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Tissue Specific Drought Stress Response in Different Varieties of Mungbean (*Vigna radiata* L Wilczek) of Rajasthan

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ABSTRACT

Abiotic stresses such as extreme temperatures, low water availability, high salt levels, mineral deficiency and toxicity are frequently encountered by plants. These environmental stress factors negatively affect growth and productivity, and plants have evolved different mechanisms to respond to such challenges. For their survival under the stress conditions, plants respond by different molecular, cellular, biochemical and physical responses. These response mechanisms help plants to survive during the stress period as well as to recover after the stress period. Therefore, aim of the present study is to evaluate the various biochemical characteristic of six varieties of *Vigna radiata* (RMG-62, RMG-975, MUM-2, MSJ-118, RMG-492 and RMG-344) under different drought stress. Plants are exposed to different levels of drought stress by withdrawing water and parameters like leaf relative water content (RWC), proline content, total chlorophyll content, malondialdehyde (MDA) content & different antioxidant effect (Ascorbate peroxidase, Catalase, Guaiacol peroxidase & Superoxide dismutase) were measured. Therefore, present study proves that, general growth, physiological and biochemical traits can be used successfully as a way to screen different sensitive and tolerant varieties of Mung bean in drought stress condition.

Highlights

- Mungbean (*Vigna radiate*) adapt to different level of drought stress via production of various biochemicals and antioxidants.
- A measure of these antioxidants in six different variety of Mungbean (*Vigna radiate*), gives idea of drought sensitive and tolerant variety tested
- Tissue (leaves and root) were compared for the production of biochemicals and antioxidants, and it was found that leaves were able to produce more biochemicals and antioxidants as compared to roots.

Keywords: Drought stress, *Vigna radiata,* Proline, Chlorophyll, Lipid Peroxidation, Antioxidants

Drought stress is one of the most important factors, which is the central problem in the agriculture of the countries where irrigation sources are deficit and receive very low annual precipitations. Drought stress influences morphological, physiological and biological characteristics of the plant in every stage of plant growth (Basu *et al.* 2016). In the arid and a semi-arid region of Rajasthan and Gujarat, water deficit is the main factor that limits crops performance. Limitation of water sources, irregular annual rainfall during growth season and lack of sources management cause severe decreasing in crops yield at these regions. Therefore, drought stress during growth season is a crucial problem that needs attention. In these regions, it is necessary to introduce the Legume cultivars (Mung Bean) tolerant to dry conditions. Due to short-term growth, nitrogen fixation capability, soil reinforcement and prevention of soil erosion, Mungbean is superior to other plants for second culture. Mungbean is the most common crop in most tropical and sub-tropical regions that is cultivated after harvesting of wheat and harvest before planting of autumn crops (Sriphadet *et al*. 2007).

Mungbean (*Vigna radiata* L. Wilczek) is an important pulse crop which is an annual legume. It is

well suited to dry areas, mainly under irrigated conditions. It has the diploid chromosome number 2n = 2x = 22 (Tomooka *et al.* 2005). It is a short duration crop and grown as sole as well, as inter and multiple cropping system. It is a source of digestible protein. It improves the nutrient status of soil through atmospheric nitrogen fixation and adds humus to the soil (Tang *et al*. 2014).

MATERIALS AND METHODS

Plant growth and stress Treatment

Seed material of six varieties of Mung bean (RMG-62, RMG-975, MUM-2, MSJ-118, RMG-492 and RMG-344) provided from Rajasthan Agriculture Research Institute (RARI) Durgapura, was evaluated in the present study. Mature seeds of each variety were surface sterilized with 0.1% mercuric chloride (w/v) for 1 min, washed thoroughly with sterile distilled water. The mature seedlings were then planted in clay pots containing soil bed moistened with distilled water and grown in normal environmental condition. Pots were divided into three sets for each six varieties. Both sets were subjected to regular watering till the seeds turned into healthy plants. Water stress treatment was commenced as the irrigation was stopped and plants were subjected to drought stress. Plant sample were collected for different intervals Day 0, Day 3, Day 6 and Day 12 respectively. Day-0 is treated as Control against stressed plants. Samples for relative water content (RWC), Proline, Chlorophyll,Lipid Peroxidation assay and antioxidant assays were taken from the youngest alternate, trifoliate leaves.

Relative leaf water content (RWC)

Relative content of leaf water (RWC) was calculated according to the following equation (Turner 1981). Leaves were weighed to obtain leaf sample fresh weight (FW). The leaf samples was immediately hydrated to full turgidity for 4 hours under normal room light and temperature and were dried by filter paper and immediately weighed to obtain fully turgid weight .Samples were then oven dried at 80^0C for 24 hours and weighed (after being cooled down in a desiccators) to determine dry weight (DW). Finally, Leaf RWC% was calculated by: RWC (%) = [(FW-DW) / (TW-DW)] × 100.

Proline

Plant material homogenized by mortar in 3% aqueous sulphosalicylic acid (0.01g/ 0.5 ml) and the residue was removed by centrifugation at 12000 g for 10 min. 1 ml of the homogenized tissue reacts with 1 ml acid-ninhydrin and 1 ml of glacial acetic acid in a test tube for 1 hour at 100°C and the reaction was terminated in an ice bath. The reaction mixture was extracted with 2 ml toluene by mixing vigorously and left at room temperature for 30 min until separation of the two phases. The chromospheres-containing toluene (1 ml, upper phase) was warmed up to room temperature and its optical density was measured at 520 nm using toluene as a blank and finally the Proline concentration was determined in terms of μmol g^{-1} FW of plant according to the Bates *et al*. 1973, from a standard curve using D-Proline, according to the following equation: Proline = [(μg proline/mL × mL toluene)]/ [115.5 μg/umole]/ [(g sample/5]

Chlorophyll

Chlorophyll estimation was done according to Richardson *et al*. 2002. 20 mg leaf material was weighed into eppendorf. Add 1.0 mL DMSO to each eppendroff and mix it in motor and pestle. Centrifuge it and then obtained extract. Then the absorbance was measured using spectrophotometer at 645 and 663 nm, keeping DMSO as blank. Chlorophyll A, B is calculated according to following formula Arnon *et al.* (1994):

Chl a (g l^{-1}) = 0.0127 A663 – 0.00269 A645, Chl b (g l^{-1}) = 0.0029 A663 – 0.00468 A645

Total Chl (g l^{-1}) = 0.0202 A663 + 0.00802 A645

Lipid Peroxidation Assay

Lipid Peroxidation assay calculated according to Health and Packer, 1968. The leaves samples were homogenized by adding 0.5 ml 0.1 % (w/v) TCA using a mortar and pestle. The homogenate was transferred to a 2 mL eppendroff tube and centrifuge for 10 min (15000 x g, 4 °C). Supernatant is collected and solutions with or without thiobarbituric acid (+TBA and –TBA) were added to 0.5 ml with 1.5 ml 0.5% TBA diluted in 20 % TCA. The -TBA solution is formed by adding 20% trichloroacetic acidand 0.01% butyratehydroxytoluene (2, 6-Di-tertbutyl-4-methylphenol) and +TBA solution is formed by

0.65% thiobarbituric acid and same chemicals as in –TBA solution. Samples then kept in water bath at 95 °C for 25 min and then cooled by incubating on ice. In case the solution is not clear, it can be centrifuged for a further 5 min (15000 × g, 4 °C). Absorbance measured at 532 and 600 nm and further calculations made according to the following equation: MDA equivalents ($nmol.cm^{-1}$) =A. (Abs 532 +TBA) – (Abs 600nm+TBA) – (Abs 532nm –TBA)-(Abs 600nm –TBA), B. (Abs 400nm + TBA) – (Abs 600nm +TBA) * 0.0571, C. (A-B/15000)*106

Determination of Antioxidant Enzymes Activity

To determine the activity of antioxidant enzymes, a crude enzyme extract was prepared by homogenising leaf sample in ice coldhomogenization buffer using a mortar and pestle. The homogenate was transferred to a 2 mL eppendroff tube and centrifuge it at 8000 rpm for 10 minutes at 4°C. Supernatant is collected and used for enzymatic assays described below. All enzyme activities were expressed as mol Unit activity per mg protein.

Ascorbate peroxidase assay calculated according to Nakano and Asado,1981. 50µl supernatant were added with 100 µl 1mM EDTA, 100µl 5mM Ascorbate and 100µl 1mM Hydrogen Peroxide in 650 µl 50mM K-P buffer. Absorbance measured at 290nm.

Catalase assay calculated according to Aebi, 1984. 40µl supernatant were added with 10 µl 3% Hydrogen Peroxide in 950 µl 0.1mM EDTA. Absorbance measured at 240nm.

Guaiacol peroxidase calculated according to Chance and Maehly,1955. 25µl supernatant was added with 50 µl 5mM Hydrogen Peroxide in 925 µl of Reaction mixture (20mM Guaiacol, 50mM K-P Buffer). Absorbance measured at 470nm.

Superoxide dismutase assay calculated according to Kono, 1978.100µl supernatant were added with 100 µl Extraction buffer in 3mL of Reaction mixture (2306 µl 50mM Na-P buffer, 40µl 13.3µM NBT (Nitroblue Tetrazolium), 4µl 0.66mM EDTA, 600µl 10mM L-Methionine and 50µl 0.0033mM Riboflavin). Blank was also prepared with 200 µl of Extraction buffer in 3mL of Reaction mixture. Absorbance of control and test were measured at 565nm and percentage inhibition was calculated.

RESULTS AND DISCUSSION

As sessile organisms plants are challenged by various abiotic stresses like drought, salinity, high and low temperatures etc. As a water deficit area drought is a common problem faced in Rajasthan, which cause loss in yield of crop. A stressed plant responds by increase in various biochemical and antioxidants which can be used as biomarkers in plants (Arbona *et al.* 2013). These biomarkers in plants can include proline, Malondialdehyde, chlorophyll and antioxidants like catalase, ascorbate peroxidase, superoxide dismutase etc (Hasanuzzaman *et al*. 2012). Various biomarkers used in this study are summarized in table I and II for leaves and root respectively.

Morphological Parameters

Root development is strongly influenced by drought stress. A decrease in shoot: root ratio is a common observation under drought-stress. In addition, a greater percentage of fine roots optimise the exploratory capabilities of the root system. Hardening of roots, as revealed by an increased percentage of brown roots, is frequent in drought-stressed plants. In drought stressed plants, more fibrous roots have been observed when compared to control plants.

Biochemical Parameters

Relative water content (RWC) is the appropriate measure of plant water status in terms of leaf hydration, leaf water deficit and physiological water status. RWC was significantly reduced with water stress in all the tested varieties from 55% to 1.7%. Varieties that are resistant to drought have more RWC as shown in other studies (Alexieva *et al*. 2001, Babu *et al*. 2003), In our study MUM-2, RMG-62, MSJ-118 have more RWC as the stress progress as compared to RMG-975, RMG-492 and RMG-344.

Chlorophyll content is positively associated with photosynthetic rate, which increases biomass production and grain yield. The effect of drought stress decreases Chlorophyll content in the leaves (Bijanzadeh and Emam 2010; Li *et al*. 2006). As the drought progress i.e., with the mild drought stress it is not significant, but as the drought increases to moderate and severe MUM-2, RMG-62 have relatively more chlorophyll as compared to other

Table 1: Effect of drought stress on biochemical (chlorophyll, RWC, proline, MDA, per gram fresh weight) and antioxidant activity (Catalase, Ascorbate peroxidase, guaiacol Peroxidase and superoxide Dismutase) in leaves of different varieties of Mung bean

	Genotypes					
Treatment	**RMG 344**	**MSJ 118**	**RMG 492**	**MUM 2**	**RMG 62**	**RMG 975**
			Total Chl (mg.g^{-1})			
Control	28.83203	19.15578	22.15984	29.32716	32.39686	20.58422
Mild	24.50632	16.57369	17.41432	18.08979	25.5732	17.57289
Moderate	3.71417	6.84492	3.903547	13.88946	5.335667	16.47158
Severe	0.356723	2.23263	2.576737	1.750473	2.78643	2.78643
			RWC (%)			
Control	55.35	53.52	48.04	45.72	76.43	45.45
Mild	4.88	11.43	10.05	15.19	8.92	10.44
Moderate	4.57	8.21	6.24	4.27	6.07	5.48
Severe	3.25	3.73	5.37	3.84	4.49	1.70
			Proline Leaves (ug^{-1})			
Control	0.003596	0.003585	0.001901	0.003191	0.002985	0.001416
Mild	0.005398	0.004002	0.004581	0.003206	0.004256	0.004181
Moderate	0.00792	0.004089	0.004971	0.003835	0.004543	0.006242
Severe	0.008109	0.006897	0.005458	0.007016	0.006735	0.009462
			Malondialdehyde MDA Content Leaves (nmol.g^{-1})			
Control	1.982796	1.470968	1.204301	1.294624	0.735483	1.073118
Mild	0.526883	1.04086	1.251613	1.182794	1.501075	0.944086
Moderate	2.40215	3.55914	2.916129	2.027957	1.911828	2.086022
Severe	2.243011	2.348387	2.268817	1.868817	1.184946	1.572043
			Specific Activity of Catalase in Leaves (molUA/mg protein)			
Control	0.013	0.177	0.01	0.0125	0.063	0.008
Mild	0.05	0.033	0.024	0.013	0.01	0.01
Moderate	0.25	0.43	0.56	0.07	0.71	0.214
Severe	0.002	0.01	0.01	0.01	0.002	0.5
			Specific Activity of Ascorbate Peroxidase in Leaves (molUA/mg protein)			
Control	1.5	1.64	0.3	1.35	0.95	0.33
Mild	0.53	0.53	0.3	0.4	0.97	0.6
Moderate	66.67	66.67	0.25	5.77	46.67	45.22
Severe	11.82	11.82	3.125	10	136.67	20
			Specific Activity of Guaiacol Peroxidase in Leaves (molUA/mg protein)			
Control	0.5	0.09	0.16	0.625	0.26	0.28
Mild	8.5	33.3	0.46	1.74	3.54	1.84
Moderate	0.67	4.57	0.11	2.28	0.14	0.57
Severe	0.05	0.05	0.03	0.025	0.025	0.02
			Specific Activity of superoxide dismutase in Leaves (molUA/mg protein)			
Control	58.2	57	66	55.2	86.6	66.2
Mild	59.74	66.8	63.73	60	73	73.25
Moderate	130	118.3	95.6	91.4	101.24	133.23
Severe	221.24	570.78	252.52	340.14	159.13	616.52

four varieties, RMG-975, MSJ-118, RMG-492 and RMG-344 showing least chlorophyll content at severe stress.

Proline functions as osmoprotectant and it contributes in enhanced drought tolerance. There has been a report by other researches of increasing of proline amount due to drought stress (Yamada *et al*. 2005; Ashraf and Foolad 2007). Our study shows that leaves accumulate little more proline as compared to roots. In leaves maximum increase in proline content from 0.00141(μmol g^{-1} FW) to 0.00942 can be seen in RMG 975 followed by RMG-62 and MUM-2. Same is the case of roots there as maximum increase in proline content from 0.000705 (μmol g^{-1} FW) to 0.00521 was observed in case of RMG 975 followed by RMG-62 and MUM-2 as compared to variety RMG-344, RMG-492, MSJ-118. Modification of cellular membrane is one of the major effects of stress in plant cell. Blum and Ebercon 1981, Fu and Huang, 2001 showed that membrane integrity is one of the major factors in abiotic stress tolerance. Due to generation of reactive oxygen species in stress conditions, there is susceptibility of membranes to attack by reactive oxygen species.

Cellular injury of membrane lipids which results in lipid peroxidation can be used as an indicator of oxidative stress and measured as malondialdehyde (MDA) content, indicating possible damage. In our study, MDA content increases with the stress but leaves are more effected as compared to roots as shown in Table 1 and 2. In case of both leaves and roots varieties MUM-2, RMG-62, RMG-975 are

Table 2: Effect of drought stress on biochemical (chlorophyll, RWC, proline, MDA, per gram fresh weight) and antioxidant activity (Catalase, Ascorbate peroxidase, guaiacol Peroxidase and superoxide Dismutase) in roots of different varieties of Mung bean

	Genotypes					
Treatment	**RMG 344**	**MSJ 118**	**RMG 492**	**MUM 2**	**RMG 62**	**RMG 975**
			Proline Roots (ug^{-1})			
Control	0.004062	0.003483	0.002146	0.000972	0.002043	0.000705
Mild	0.004749	0.003488	0.004652	0.00306	0.003353	0.004256
Moderate	0.004954	0.004175	0.004846	0.004527	0.003883	0.004424
Severe	0.005609	0.004652	0.00654	0.005571	0.004679	0.005214
			Malondialdehyde MDA Content in Roots ($nmol.g^{-1}$)			
Control	0.290323	0.189247	0.10323	0.215054	0.135484	0.19785
Mild	0.066668	0.372043	0.184946	0.346237	0.2	0.24086
Moderate	0.068817	0.621505	0.03011	0.262366	0.00248	0.262399
Severe	1.578595	0.477421	0.350538	0.329032	0.316129	0.44086
			Specific Activity of Catalase in Roots (molUA/mg protein)			
Control	0.006	0.013	0.005	0.005	0.004	0.004
Mild	0.001	0.042	0.004	0.005	0.004	0.002
Moderate	0.08	0.17	0.12	0.08	0.08	0.125
Severe	0.033	0.05	0.014	0.025	0.025	0.014
			Specific Activity of Ascorbate peroxidase in Roots (molUA/mg protein)			
Control	0.38	1.64	0.2	0.9	1.44	0.3
Mild	0.24	6.4	0.12	0.32	0.48	0.23
Moderate	2.86	1.45	3.125	0.6	4	6.67
Severe	0.75	1.5	0.375	1.5	0.7	0.25
			Specific Activity of Guaiacol peroxidase in Roots (molUA/mg protein)			
Control	1.43	3.22	0.82	1.22	0.73	0.82
Mild	2.08	1.6	0.7	4.14	2.8	0.7
Moderate	3.75	10.67	2.24	2.83		2.24
Severe	0.33	1.5	0.3	0.25	0.2	0.3
			Specific Activity of Superoxide dismutase in Roots (molUA/mg protein)			
Control	64	64	62.1	60.24	62	65.27
Mild	124.66	160.3	123.6	117.51	100	118
Moderate	125	146.2	112.11	135.1	113.2	120
Severe	342.5	230.4	356.12	337.8	411.2	919.12

found to be tolerant because of less content of MDA till severe stress whereas varieties MSJ-118, RMG-344, RMG-492 are found to be sensitive towards drought.

Antioxidant Parameters

One of the reactions of drought stress isexcessive generation of reactive oxygen species (ROS), such as singlet oxygen, superoxides, and hydrogen peroxide. These ROS, plays very important role in signalling but uncontrolled production of ROS can be harmful to the cell. So to protect the cell from oxidative damage by higher levels of antioxidants are produced to scavenge these ROS (Gill and Tuteja 2010). We studied four antioxidant enzymes, i.e. Catalase, ascorbate peroxidase, Guaiacol peroxidase and superoxide dismutase. These are key antioxidant enzyme and are upregulated in stress conditions as shown by other studies too (Anjum *et al.* 2011; Siddiqui *et al*. 2015).

In case of Mung bean, as the drought stress increase, from mild to severe, all the antioxidants are also increased in leaves as well as in roots. Overall it can be seen from table that leaves of RMG-62, RMG-975, have more of superoxide dismutase and ascorbate peroxidase till severe drought as compared to varieties MUM-2, MSJ-118, RMG-492 and RMG-344 and in caseguaicol peroxidase maximum activity is shown in MSJ118 as compared to other varieties where as in case of catalase maximum activity is shown by RMG-62, RMG-975 as compared to other tested varieties till severe stress.

In case of roots, like leaves levels of all antioxidants are increasedas the stress is increased.RMG-62, RMG-975, have maximum superoxide dismutase as compared to other varieties till severe stress. In case of ascorbate peroxidase RMG-975, RMG-62 is increased till moderate stress; level of superoxide dismutase in MSJ 118 is comparable to other two varieties but is increased till mild stress only. In case of guaiacol peroxidase MSJ 118 have maximum enzymes till moderate stress as compared to other varieties. In case of catalase, RMG 344, RMG 975, RMG 62 shows maximum activity till mild stress only, in severe stress its activity falls own abruptly.

When compared at the level of tissue its well clear that leaves produce more of enzymes and biochemicals to fight with the drought stress as compared to roots. Possible reason can be that leaves are in direct contact with sun as compared to roots and roots only absorb water for transporting it to leaves. Another reason can be that cell wall of roots are more hard in comparison to leaves and being below the ground do not face direct stress, therefore produce less of biochemicals and antioxidants (de Azevedo Neto *et al*. 2006; Bian *et al*. 2009).

CONCLUSION

In present work, the sharp increase in Proline content attribute to the genes for synthesis and degradation of Proline. Drought stress increases oxidative stress indicated as lipid peroxidation. In most stress tolerant cultivars, chlorophyll levels were increased and caused to a more stress tolerance of these cultivars. Our data interpreted that RMG 975 is the drought tolerant variety. Leaves are more affected from drought rather than Roots and Superoxide dismutase is the enzyme which helps varieties to survive even in severe conditions.Overall it can be concluded that RMG 975, RMG 62 are more tolerant variety as compared to others and RMG 492 and RMG 344 being sensitive. Therefore, in present study proves that, general growth, physiological and biochemical traits can be used successfully as way to screen different sensitive and tolerant varieties of Mung beanin drought stress condition.

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REFERENCES

Aebi H 1984. Catalase *in vitro*. *Methods in enzymology*, **105**, 121-126.

Alexieva V, Sergiev I, Mapelli S, Karanov E 2001. The effect of drought and ultraviolet radiation on growth and stress

markers in pea and wheat. *Plant, Cell & Environment*, **24**(12): 1337-1344.

Anjum SA, Xie XY, Wang LC, Saleem MF, Man C Lei W 2011. Morphological, physiological and biochemical responses of plants to drought stress. *African Journal of Agricultural Research*, **6**(9): 2026-2032.

Arbona V, Manzi M, Ollas CD, Gómez-Cadenas A. 2013. Metabolomics as a tool to investigate abiotic stress tolerance in plants. *International journal of molecular sciences*, **14**(3): 4885-4911.

Arnon DI 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. *Plant physiology*, **24**(1): 1.

Ashraf M, Foolad M 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany*, **59**(2): 206-216.

Babu RC, Nguyen BD, Chamarerk V, Shanmugasundaram P, Chezhian P, Jeyaprakash, P, ... Wade LJ 2003. Genetic analysis of drought resistance in rice by molecular markers. *Crop Science*, **43**(4): 1457-1469.

Basu S, Ramegowda V, Kumar A, Pereira A 2016. Plant adaptation to drought stress. *F1000 Research*, **5**.

Bates LS, Waldren RP, Teare ID 1973. Rapid determination of free proline for water-stress studies. *Plant and soil*, **39**(1), 205-207.

Bian S, Jiang Y 2009. Reactive oxygen species, antioxidant enzyme activities and gene expression patterns in leaves and roots of Kentucky bluegrass in response to drought stress and recovery. *Scientia Horticulturae*, **120**(2): 264-270.

Bijanzadeh E, Emam Y 2010. Effect of defoliation and drought stress on yield components and chlorophyll content of wheat. *Pakistan Journal of Biological Sciences*, **13**(14): 699.

Blum A, Ebercon A 1981. Cell membrane stability as a measure of drought and heat tolerance in wheat. *Crop Science*, **21**(1): 43-47.

Chance B, Maehly AC 1955. Assay of catalases and peroxidases. *Methods in enzymology*, **2**: 764-775.

de AzevedoNeto AD, Prisco JT, Enéas-Filho J, de Abreu CEB, Gomes-Filho E 2006. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. *Environmental and Experimental Botany*, **56**(1): 87-94.

Fu J, Huang B 2001. Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. *Environmental and Experimental Botany*, **45**(2): 105-114.

Gill SS, Tuteja N 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant physiology and biochemistry*, **48**(12): 909-930.

Hasanuzzaman M, Hossain MA, da Silva JA T, Fujita M. 2012. Plant response and tolerance to abiotic oxidative stress: antioxidant defence is a key factor. In Crop stress and its management: Perspectives and strategies (pp. 261-315). Springer Netherlands.

Heath RL, Packer L 1968. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of biochemistry and biophysics*, **125**(1): 189-198.

Kono Y. 1978. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. *Archives of Biochemistry and Biophysics*, **186**(1): 189-195.

Li RH, Guo PG, Michael B, Stefania G, Salvatore C 2006. Evaluation of chlorophyll content and fluorescence parameters as indicators of drought tolerance in barley. *Agricultural Sciences in China*, **5**(10): 751-757.

NakanoY, Asada K 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and cell physiology*, **22**(5): 867-880.

Richardson AD, Duigan SP, Berlyn GP 2002. An evaluation of noninvasive methods to estimate foliar chlorophyll content. *New phytologist*, **153**(1): 185-194.

Sriphadet S, Lambrides CJ, Srinives P 2007. Inheritance of agronomic traits and their interrelationship in mungbean (*Vigna radiata* (L.) Wilczek). *Journal of Crop Science and Biotechnology*, **10**(4): 123-132.

Tang D, Dong Y, Ren H, Li L, He C 2014. A review of phytochemistry, metabolite changes, and medicinal uses of the common food mung bean and its sprouts (*Vigna radiata*). *Chemistry Central Journal*, **8**(1): 4.

Tomooka, N., Vaughan, D. A., & Kaga, A. 2005. Mungbean [*Vigna radiata* (L.) Wilczek] Genetic Resources, Chromosome Engineering, and Crop Improvement: *Grain Legumes*, **1**: 325-45.

Turner N.C. 1981. Techniques and experimental approaches for the measurement of plant water status. *Plant and soil*, **58**(1): 339-366.

Yamada M, Morishita H, Urano K, Shiozaki N, Yamaguchi-Shinozaki K, Shinozaki K, Yoshiba Y 2005. Effects of free proline accumulation in petunias under drought stress. *Journal of Experimental Botany*, **56**(417): 1975-1981.