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 radiata*) 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 radiata*), 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 subtropical 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 (DW). Samples were then oven dried at 80°C 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 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 eppendorf 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 acid and 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 532 nm –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 cold homogenization 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 | 2.57637 | 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 was observed in case of 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.

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 (μg\(^{-1}\)) | Control | 0.004062 | 0.003483 | 0.002146 | 0.000972 | 0.002043 | 0.000705 |
| | Mild | 0.004749 | 0.003488 | 0.004652 | 0.000306 | 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 | 0.125054 | 0.135484 | 0.19785 |
| | Mild | 0.066668 | 0.372043 | 0.184946 | 0.346237 | 0.00248 | 0.24086 |
| | Moderate | 0.068817 | 0.621505 | 0.03011 | 0.262366 | 0.00248 | 0.262399 |
| | Severe | 1.578595 | 0.477421 | 0.350538 | 0.392032 | 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 is excessive generation of reactive oxygen species (ROS), such as singlet oxygen, superoxides, and hydrogen peroxide. These ROS, play a 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 case guaiacol 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 increased as 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 bean in drought stress condition.

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