The Bioscan 11(4): 2245-2248, 2016
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.com

EFFECT OF DROUGHT AND SALINITY STRESS ON ANTIOXIDATIVE ENZYMES ACTIVITY AND PHENOL CONTENT IN COTYLEDON AND EMBRYONIC AXIS OF MUNGBEAN [*VIGNA RADIATA* (L.) WILCZEK]

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KEYWORDS
Mungbean
Antioxidative Enzyme
Drought
Salinity Stress

Received on :
14.07.2016

Accepted on :
26.10.2016

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ABSTRACT
A laboratory experiment was undertaken on mungbean (cultivar PUSA 9531) at Bidhan Chandra Krishi Viswavidyalaya, West Bengal; seeds were subjected to drought and salinity by using PEG-6000 (12%) and NaCl (100mM) respectively. The result showed that PEG 12% registered higher activity of SOD, CAT and Phenol content at all stages over control in comparison with the NaCl 100 mM in both cotyledon and embryonic axis. However, at 72 hours of germination PEG 12% recorded higher activity of SOD (12.364, 18.445 unit min^{-1} g^{-1} fresh weight), against under NaCl 100 mM in both cotyledon and embryonic axis. While GPOX activity was decrease under PEG and NaCl, respectively. Similarly, decrease the activity of CAT (19.84% over control) in both organs under NaCl, while the phenol content in cotyledon decreases at 72 hrs of germination. The objective of the present investigation was to study the effect of drought and salinity stress on antioxidative enzyme and phenol content in cotyledon and embryonic axis in mungbean.

INTRODUCTION

Mungbean [*Vigna radiata* (L.) Wilczek] is an important leguminous crop. It is considered as one of the cheap sources of dietary proteins in our country. Mungbean seeds contain 22-28 % protein, 60-65 % carbohydrates, 1-1.5 % fat, 3.5-4.5 % fibres and 4.5-5.5 % ash (Mohamed and Kramany, 2005). Plants are frequently exposed to a variety of stress conditions such as drought, salinity, low temperature, flooding, heat, extremes of soil pH and heavy metal toxicity. Salt stress and drought stress show a high degree of similarity with respect to physiological, biochemical and molecular events. During abiotic stress, over production of ROS damages macromolecules in cells such as proteins, nucleic acids and lipids (Imlay 2003). In order to prevent excessive accumulation of ROS, plant species are endowed with enzymatic as well as non-enzymatic antioxidative systems. Key enzymes involved in detoxification are superoxide dismutase (SOD EC 1.15.1.1.), catalase (CAT EC 1.11.1.6.) and peroxidases *i.e.* Guaiacol peroxidase while non-enzymatic antioxidants include small molecules such as ascorbate, glutathione as well as tocopherol, phenol flavonids and carotenoids (Appel and Hirt 2004). The correct functioning of ROS scavenging mechanisms is essential for successful germination. These scavenging mechanisms also help the growing embryonic axis to overcome different stressful conditions during germination. Moreover, it has been found that water distribution in dry seeds is not homogeneous and embryo axes and cotyledons hydrate to different extent (Wojtyla *et al*., 2006). Thus, it is conceivable that the two organs show different metabolic activity during imbibition and germination. Evidence collected from various species suggests that stress response is a developmentally regulated, stage-specific phenomenon, so that tolerance at one stage of development may not be correlated with tolerance at other developmental stages (Harb, 2013). There fore, specific stages throughout the ontogeny of the plant, such as germination and emergence, seedling survival and growth, should be evaluated separately during the assessment of germplasm for stress tolerance. Different developmental stages of this crop are sensitive to drought and salinity stress. So far, some research works have already been carried out on the effect of drought stress (Allahmoradi *et al*., 2011; Uddin et *al*., 2013) and salinity stress (Saha *et al*., 2010 and Dutta and Bera, 2014) on morpho-physiological and biochemical characteristics of mungbean and other crops (Sharma *et al*., 2016). The information regarding the differential activity of different ROS scavenging enzymes as well as solute accumulation in cotyledon and growing embryonic axis is especially lacking in mungbean. So, the experiment was conducted to study the differential activities of antioxidative enzyme along with phenol content in cotyledon and embryonic axis of germinating seed under drought and salinity stress at different time interval.

MATERIALS AND METHODS

Plant material, treatment and plant growth conditions

A laboratory experiment was conducted in the Department of Plant Physiology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, by taking mungbean genotype PUSA 9531 obtained from Instructional Farm, seeds were surface sterilized and twenty five seeds of cultivar Pusa 9531 was set to germinate in petridishes and moistened with solution of NaCl (100 mM) and PEG (12%) separately. Seeds were also germinated in distilled water (control) for comparsion of performance. Each treatment consisted of three replications in a complete randomized design (CRD). Germination count was done at each interval (12hrs, 24hrs, 48hrs and 72hrs) after germination. The germinated seeds were dissected with the help of sharped needle and then embryonic axis and cotyledons were kept separately and were used for biochemical analysis at 12, 24, 48 and 72 hours of germination.

Sampling and analysis

The samples were analyzed at 12, 24, 48 and 72 hours of germination for the changes in the accumulation of biochemicals. Superoxide dismutase activity was assayed by the method of Giannopolitis and Ries (1977). Catalase activity was measured according to the method of Goth (1991). Guaiacol peroxidase activity was measured by a method of Siegel and Galston (1967). Total phenol in the germinating seeds was estimated following the method of McDonald *et al*., (2001).

Statistical analysis

The data were taken in triplicates and the mean data in all the cases were subjected to statistical analysis following two factor factorial design using INDOSTAT version 7.1 soft ware.

RESULTS AND DISCUSSION

Superoxide dismutase (SOD)

The result shows that the activity of superoxide dismutase (SOD) indicated significant differences among stages, treatments and treatment x stage interaction in both embryonic axis and cotyledon (Table 1). Perusal of the data indicated that the embryonic axis in control seeds exhibited little change in SOD activity over the stages of germination. The activity increased upto 48 hours of germination followed by a decline. On the contrary, both the treatments showed same pattern of changes and the activity of the enzyme increased continuously over the stages. Of the two treatments, PEG 12% registered higher activity of SOD at all stages in comparison with the NaCl 100 mM treatment in the embryonic axis. At 72 hours of germination PEG 12% recorded an activity of 18.445 unit min^{-1} g^{-1} fresh weight (321.50% increase over control) against the corresponding value of 12.556 unit min^{-1} g^{-1}(186.93%) under NaCl treatment. The SOD activity in the cotyledon showed continuous increase over all the stages of germination under control as well as stress treatments. However, the cotyledon of the control seeds exhibited little change in SOD activity with the progress of germination. Of the two stress treatments, the PEG treated cotyledons showed higher activity of SOD enzyme in almost all stages of germination. At 72 hours of germination, the activity was found to be 12.364 unit min^{-1} g^{-1} fresh weight (144.73% increase over control) against 8.432 unit min^{-1} g^{-1} fresh weight under NaCl 100 mM (66.90% increase over control). Overall, the embryonic axis registered higher activity of SOD than the cotyledon in the present experiment. This finding was well consistent with the early reports of Wojtyla *et al*. (2006).

Guaiacol peroxidise (GPOX)

The GPOX activity in the embryonic axis showed continuous increase over the stages under control condition with the slope being very steep in between 24 and 48 hours (Table 2). The result was more or less consistent with the early finding of Bellani *et al*. (2002). Thereafter there was a slight increase. The activity of the enzyme mostly decreased under stress conditions as compared to the control in the present experiment. At 48 hours of germination the PEG 12% and

Table 1: Effect of drought and salinity stress on activity of superoxide dismutase (SOD) enzyme in embryonic axis and cotyledon of mungbean cultivar Pusa 9531

Stage(S)	SOD Embryonic axis*					SOD cotyledon*				
Treatment(T)	12hrs	24hrs	48hrs	72hrs	Mean	12hrs	24hrs	48hrs	72hrs	Mean
Control	4.805	5.156	5.352	4.376	4.922	3.226	4.303	4.989	5.052	4.392
PEG 12%	6.875(43.08)	8.235(59.72)	12.667(136.68)	18.445(321.50)	11.556	4.225(30.97)	5.862(36.23)	8.443(69.23)	12.364(144.73)	7.724
NaCl 100mM	5.853(21.81)	7.698(49.30)	10.335(93.11)	12.556(186.93)	9.111	4.331(34.25)	5.221(21.33)	6.706(34.42)	8.432(66.90)	6.173
Mean	5.844	7.030	9.451	11.792		3.927	5.129	6.713	8.616	
	S.E. m(±)			C.D.(P=0.05)		S.E. m(±)			C.D.(P=0.05)	
Treatment(T)	0.029			0.083		0.028			0.081	
Stage(S)	0.033			0.096		0.032			0.093	
T X S	0.057			0.167		0.055			0.162	

Data in parentheses indicate percent increase (+) or decrease (-) over control; *Data expressed as Unit min^{-1} g^{-1} fresh weight

Table 2: Effect of drought and salinity stress on activity of guaiacol peroxidase (GPOX) enzyme in embryonic axis and cotyledon of mungbean cultivar Pusa 9531

Stage(S)	GPOX Embryonic axis*					GPOX cotyledon*				
Treatment (T)	12hrs	24hrs	48hrs	72hrs	Mean	12hrs	24hrs	48hrs	72hrs	Mean
Control	13.840	14.160	39.040	40.640	26.920	0.800	2.038	2.518	46.640	12.999
PEG 12%	16.560(19.65)	10.368(-26.78)	10.496(-73.11)	43.600(7.28)	20.256	3.936(392.00)	1.840(-9.72)	4.016(59.49)	20.880(-55.23)	7.668
NaCl 100mM	12.944(-6.47)	12.976(-8.36)	7.360(-81.15)	28.000(-31.10)	15.320	7.040(780.00)	3.120(53.09)	3.850(52.90)	2.816(-93.96)	4.208
Mean	14.448	12.501	18.965	37.413		3.925	2.333	3.463	23.445	
	S.E. m(±)			C.D.(P=0.05)		S.E. m(±)			C.D.(P=0.05)	
Treatment(T)	0.028			0.081		0.028			0.082	
Stage(S)	0.032			0.093		0.032			0.095	
T X S	0.055			0.162		0.056			0.164	

Data in parentheses indicate percent increase (+) or decrease (-) over control; *Data expressed as ÄA470 min^{-1} g^{-1} fresh weight

Table 3: Effect of drought and salinity stress on activity of catalase (CAT) enzyme in embryonic axis and cotyledon of mungbean cultivar Pusa 9531

Stage (S)	Catalase Embryonic axis*					Catalase cotyledon*				
Treatment(T)	12hrs	24hrs	48hrs	72hrs	Mean	12hrs	24hrs	48hrs	72hrs	Mean
Control	318.326	235.760	581.646	364.073	374.951	467.838	528.089	605.077	596.151	549.289
PEG 12%	456.681(43.46)	355.146(50.64)	542.594(-6.71)	487.922(34.02)	460.586	601.729(28.62)	451.102(-14.58)	588.340(-2.77)	555.983(-6.74)	549.289
NaCl 100mM	474.533(49.07)	307.169(30.29)	505.774(-13.04)	338.410(-7.05)	406.471	572.720(22.42)	429.902(-18.59)	574.951(-4.98)	477.880(-19.84)	513.863
Mean	416.513	299.358	543.338	396.802		547.429	469.698	589.456	543.338	
	S.E. m(±)			C.D.(P=0.05)		S.E. m(±)			C.D.(P=0.05)	
Treatment(T)	0.028			0.082		0.028			0.082	
Stage(S)	0.036			0.095		0.032			0.094	
T X S	0.056			0.165		0.056			0.163	

Data in parentheses indicate percent increase (+) or decrease (-) over control; *Data expressed as μmol H_2O_2 min^{-1} g^{-1} fresh weight

Table 4: Effect of drought and salinity stress on phenol content in embryonic axis and cotyledon of mungbean cultivar Pusa 9531

Stage(S)	Phenol embryonic axis*					Phenol cotyledon*				
Treatment(T)	12hrs	24hrs	48hrs	72hrs	Mean	12hrs	24hrs	48hrs	72hrs	Mean
Control	4.028	6.063	8.897	1.821	5.202	2.548	2.245	3.448	2.626	2.717
PEG 12%	1.746(-56.65)	6.009(-0.89)	13.160(47.92)	2.927(60.74)	5.961	2.853(11.97)	3.066(36.57)	3.373(-2.18)	2.875(9.48)	3.042
NaCl 100mM	1.574(-60.92)	5.794(-4.44)	9.499(6.77)	2.090(14.77)	4.739	3.336(30.93)	4.12(83.52)	2.761(-19.92)	1.945(-25.93)	3.040
Mean	2.449	5.955	10.519	2.279		2.912	3.144	3.194	2.482	
	S.E. m(±)		C.D.(P=0.05)			S.E. m(±)		C.D.(P=0.05)		
Treatment(T)	0.028		0.082			0.028		0.083		
Stage(S)	0.032		0.094			0.033		0.096		
T X S	0.056		0.163			0.057		0.166		

Data in parentheses indicate percent increase (+) or decrease (-) over control, *Data expressed as millimol of gallic acid g^{-1} fresh weight

NaCl 100 mM registered a decrease of 73.11% and 81.15% over control. Thereafter at 72 hours of germination, NaCl treatment still registered decrease in the enzyme activity while PEG recorded 7.28% increase over control. Earlier Mandal and Singh (2000) observed a reduction in peroxidase activity of the embryonic axis under salinity stress in rice. Some what different pattern of changes in GPOX activity in cotyledon was recorded. Under control and PEG 12% the activity changed very little upto 48 hours after which there was are markable increase at 72 hours. Under salinity stress, the activity of GPOX registered very little change at all stages of germination. Both the stress treatments showed remarkable increase in the activity of the enzyme over control at 12 hours of germination. Thereafter, the activity decreased under PEG treatment while the treatment registered 53.09% increase over control. However, the GPOX activity was found to exhibit a decrease of 55.23% and 93.96% under PEG and NaCl, respectively, over control at 72 hours of germination. Overall, the embryonic axis recorded higher activity of GPOX than cotyledon in the present experiment.

Catalase (CAT)

The activity of catalase enzyme (CAT) showed significant differences among the treatments, stages and interaction of treatment x stage (Tables 3). Unlike SOD and GPOX, the CAT activity was higher in cotyledon than in embryonic axis. No definite pattern of changes in the activity was noted in the embryonic axis over the stages of germination. The highest activity was observed at 48 hours of germination. At this stage the PEG and NaCl treatment registered a decrease of 6.71% and 13.04%, respectively, over that of control. Both the stress treatments showed considerable increase over control at 12 and 24 hours of germination. However, at 72 hours the activity of CAT under PEG was 34.02% higher than control, while that of NaCl revealed a decrease of 7.05% as compared to control. Like the embryonic axis the activity of CAT in the cotyledon also did not reveal any definite pattern of changes over the stages of germination. Both the stresses showed substantial increase over control at 12 hours of germination followed by decrease over control in all the subsequent stages. The maximum reduction in the CAT activity in comparison to control was recorded under NaCl 100 mM treatment at 72 hours of germination (19.84% reduction over control).

Phenol content

In the embryonic axis, the phenol content showed a linear increase upto 48 hours of germination followed by a decline in all the conditions. The phenol content under PEG 12% showed decrease over control at 12 and 24 hours of germination and then 47.92% and 60.74% increase at 48 and 72 hours of germination, respectively. The phenol content under NaCl 100 mM indicated a decrease over control upto 48 hours of germination followed by an increase of 14.77% over control at 72 hours (Table 4). The phenol content in the cotyledon showed little change over the stages as compared to that in embryonic axis. In the initial hours of germination (12 and 24 hours), the phenol under both the stresses revealed increase over control. The salinity stress induced an increase of 83.52% over control at 24 hours while the corresponding increase under drought stress was 36.57%. This was followed by considerable decrease in salinity stress at both 48 and 72 hours but a slight decrease in drought stress followed by an increase at 72 hours. However, the content of phenol in the embryonic axis was found to be mostly higher than that in the cotyledon.

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