectively (Fig. 2). Remaining PBZ treatments were also responded significantly on PPO activities in both HUM-16 and HUM-12 genotypes under flooding stress.

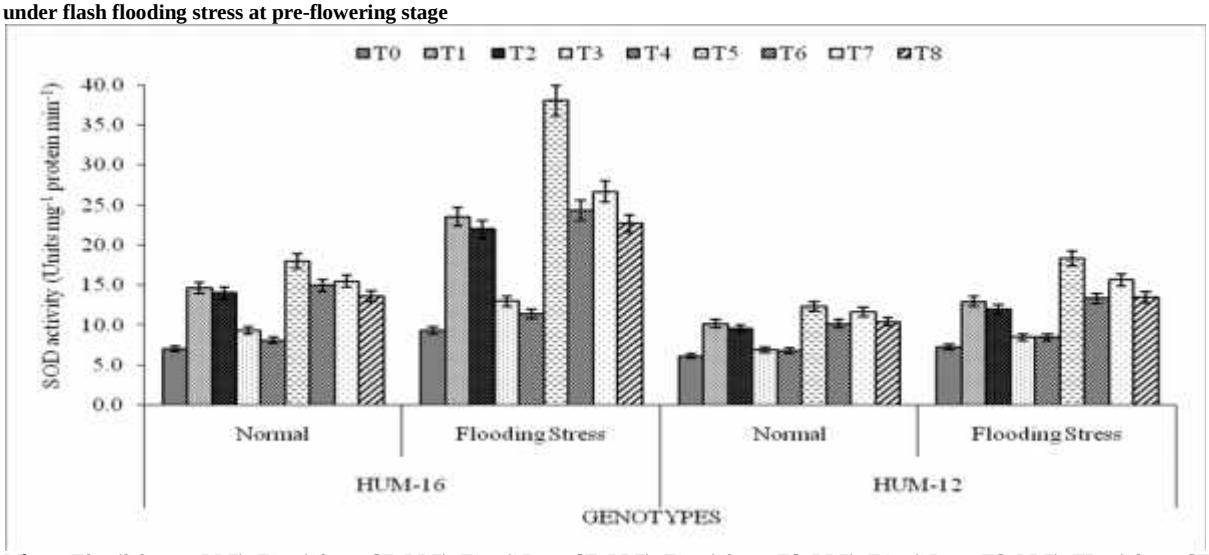
Fig. 2 Effect of paclobutrazol on polyphenol oxidase activity (Units mg^{-1} protein min^{-1}) in mungbean (*Vigna radiata* L.) genotypes under flash flooding stress at pre-flowering stage



Where, T0= (0.0 ppm, PBZ), T1= (10ppm ST, PBZ), T2= (25ppm ST, PBZ), T3= (10ppm FS, PBZ), T4= (25ppm FS, PBZ), T5= (10ppm ST + 10ppm FS, PBZ), T6= (10ppm ST + 25ppm FS, PBZ), T7= (25ppm ST + 10ppm FS, PBZ), T8= (25ppm ST + 25ppm FS, PBZ), ST= Seed treatment, FS= Foliar spray, PBZ= Paclobutrazol

Superoxide Dismutase Activity: Specific activity of superoxide dismutase (SOD) was greatly influenced by flash flooding stress in both tolerant (HUM-16) and susceptible (HUM-12) mungbean genotypes. Paclobutrazol treatment acquired higher SOD activity with treatment T_5 (38.00 and 18.33 units mg^{-1} protein min^{-1}) and lesser with control T_0 (9.33 and 7.30 units mg^{-1} protein min^{-1}) in both tolerant and susceptible genotypes, respectively under flooding stress (Fig. 3). In order to per cent increase in SOD activity, PBZ treatment T_5 (10ppm ST + 10ppm FS) revealed 111.2% and 48.8% in both tolerant and susceptible genotypes, respectively. However, the minimum per cent increment in SOD activity was recorded with control T_0 (without PBZ), i.e., 33.1 and 18.8% in both tolerant and susceptible genotypes, respectively under flooding stress over their respective normal plants.

Fig. 3 Effect of paclobutrazol on superoxide dismutase activity (Units mg^{-1} protein min^{-1}) in mungbean (*Vigna radiata* L.) genotypes under flash flooding stress at pre-flowering stage



Where, T0= (0.0 ppm, PBZ), T1= (10ppm ST, PBZ), T2= (25ppm ST, PBZ), T3= (10ppm FS, PBZ), T4= (25ppm FS, PBZ), T5= (10ppm ST + 10ppm FS, PBZ), T6= (10ppm ST + 25ppm FS, PBZ), T7= (25ppm ST + 10ppm FS, PBZ), T8= (25ppm ST + 25ppm FS, PBZ), ST= Seed treatment, FS= Foliar spray, PBZ= Paclobutrazol

Yield Parameters

Number of Pods Plant^{-1}: Flash flooding stress significantly declined the number of pods plant^{-1} in both HUM-16 and HUM-12 mungbean genotypes. Genotypic differences were evident, tolerant (HUM-16) genotype produced greater number of pods plant^{-1} than the susceptible (HUM-12) under normal and flooding stress conditions. In response to flash flooding stress, the number of pods plant^{-1} were higher with PBZ treatment T_5 (18.67 and 13.00) and minimum with control T_0 (9.67 and 7.33) in both HUM-16 and HUM-12 genotypes, respectively (Table 3). In view of per cent reduction on number of pods plant^{-1}, it was maximum with control T_0 (19.4% and 26.7%) and minimum with PBZ T_5 (5.1% and 13.3%) in both HUM-16 and HUM-12 genotypes, respectively, under flooding stress over their respective normal plants.

Table 3 Effect of paclobutrazol on number of pods plant^{-1} in mungbean (*Vigna radiata* L.) genotypes under flash flooding stress at pre-flowering stage

TREATMENTS		GENOTYPES					
		HUM-16			HUM-12		
		Normal	Flooding stress	Mean	Normal	Flooding stress	Mean
T_0 (0.0 ppm PBZ)		12.00	9.67 (-19.4)	10.83	10.00	7.33 (-26.7)	8.67
T_1 (10 ppm ST PBZ)		15.67	13.67 (-12.8)	14.67	12.67	10.00 (-21.1)	11.33
T_2 (25 ppm ST PBZ)		14.33	12.67 (-11.6)	13.50	13.00	10.33 (-20.5)	11.67
T_3 (10 ppm FS PBZ)		13.33	11.33 (-15.0)	12.33	11.00	8.33 (-24.2)	9.67
T_4 (25 ppm FS PBZ)		12.33	10.33 (-16.2)	11.33	10.67	8.33 (-21.9)	9.50
T_5 (10 ppm ST + 10 ppm FS PBZ)		19.67	18.67 (-5.1)	19.17	15.00	13.00 (-13.3)	14.00
T_6 (10 ppm ST + 25 ppm FS PBZ)		15.67	14.00 (-10.6)	14.83	12.00	10.00 (-16.7)	11.00
T_7 (25 ppm ST + 10 ppm FS PBZ)		16.33	15.00 (-8.2)	15.67	13.33	11.33 (-15.0)	12.33
T_8 (25 ppm ST + 25 ppm FS PBZ)		13.00	11.33 (-12.8)	12.17	13.00	10.67 (-17.9)	11.83
Mean		**14.70**	**12.96**		**12.30**	**9.93**	
Factors	**G**	**C**	**T**	**G × C**	**G × T**	**C × T**	**G × C × T**
SEm±	0.22	0.22	0.46	0.31	0.65	0.65	0.92
CD at 5 %	0.61	0.61	1.29	0.86	1.83	1.83	2.58

Where, ST= Seed treatment, FS= Foliar spray, PBZ= Paclobutrazol, G= Genotype, C= Condition (Normal and Flooding Stress), T= Treatment

Values in parentheses indicate per cent decrease under flash flooding stress over normal

Seed Yield Plant^{-1} : Flash flooding significantly declined the seed yield plant^{-1} in both HUM-16 and HUM-12 genotypes. Paclobutrazol treatments significantly enhanced the seed yield plant^{-1} (g) which was higher with PBZ treatment T_5 (5.07 and 3.57 g) and lesser with control T_0 (2.64 and 2.05 g) in both tolerant and susceptible genotypes, respectively, under flash flooding stress. PBZ treatment minimized the per cent loss on seed yield in both tolerant and susceptible genotypes, while it was more efficient in tolerant genotype. Control (without PBZ) plants exhibited higher per cent on yield loss i.e., 20.4% and 25.9%, while it was minimum with PBZ treatment T_5 i.e., 5.6% and 14.4% in both tolerant and susceptible genotype, respectively under flooding stress (Table 4). As compared to control plants, remaining PBZ treatments were also performed well on normal (non-flooded) and flooded plants in response to seed yield plant^{-1}.

Table 4 Effect of paclobutrazol on seed yield plant^{-1} in mungbean (*Vigna radiata* L.) genotypes under flash flooding stress at pre-flowering stage

TREATMENTS		GENOTYPES					
		HUM-16			HUM-12		
		Normal	Flooding stress	Mean	Normal	Flooding stress	Mean
T_0 (0.0 ppm PBZ)		3.32	2.64 (-20.4)	2.98	2.77	2.05 (-25.9)	2.41
T_1 (10 ppm ST PBZ)		4.33	3.75 (-13.5)	4.04	3.47	2.75 (-20.7)	3.11
T_2 (25 ppm ST PBZ)		3.92	3.45 (-11.9)	3.68	3.57	2.85 (-20.1)	3.21
T_3 (10 ppm FS PBZ)		3.65	3.13 (-14.2)	3.39	3.00	2.32 (-22.8)	2.66
T_4 (25 ppm FS PBZ)		3.35	2.83 (-15.4)	3.09	2.93	2.33 (-20.5)	2.63
T_5 (10 ppm ST + 10 ppm FS PBZ)		5.37	5.07 (-5.6)	5.22	4.17	3.57 (-14.4)	3.87
T_6 (10 ppm ST + 25 ppm FS PBZ)		4.25	3.87 (-9.0)	4.06	3.28	2.77 (-15.7)	3.03
T_7 (25 ppm ST + 10 ppm FS PBZ)		4.43	4.13 (-6.8)	4.28	3.67	3.12 (-15.0)	3.39
T_8 (25 ppm ST + 25 ppm FS PBZ)		3.55	3.15 (-11.3)	3.35	3.55	2.95 (-16.9)	3.25
Mean		**4.02**	**3.56**		**3.38**	**2.74**	
Factors	**G**	**C**	**T**	**G × C**	**G × T**	**C × T**	**G × C × T**
SEm±	0.06	0.06	0.12	0.08	0.18	0.18	0.25
CD at 5 %	0.17	0.17	0.35	0.23	0.50	0.50	0.70

Where, ST= Seed treatment, FS= Foliar spray, PBZ= Paclobutrazol, G= Genotype, C= Condition (Normal and Flooding Stress), T= Treatment

Values in parentheses indicate per cent decrease under flash flooding stress over normal

Discussion

Proteins are an important part of perception and response to abiotic stresses [19]. Flooding stress leads root hypoxic conditions and increased anaerobic fermentation, which adversely impacts various physiological and

biochemical events, viz., photosynthesis, energetic metabolism, redox status, programmed cell death, RNA processing, as well as protein synthesis or degradation [20]. In the present investigation, flash flooding declined protein content in both tolerant (HUM-16) and susceptible (HUM-12) genotypes, though the amount was reasonably higher in flooding tolerant genotype. Among all the treatments, PBZ treatment T_5 registered greater amount of protein along with lesser per cent reduction in both HUM-16 and HUM-12 genotypes under flash flooding stress. Higher level of protein content in tolerant genotype might be due to the higher rate of photosynthesis and slow degradation under flooding stress. According to the protein contents were negatively influenced by flooding in red clover plants [21]. It has been reported that under waterlogging/flooding stress condition in Rape (*Brassica napus* L.) uniconazole (a trizole) treatment significantly enhanced the protein content [22].

Although reactive oxygen species (ROS) (H_2O_2, $^1O_2$, OH•, O_2^-) are considered to be very toxic compound for the plant, particularly at high concentrations, those also play an important role in signaling and are involved in the activation of defense responses under flooding stress [23]. Various stress both abiotic and biotic caused excessive generation of reactive oxygen species [24]. Present experiment revealed that flash flooding enhanced H_2O_2 production in both tolerant and susceptible genotypes, while the extent was fairly higher in susceptible genotype. These findings indicated that tolerant genotype has greater ability to minimize the H_2O_2 accumulation with PBZ treatment T_5 due to higher antioxidant enzyme activities than the susceptible genotype under flooding stress. Therefore, in this study, it was further proved that the H_2O_2 production during O_2 deprivation was observed in the plant cells [25] and its degradation was found to play an important role in flooding tolerance in egg plants and tomato [26]. Plants have vital capacity to detoxify the detrimental effects of ROS through enzymatically and non-enzymatically. Enzymatic antioxidants include catalase (CAT), polyphenol oxidase (PPO), superoxide dismutase (SOD), peroxidase (POD) and glutathione reductase (GR), whereas ascorbic acid, glutathione, tocopherols and carotenoids are included in non-enzymatic antioxidants [27].

Alteration of catalase (CAT) activities were observed in flooded plants between HZ32 (tolerant) and K12 (susceptible) genotypes, and flooding stress severely affected catalase activity of HZ32 than that of K12 genotype [28]. Our study also revealed significantly enhanced catalase activity by flash flooding more in tolerant genotype than the susceptible genotype. Further, reported that, under flooding conditions, the CAT activity was higher in flooding resistant pigeonpea genotype (ICPL-84023) as compared to susceptible genotype (MAL-18) [29]. In these experiments, PBZ treatment T_5 acquired higher per cent increment (101.1% and 65.5%) in both HUM-16 (tolerant) and HUM-12 (susceptible) genotypes, respectively in response to flash flooding. Data show that at cellular level, tolerant mungbean genotype has greater efficiency to remove H_2O_2 produced under flooding stress as compared to susceptible genotype through enhancing CAT activity, because it converts H_2O_2 into $O_2 + H_2O$, which might be helpful to survive under flooding stress. Reported that catalase is the most important enzyme involved in regulation of intracellular level of H_2O_2 [30]. Consequently, PBZ treated plants exhibited about 50% higher catalase activity than the control plants of mungbean [31].

Polyphenol oxidase (PPO) activities has been purified and characterized from a large number of plant species [32]. Confirmed that, PPO activity increased greatly as a consequence of flooding stress and was 487.2% higher in genotype ICPL-84023 (tolerant), while 444.2% in MAL-18 (susceptible) [29]. In the present investigation, PPO enzyme activity was provoked by flash flooding stress in both HUM-16 and HUM-12 genotypes. Results showed that the tolerant genotype (HUM-16) had higher PPO activity than the susceptible (HUM-12) one under flash flooding stress. PBZ treatment with T_5 revealed about 3 fold more PPO activity in tolerant genotype as compared to their control (without PBZ) plants under flooding stress. In view of per cent increase on PPO activity, PBZ treatment T_5 exposed higher PPO activity i.e., 67.8% and 38.3% and minimum with control T_0 i.e., 23.1% and 6.3% in both tolerant and susceptible genotypes, respectively under flooding stress over their respective normal plants. The higher PPO activity in tolerant genotype might be responsible for decreased level of H_2O_2. It may help genotype HUM-16 to maintain better plasma membrane integrity and, thus, conferring flooding stress tolerant in this genotypes. At cellular level, both CAT and PPO enzymes have similar functions, the conversion

of H_2O_2 into O_2 and H_2O, as produced during oxidative stress, but the efficiencies were greatly differed on species to species. Polyphenol oxidase synthesis and accumulation in plants are generally stimulated in response to abiotic stresses [33-35]. Significant effects of PBZ treatment under flooding exposure has been reported on antioxidant enzymes activity in sweet potato [36].

Superoxide dismutase (SOD) enzyme activity helps converting superoxide (O_2^-) to hydrogen peroxide (H_2O_2), is usually considered as the first line of defense against oxidative stress. Increased SOD activity was correlated with increased protection from damage associated with oxidative stress [37]. High levels of SOD should be followed by the scavenging of H_2O_2 catalyzed by PPO and CAT enzymes. Present examinations were revealed that flash flooding significantly enhanced SOD activities in both tolerant and susceptible genotypes, however, the per cent increment in SOD activity was quite higher with PBZ treatment T_5 (111.2%) in tolerant genotype than the susceptible (48.8%) one. These results further confirmed that the tolerant genotype had two folds higher SOD activity than the susceptible genotype under the exposure of flash flooding. Similarly, observed that increased SOD activity in maize leaves was more significant in HZ32 (flooding tolerant) [28], while the reduction was greater in genotype K12 (flooding sensitive), suggesting that the flooding tolerant genotype has a better superoxide (O_2^-) radical scavenging ability. In this study, SOD activity increased on account of flash flooding stress and was higher in tolerant genotype HUM-16; higher SOD activity might be associated with increased the ROS scavenging efficiency in HUM-16 leading to flooding resistance. Increase in superoxide dismutase activity was also demonstrated in Barley [38], Citrus [39] and Pigeonpea [40-41] during flooding stress. Found that spraying paclobutrazol increased SOD activity of rice plant at late growth stage [42]. This further reiterates that PBZ confers protection to plants by reducing oxidative damage via the elevation of antioxidants or the reduction of oxidative enzyme activity.

Flooding stress declined the number of pods, however, one day flooding stress had no significant effect on pods plant^{-1} in mungbean genotype [43]. Present findings exposed that flash flooding reduced number of pods plant^{-1} in both tolerant (HUM-16) and susceptible (HUM-12) genotypes, while the reduction was more prominent in susceptible genotype. Also reported that flooding stress remarkably reduced pods plant^{-1} in mungbean [44] and recorded 36% more pods in non-flooded plants. In our study, PBZ treatment T_5 (10ppm ST+10ppm FS) revealed moderately higher number of pods plant^{-1} in tolerant genotype than the susceptible under flash flooding stress. Our work is also supported [45] who reported higher number of pods in tolerant cultivars probably due to greater availability of the source to the reproductive sinks. Observed that PBZ changed the pattern of assimilate distribution towards reproductive parts, especially to the terminal and upper branches of plants thus, increasing their sink capacity leading to higher number of pod formation [46]. In our case too, the increased availability of assimilates due to increased photosynthesis, lower H_2O_2 production along with higher antioxidative enzymes activity (CAT, PPO and SOD) particularly in tolerant genotype might be associated for more number of pods on branches and ultimately resulted in higher seed yield in PBZ treated mungbean plants.

Several physiological and biochemical attributes reported which are directly linked with plant productivity and yield. Observed a 45% reduction [47], and found a 44% decrease in wheat yield from flooding stress [48]. Present data indicated that flooding stress significantly reduced the seed yield in both tolerant and susceptible genotypes, though tolerant genotype acquired moderately higher seed yield plant^{-1}. The higher yield in tolerant cultivars resulted with increase in the number of pods plant^{-1}. In response to flash flooding, PBZ @10ppm ST+10ppm FS (T_5) showed minimum per cent reduction on seed yield (5.6% and 14.4%) in HUM-16 and HUM-12 genotypes, respectively. Yield reduction up to 10% [49] and 40% in severe cases [50] may result. Yield loss may reach up to more than 60% in soybean applied with periodical flooding stress [51]. Results suggest that the effect of PBZ on seed yield improvement might be linked with improved CO_2 assimilation physiology, enhanced sink activity, better partitioning coefficient, and quantitative enhancement of yield determining growth traits and rooting vigour [52-54]. Uniconazole (a trizole) improved flooding plant performance and increased seed yield, possibly because of improved antioxidant defense mechanisms that retarded lipid peroxidation and membrane deterioration [55]. Paclobutrazol showed

quantitative enhancement in most of the yield attributing traits, which contributed towards overall seed yield in *Camelina* [56].

In conclusion, present study revealed that flash flooding stress enhanced H_2O_2 accumulation in susceptible mungbean genotype (HUM-12), which is directly associated with oxidative damage by sinking antioxidant defense system that might be further affecting the seed yield. On the other hand, paclobutrazol treatments in tolerant genotype (HUM-16) reduced the H_2O_2 accumulation via enhancing antioxidativwe enzymes activity viz., CAT, PPO and SOD that reduced the yield losses under stress by making strong relation between antioxidant defense system and flash flooding stress tolerance.

Author Contribution Statement: Dinesh Kumar Yadav: data collection and its arrangement, statistical analysis along with preparation of whole manuscript; Professor A. Hemantaranjan: for proper execution of this research work and also was involved in the manuscript preparation.

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References

1. Keatinge, J., Easdown, W., Yang, R., Chadha, M., Shanmugasundaram, S. (2011). Overcoming chronicmalnutrition in a future warming world: the key importance of mungbean and vegetable soybean. *Euphytica*, 180: 129–141.
2. Rahmianna, A.A., Tadisarwanto, T., Kirchof, G., So, H.B. (2000). Crop establishment of legumes in rain-fed lowland rice-based cropping system. *Soil Tillage Res*, 56: 67–82.
3. Islam, M.R., Hamid, A., Kaliq, Q.A., Ahmed, J.U., Haque, M.M., Karim, M.A. (2007). Genetic variability in flooding tolerance of mungbean (*Vigna radiata* L. Wilczek) genotypes. *Euphytica* 156: 247–255.
4. Irfan, M., Hayat, S., Hayat, Q., Afroz, S., Ahmad, A. (2010). Physiological and biochemical changes in plants under waterlogging. *Protoplasma*, 241: 3–17.
5. Ramakrishna, A., Ravishankar, G.A. (2011). Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signal Behav*, 6: 1720–1731.
6. Ashraf, M. (2009). Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotech Adv*, 27: 84–93.
7. Ashraf, M.A., Ashraf, M., Ali, Q. (2010). Response of two genetically diverse wheat cultivars to salt stress at different growth stages: Leaf lipid peroxidation and phenolic contents. *Pak J Bot.*, 42:559–565.
8. Banti, V., Beatrice, G., Silvia, G., Elena, L., Leonardo, M., Giacomo, N., Eleonora, P., Sandro, P., Chiara, P., Antonietta, S., Pierdomenico, P. (2013). Low oxygen response mechanisms in green organisms. *Int J Mol Sci.*, 14: 4734–4761.
9. Vashisht, D., Hesselink, A., Pierik, R., Ammerlaan, J.M.H., Bailey-Serres, J., Visser, E.J.W., Sasidharan, R. (2011). Natural variation of submergence tolerance among Arabidopsis thaliana accessions. *New Phytol*, 190: 299–310.
10. Bailey-Serres, J., Lee, S.C., Brinton, E. (2012). Water proofing crops: effective flooding survival strategies. *Plant Physiol.*, 160: 1698–1709.
11. Sairam, R.K., Kumutha, D., Ezhilmathi, K., Deshmukh, P.S., Srivastava, G.C. (2008). Physiology and biochemistry of waterlogging tolerance in plants. *Biol Plant*, 52: 401– 412.
12. Gill, S.S., Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol Biochem*., 48: 909–930.
13. Lowry, O.H., Rosebrough, N.J., Farr, A.J., Randall, R.J. (1951). Protein measurement with the folin-phenol reagents. *J Bio Chem.*, 193: 265–275.
14. Mukherjee, S.P., Choudhari, M.A. (1983). Implications of water stress induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. *Physiol Planta*, 58: 116–170.
15. Aebi, H.E. (1983). Catalase. In: Weinheim VCH (ed) Method of Enzymatic analysis, 3rd edn. Deerfield, Germany, pp 273-286.
16. Kar, M., Mishra, D. (1976). Catalase, peroxidase and polyphenoloxidase activities during rice leaf senescence. *Plant Physiol.*, 57: 315–319.
17. Dhindsa, R.S., Plumb-Dhindsa, P., Thorpe, T.A. (1981). Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J Exp Bot*., 32: 93–101.
18. Gomez, K.A., Gomez, A.A. (1984). Statistical procedures for agricultural research. A Willy-Inter sci Pub, New York.
19. Hashiguchi, A., Ahsan, N., Komatsu, S. (2010). Proteomics application of crops in the context of climatic changes. *Food Res Int.*, 43: 1803–1813.
20. Jackson, M.B. (2003). The impact of flooding stress on plants and crops. School of Biol Sci, University of Bristol, *Bristol,*, 1–15.

21. Veselin, S., Lyudmila, S.S., Irina, V., Anelia, K., Rosa, N., Urs, F., Demirevska, K. (2013). Protein changes and proteolytic degradation in red and white clover plants subjected to waterlogging. *Acta Physiol Plant*, 35: 1925–1932.
22. Yang, D., Yang, J., Hu, Y. (1994). Effects of S3307 on some physiological characteristics of rape seedlings. *Plant Physiol Commun.*, 30: 182–185.
23. Dat, J., Vandenabeele, S., Vranova, E., Van Montagu, M., Inze, D., Van Breusegem, F. (2000). Dual action of the active oxygen species during plant stress responses. *Cell Mol Life Sci.*, 57: 779–795.
24. Polle, A. (2001). Dissecting the superoxide dismutase ascorbate peroxidase glutathione pathway in chloroplasts by metabolic modeling, Computer simulations as a step towards flux analysis. *Plant Physiol.*, 126: 445–462.
25. Blokhina, O.B., Chirkova, T.V., Fagerstedt, K.V. (2001). Anoxic stress leads to hydrogen peroxide formation in plant cells. *J Exp Bot.*, 52: 1179–1190.
26. Lin, K.H., Wang, C.C., Loa, H.F., Chen, J.T. (2004). *Plant Science*, 167: 355–365.
27. Gupta, K.J., Stoimenova, M., Kaiser, W.M. (2005). In higher plants, only root mitochondria, but not leaf mitochondria reduce nitrite to NO, *in vitro* and in situ. *J Exp Bot.*, 56: 2601–2609.
28. Tang, B., Xu, S., Zou, X., Zheng, Y., Qiu, F. (2010). Changes of antioxidative enzymes and lipid peroxidation in leaves and roots of waterlogging tolerant and waterlogging sensitive maize genotypes at seedling stage. *Agri Sci China*, 9 (5): 651–661.
29. Bansal, R., Srivastava, J.P. (2012). Antioxidative defense system in pigeonpea roots under waterlogging stress. *Acta Physiol Plant*, 34: 515–522.
30. Prasad, T., Anderson, M., Steward, C. (1995). Localization and characterization of peroxidases in the mitochondria of chilling acclimated maize seedlings. *Plant Physiol.*, 108: 1597–1605.
31. Kanti, K.B., Ganesh, R., Mathur, S.R. (2007). Paclobutrazol delayed dark induced senescence of mungbean leaves. *Biol Bratislava*, 62 (2): 185–188.
32. Mayer, A.M. (2006). Polyphenol oxidases in plants and fungi: going places: A review. *Phytochem.*, 67: 2318–2331.
33. Yaginuma, S., Shiraishi, T., Ohya, H., Igarashi, K. (2002). Polyphenol increases in safflower and cucumber seedlings exposed to strong visible light with limited water. *Bio Biotech Biochem.*, 66: 65–72.
34. Ksouri, R., Kreuzwieser, J., Hauberg, J., Howell, K.A., Carroll, A., Rennenberg, H., Megdiche, W., Debez, A., Falleh, H., Grignon, C., Abdelly, C. (2007). Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte Cakile maritime. *Plant Physiol Biochem.*, 45: 244–249.
35. Giorgi, A., Mingozzi, M., Madeo, M., Speranza, G., Cocucci, M. (2009). Effect of nitrogen starvation on the phenolic metabolism and antioxidant properties of yarrow (Achillea collina Becker ex Rchb). *Food Chem.*, 14: 204–211.
36. Lin, K.H., Tsou, C.C., Hwang, S.Y., Chen, L.F., Lo, H.F. (2008). Paclobutrazol leads to enhanced antioxidative protection of sweet potato under flooding stress. *Bot Studies*, 49: 9–18.
37. Asada, K. (1999). The water–water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. *Ann Rev Plant Physiol Plant Mol Biol.*, 50: 601–639.
38. Yordanova, R.Y., Alexieva, V.S., Popova, L.P. (2003). Influence of root oxygen deficiency on photosynthesis and antioxidants in barley plants. *Russ J Plant Physiol.*, 50: 163–167.
39. Hossain, Z., Lopez-Climent, M.F., Arbona, V., Perez-Clemente, R.M., Gomez-Cadenas, A. (2009). Modulation of the antioxidant system in citrus under waterlogging and subsequent drainage. *J Plant Physiol.*, 166 (13): 1391–1404.
40. Kumutha, D., Ezhilmathi, K., Sairam, R.K., Srivastava, G.C., Deshmukh, P.S., Meena, R.C. (2009). Waterlogging induced oxidative stress and antioxidant activity in pigeonpea genotypes. *Biol Planta*, 53 (1): 75–84.
41. Singh, V.P. (2010). Physiological and biochemical changes in pigeonpea [*Cajanus cajan* (L.) Millsp.] genotypes to waterlogging stress at early stage. Ph.D. Thesis, Banaras Hindu University, Varanasi.
42. Pan, S., Fahd, R., Wu, L., Hua, T., Zhaowen, M., Meiyang, D., Xiangru, T. (2013). Roles of plant growth regulators on yield, grain qualities and antioxidant enzyme activities in super hybrid rice (*Oryza sativa* L.). *Rice*, 6: 9.
43. Islam, M.R., Hamid, A., Karim, M.A., Haque, M.M., Khaliq, A.Q., Jalal, U.A. (2008). Gas exchanges and yield responses of mungbean (*Vigna radiata* L. Wilczek) genotypes differing in flooding tolerance. *Acta Physiol Plant*, 30: 697–707.
44. Islam, M.T. (1994). Eco-physiological studies on photosynthesis and dry matter production in mungbean (*Vigna radiata* (L.) Wilczek), Ph.D. thesis, Kyushu University, Fukuoka Wein C, Lal R, Pulver EL (1979): Effects of transient flooding on growth and yield of some tropical crops. In: Lal R, Greenland DJ (ed) Soil physical properties and crop production in the tropics, Wiley, Chichester, 234–245.
45. Kumar, P., Madan, P., Rohit, J., Sairam, R.K. (2013). Yield, growth and physiological responses of mung bean [*Vigna radiata* (L.) Wilczek] genotypes to waterlogging at vegetative stage. *Physiol Mol Biol Plants*, 19 (2): 209–220.

46. Addo-Quaye, A.A., Daniels, R.W. Scarisbrick, D.H. (1985). The influence of paclobutrazol on the distribution and utilization of 14C-labelled assimilate fixed at anthesis in oilseed rape (*Brassica napus* L.). *J Agric Sci.*, 105: 365–373.
47. Musgrave, M.E., Ding, N. (1998). Evaluating wheat cultivars for waterlogging tolerance. *Crop Sci.*, 38: 90–97.
48. Collaku, A., Harrison, S.A. (2002). Losses in wheat due to waterlogging. *Crop Sci*., 42: 444–450.
49. Bange, M.P., Milroy, S.P., Thongbai, P. (2004). Growth and yield of cotton in response to waterlogging. *Field Crops Res*, 88: 129–142.
50. Hodgson, A.S., Chan, K.Y. (1982). The effect of short term waterlogging during furrow irrigation of cotton in cracking grey clay. *Aus J Agri Res.*, 33: 109–116.
51. Kuswantoro, H. (2011). Response of soybean genotypes to waterlogging. J Agron Indonesia 39: 19–23.
52. Berova, M., Zlatev, Z. (2000). Physiological response and yield of paclobutrazol treated tomato plants (*Lycopersicon esculentu* Mill.). *Plant Growth Regul*., 30: 117–123.
53. Senoo, S., Isoda, A., Nojima, H., Takasaki, Y. (2001). Effects of paclobutrazol on flowering and seed-setting habit and physiological, morphological characters in peanut. *Jpn J Crop Sci.*, 70: 188–189.
54. Senoo, S., Isoda, A. (2003). Effects of paclobutrazol on dry matter distribution and yield in peanut. *Plant Prod Sci.*, 6: 90–94.
55. Leul, M., Zhou, W. (1999). Alleviation of waterlogging damage in winter rape by uniconazole application: effects on enzyme activity, lipid peroxidation, and membrane integrity. *J Plant Growth Regul.*, 18: 9–14.
56. Sumit, K., Ghatty, S., Satyanarayana, J., Guha, A., Chaitanya, B.S.K., Reddy, A.R. (2012). Paclobutrazol treatment as a potential strategy for higher seed and oil yield in field grown *Camelina sativa* L. Crantz. *B.M.C. Research Notes*, 5: 137.