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Responses of Mungbean to Water Deficit, Water use Efficiency and Drought Resistance

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ABSTRACT

Backround: Legumes are the second important agricultural crop of great prominence to humans. Among 20000 legume species the mungbean is one of the most important grain cultivated in India. Drought is a major environmental stress that affects mungbean in the sub-humid, dry and intermediate zones of India. The present study records the response of mung bean varieties to water stress during its growth stage.

Methods: The impact of drought stress imposed on the crop was evaluated by measuring the water relation parameters and the biochemical progresses like osmolyte accumulation, nitrate assimilation and antioxidant system in Mung bean during 2017-2018 in Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore.

Result: Drought stress altered the water status of the crop by reducing the RWC, which was enhanced in drought susceptible varieties. Increased amount of proline denotes the osmoregulatory mechanism in the crop to bring about resistance and the elevated levels of antioxidant enzymes shows the protective mechanism in the crop at cellular level.

Key words: Antioxidant enzymes, Chlorophyll stability index, Drought stress, Mung bean, Nitrate reductase enzyme, Proline accumulation, Relative water content.

INTRODUCTION

Mungbean is one of the main pulse crops in India. It is popularly known as “Moong Dal or Green gram” and is basically a tiny circular shaped bean that is green in colour. It is rich source of proteins, vitamins and minerals for the poor’s vegetarian diet in developing and under developing countries.

In India mungbean is grown in two seasons, July-August (*kharif*) and March-April (summer). And it is mostly grown in *kharif* crop in Rajasthan, Maharashtra, Gujarat, Karnataka, Andra Pradesh, Madhya Pradesh and Uttar Pradesh *etc.* But in Tamil Nadu, Punjab, Haryana and Bihar it is grown as a summer crop. Green gram is widely cultivated throughout the Asia, including India (Lata and Kushwaha (2020), Kumar *et al.,* 2020). In India, Rajasthan (30%) and Maharashtra (17.73%) are the two major Moong producer of country contributes about 50% of total production. Andhra Pradesh (10.32%), Bihar (8.59%), Uttar Pradesh (4.39%), Tamil Nadu (3.85%) and Madhya Pradesh (2.58%) are other major mungbean producing states of the country. India is the largest producer and consumer of mungbean and accounts for about 65% of the world acreage and 54% of the world production of this crop. It is the third most important pulse crop in India, occupying nearly 3.72 million ha area with 1.56 million tons production.

The dried beans are prepared by cooking or milling. They are eaten whole or split. Green gram is sometimes specifically grown for hay, green manure or as a cover crop. Mungbean has a major impact on immunity; its regular diet can enhance the immune power. Germinated seeds of Mungbean contain anti-carcinogenic, antibacterial and antifungal properties which neutralize the toxicity. Since, it is an important ingredient in several protein supplements and nutraceutical formulation. Therefore it can be used for the welfare of human beings. It has a fantastic property to fix the atmospheric nitrogen by forming symbiotic relation with *Rhizobium* bacteria which also beneficial for the crop succeeding (Ali, 2010). Water and salt stress are the main environmental factors which act as major constraints for Mungbean. Stress changes the growth pattern by affecting the major physiological and biochemical parameters (Prakash *et al.* 2017).

Drought is temporary natural climatic feature and takes place when there is more moisture loss from soil surface and it is one of the abiotic environmental stresses which drastically affect all agricultural crops by reducing the yield. Similarly mungbean also gets effected by Moisture stress and reduction in pod filling and pod yield has been recorded.

During the drought conditions water potential and turgor are decreased which consequently disturbs the normal functioning of plant body (Hsiao, 1974). Drought is a

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worldwide problem and dangerous for food productivity. Globally, crop yield is decreased by biotic or abiotic stresses and these adverse conditions affects the plant's morphological, physiological and biochemical activities. Worldwide environmental issues has focused on detailed studies on the abiotic stress among which drought stress has major impact on crop plants. A two-fold reduction in the overall plant growth is seen under drought and along with this its impact on the various physiological and biochemical parameters is extremely potential to such an extent where the crop yield and production is highly reduced (Parvaze *et al.,* 2018).

Drought stress results when the plant's water content is reduced enough to interfere with normal plant process and when water loss from the plant exceeds the ability of the plant's roots to absorb water. There are many physiological effects of drought is the decrease of photosynthesis. Drought symptoms include leaves drooping and loss of turgor in needles, yellowing wilting and premature leaf fall are frequently connected with moisture stress. Drought stress leads to stomatal closure and limitation of gas exchange. Drought stress resulted reduction of water content, closure of stomata and decrease in cell enlargement, growth diminished leaf water potential and turgor loss. Severe water stress may result in the hold of photosynthesis, stoppage of metabolism and finally the death of plant. In leguminous plants, drought also reduces nitrogen fixation and its related traits. This phenomenon has been observed in mungbean (*Vigna radiata* L.).

Stress changes the growth pattern by affecting the major physical and biochemical parameters of mungbeans. Insufficient water levels limit the plant growth and crop productivity. Drought stress being a global issue the present experiment was conducted to evaluate the various physiological and biochemical changes taking place in selected mungbeans varieties under stress.

MATERIALS AND METHODS

A pot culture experiment was conducted at Department of Botany, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamil Nadu, during 2017-2018, utilizing four mungbean (*Vigna radiate* L.) verities, such as CO6, CO7, CO8, VBN2. Seeds were collected from Tamil Nadu Agricultural University, Coimbatore and sown in pots filled with red soil and sand (3:1). The experiment was triplicated and randomly selected plants were used for further studies.

Morphological parameters

At the time of harvest the morphological features *viz*., shoot and root length, numbers of leaves/ plant and leaf area index were measured using standard method.

Physiological parameters

Relative water contents (RWC)

Relative water content of leaf relative water content was estimated according to the method of Castillo (1996) for each treatment. Samples (0.5 g) were saturated in 100 ml distilled water for 48 h at 4°C in dark and their turgid weights were recorded. Then they were oven-dried at 65°C for 48 h and their dry weights were recorded. RWC was calculated as follows:

$$RWC\ (\%) = [(FW - DW) / (TW - DW)] \times 100$$

Where

FW, DW and TW are fresh weight, dry weight and turgid weight, respectively.

Cellular membrane thermostability

Leaf discs (0.5 cm diameter) weighing 0.2 g were cut and washed with distilled water and infiltrated in 20 ml of distilled water in test tubes covered with plastic wrap. The control tubes were kept at 10°C for 15 to 22 hours. An identical set of tubes were treated with 43% poly ethylene glycol (PEG) which gives an osmotic stress of about -18 bars and kept separately for 24 hours. Later PEG solution was drained off and the discs were washed and immersed in deionized water for 24 hours for electrolyte leakage. The initial leakage was measured through EC meter and the discs were heated at 110°C for 20 minutes and the final leakage was measured and the membrane thermostability was calculated using the formula of Martineau *et al*., (1979):

$$1-[(1-T_1/T_2) / (1- C_1/ C_2)] \times 100$$

Where,

T and C refer to the treatment and control samples, while the subscripts 1 and 2 denote the initial and final electrical conductivities, respectively.

Chlorophyll stability index (CSI)

0.2 g of fresh leaf discs were immersed in 10 ml distilled water in test tubes. One set was kept at room temperature and the other set was incubated in a water bath at 60°C for one hour. The water was decanted and the tissue was extracted for chlorophyll in 80% acetone and centrifuged. After centrifugation the supernatant containing the pigments (dissolved in acetone) was saved and its absorbance was read at 663 nm against acetone blank. The difference between the control and treatment was calculated as the chlorophyll stability index (Kaloyereas, 1958).

Biochemical parameters

Estimation of protein

Five hundred mg of plant material (leaf) were weighed and macerated in the pestle and mortar with 10 ml of 20% Trichloroacetic acid. The homogenate was centrifuged for 15 minutes at 600 g. the supernatant was discarded. The pellet, 5 ml of 0.1 N NaOH was added and centrifuged for 5 minutes. The supernatant was saved and made up to 10 ml of 0.1 n NaOH. This extract was used for protein estimation. One ml of extract was taken in a 10 ml test tube and 5 ml of reagent C was added the solution was mixed and kept in darkness for 10 minutes. Later 0.5 ml of reagent folin- phenol reagent was added and the mixture was kept in dark for 30 minutes. The sample was read at 660 nm in a UV spectrophotometer.

Estimation of carbohydrate

100 mg of sample were hydrolysed in a boiling test tube with 5 ml of 2.5 N HCL in a boiling water bath for a period of 3 hours. It was cooled at room temperature and solid sodium carbonate was added until effervescence created. The content was centrifuged and the supernatant was made to 100 ml by using distilled water. From this 0.2 ml sample was pipetted out and made up the volume to one ml of distilled water. Then 4 ml of anthrone reagent was added and heated for eight minutes in a boiling water bath. Then it was cooled rapidly and the green colour developed was read at 630 nm.

Osmolytes

Proline accumulation

The proline content was estimated by the method of Bates *et al*., (1973). 1 g leaf tissue was homogenized with 10 ml of 3% sulphosalysilic acid and filtered through Whatman No. 2 filter paper. To an aliquot of 2 ml of filtrate, 2 ml glacial acetic acid and 2 ml of acid ninhydrin reagent (1.5 g ninhydrin dissolved in 20 ml orthophosphoric acid and 30 ml glacial acetic acid) was added and the tubes were incubated in boiling water bath for one hour. The heating was terminated by immediately transferring the tubes to ice bath and after adding 4 ml toluene and the reaction mixture was vortexed to bring the chromophore to the toluene layer, which was separated using separatory funnels. The absorbance of the chromophore was read at 520 nm. L- Proline standard was used for quantification and the proline content in the sample was calculated using the formula:

$$\frac{\mu g \text{ proline} \times \text{ml toluene} \times 5}{115.5 \times \text{g sample frwt.}} = \mu \text{ mole of proline/g}$$

Antioxidants

Lipid peroxidation

Fresh leaf material (0.5 g) was ground with 10 ml of distilled water, filtered through 4 layers of muslin cloth and centrifuged at 7000 rpm for 10 minutes. The supernatant was collected and an aliquot of 2 ml was taken to which 5 ml of 0.5% (w/v) thiobarbituric acid (TBA) in 20% (w/v) trichloroacetic acid (TCA) was added and the mixture was heated at 100°C for 30 minutes. The samples were cooled and the absorbance was read at 532 nm and 600 nm using a UV spectrophotometer (Shimadzu Corporation, Japan). The manlondialdehyde (MDA) was quantified by utilizing the extinction coefficient of 155 mM^{-1} cm^{-2} (Heath and Packer, 1968) and expressed in nmol g f wt^{-1}.

Peroxidase enzyme activity

Peroxidase activity was assessed following the oxidation of O-dianisidine following the method of Malik and Singh (1980).

1 g of leaf tissue was homogenized in 5 ml of phosphate buffer (pH 7.0) using a pre-cooled mortar and pestle. The homogenate was centrifuged at 10,000 rpm for 10 minutes and the supernatant was saved for further assay. For the assay, 3.5 ml of phosphate buffer (pH 6.5) was taken in a clean dry cuvette and to it 0.2 ml of enzyme extract and 0.1 ml of freshly prepared O-dianisidine solution was added. The assay mixture was brought to room temperature and placed in the spectrophotometer at 430 nm. Then 0.2 ml 0.2 M H_2O_2 was added and immediately the stopwatch was started. Absorbance was read at every 30 seconds interval up to 3 minutes. Increase in absorbance was plotted against time and from the linear phase the change in absorbance per minute was read. Enzyme activity was expressed in terms of rate of increased absorbance per unit time per mg protein or tissue weight. Water was used as a blank for the assay.

Superoxide dismutase activity (SOD)

Superoxide dismutase (EC 1.15.1.1) was assayed by monitoring the inhibition of photo reduction of nitro blue tetrazolium (NBT) according to the method of Beauchamp and Fridovich (1971). Leaf samples were homogenized in four volumes (w/v) of an ice -cold buffer containing 0.1 M Tris-HCl, 1 M EDTA and 0.05% Triton-X 100. The homogenates were filtered through four layers of cheese cloth and centrifuged at 4°C for 30 minutes at 15000 rpm. The supernatant collected was used for SOD assay. The reaction mixture contained 50mM phosphate buffer (pH 7.8), 0.053 mM NBT, 10 mM methionine, 0.053 mM riboflavin and an appropriate aliquot of enzyme extract. The reaction was started by switching on the light and allowing running for 7 minutes. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT as monitored at 560 nm.

Nitrate reductase activity (NRA)

Nitrate reductase was assayed *in vivo* following the method of Hageman and Huckles (1971). 0.5 g leaf tissue of was cut into fragments and suspended in 5 ml of phosphate buffer pH 7.0 containing 0.1 M potassium nitrate in test tubes. The tubes were vacuum in filtered and incubated at 30°C for 30 minutes. After incubation, the contents of the tubes were filtered through Whatman's filter discs (No.1) to remove the leaf fragments and the buffer with the in filtered enzyme was saved for assay. The nitrate formed was quantified using 2.5 ml sulphanilamide reagent (1% in 25% HCl) and 2.5 ml 1- napthylethylenediamine dihydrochloride (NEDH). The absorbance of pink color solution was read at 540nm. Standard curve was prepared by using known concentrations of sodium nitrite and the activity was expressed as mol NO_2 g^{-1} fr.wt^{-1} h^{-1}.

RESULTS AND DISCUSSION

Mungbean (*Vigna radiata* L.) is an important leguminous crop of family Fabaceae and possess 2n = 22 chromosomes with self-pollination as a mode of reproduction and environment friendly food legume of dry land agriculture with rich source of protein.

Morphological parameter

Shoot and root length

Crop tolerance to abiotic stress factors very complex at cellular levels drought is generally characterized root and shoot growth. There is significant reduction in shoot and root length. This in turn affects the growth of the crop. This inhibition of growth relates to the cell elongation process which is impaired during drought. Under stress the plant showed morphological changes and it was evidently seen in our research. Water stress decreased plant height in all varieties through the magnitude of the effect of stress. The maximum shoot length was observed CO7 (12.33±1.05) when compared to other varieties (Fig 1). Whereas root length was reduced uniformly under water stress (9.00: CO6, 7.63: CO7, 6.73: CO8, 6.73: VBN2). The above results conforms that the plant height was declined by drought stress.

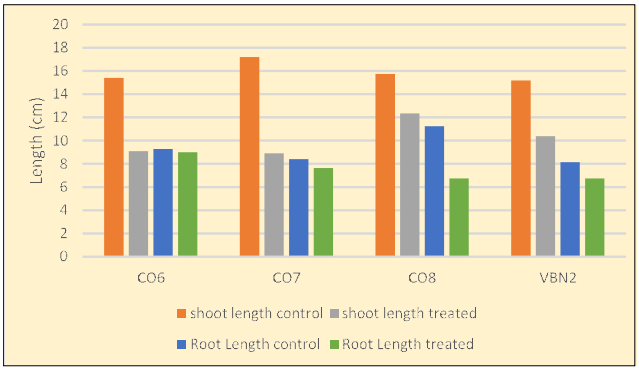

Fig 1: Effect of drought stress on shoot and root length.

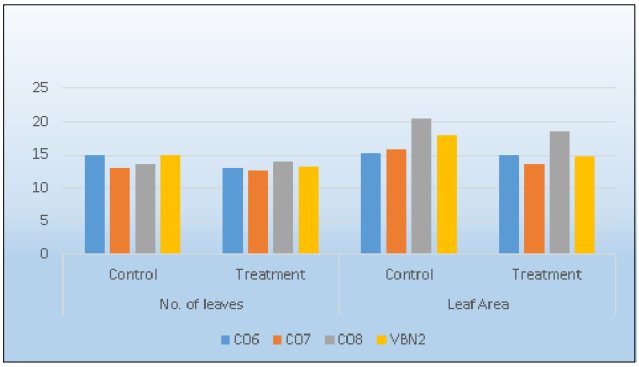

Fig 2: Effect of drought stress on number of leaves and leaf area (cm^2).

In plants, drought stress leads to a rapid decrease or increase in the root length. Comparatively, the root length becomes less affected under drought stress as compared with its shoot length. The decrease in shoot length may be either due to decrease in cell elongation resulting from by Choi and Park *et al.,* (2000). The reduction of shoot length loss of water by mechanisms of drought stress.

Koehler *et al.,* (1982) also observed that stalk elongation as expressed by plant height in drought stressed plants was less than 80% of the plants in well-watered plots. A strong and positive relationship between stalk elongation and water content was reported by Shih and Gascho (1980). In the case of sugarcane, the reduction in shoot height indicated the reduction in final sink size. The cell expansion rather than the cell division appeared to be sensitive to water stress, which might be the cause of reduction in plant height.

Number of leaves

The data on number of leaves revealed that there was significant decrease in number of leaves due to water stress compared to control. In control plants the number of range from 13 (CO6) to 15, where as in stressed plants from 12 (CO7) to 14 in CO8. In control plants the maximum leaf number in VBN2 (15 nos.) and minimum leaf number in CO7 (13 nos.). Where as in stressed plant the maximum leaf number in CO8 (14 nos.) and minimum in CO6 (13 nos.) (Fig 2).

Reduction in leaf number due to water stress was earlier reported by Ranawake *et al.* 2011 and Mahdi *et al.* 2013 in mungbean and by Turk and Hall (1980) in cowpea.

Leaf area

The effect of drought stress on the leaf area of mungbean genotypes was observed at control and treatment stages and effect was pronounced in the control, while the effect was less in the treatment. The leaf area range from 15.31 cm^2 (CO6) to 20.46 cm^2 (CO8) in control plants (Fig 2). Where as in stressed plants the leaf are range from 13.75 cm^2 (CO7) to 18.63 cm^2 (CO8) was recorded in green gram plants.

The results showed that the number of leaves and leaf area was reduced. This might be due to the lateral adaptive mechanism of plants to reduce the loss of water, hence to avoid more damage to cell.

Table 1: Effect of drought stress on relative water content (%), membrane injury (%) and chlorophyll stability index.

Genotype	Relative water content (%)		Membrane injury(%)		Chlorophyll stability index	
	Control	Treatment	Control	Treatment	Control	Treatment
CO6	93.5±2.78	80.7±9.95	13.06±4.2	26.8±5.10	0.21±0.01	0.27±0.02
CO7	68.2±10.98	67.4±5.36	28.08±3.1	60.3±1.88	0.11±0.01	0.12±0.01
CO8	82.1±6.82	70.7±5.44	23.8±4.13	71.2±4.05	0.02±0.00	0.05±0.01
VBN2	83.3±6.52	72.2±4.04	19.1±4.24	63.7±4.51	0.13±0.01	0.39±0.02
SEd	5.70689		3.28227		0.00886	
CD (p<0.05)	12.09824		6.95819		0.01878	

Values are mean ± SD of three samples.

Physiological parameters

Relative leaf water content

One of the early symptoms of drought stress is the decrease of RWC which is considered to be the best integrated measure of plant water status, representing variation in the water potential, turgor potential and osmotic adjustment in plant in plant tissues (Rampino *et al.,* 2006; Eva *et al.,* 2010). The relative water content was reduced uniformly under drought in all four varieties. Variety CO7 (67.36%) recorded a higher reduction in RWC under water stress, while variety CO6 (80.70%) recorded a least reduction (Table 1). Variety CO6 maintained a high RWC than others both in control and treatment (93.54% and 80.7%). Decrease in relative water content (RWC) was a main factor resulting in reduced growth in response to osmotic stress inpea (Alexieva *et al.,* 2001). The RWC indirectly relates to the tolerance level of any crop, when varieties which showed minimum reduction in RWC showed reduced pigments damage and increased levels of osmolytes which finally lead to tolerance and give maximum yield under drought. A similar report of reduced RWC was noted by Chang *et al.* (2016) in sugarcane plants and in maize by Ahmad *et al.* (2016).

Membrane injury (%)

Membrane injury randomly increased in both control and drought plants. In control plants the membrane injury ranged from CO6 (13.06%) to CO7 (28.07%), while it varied from CO6 (26.78%) to 71.16% under stress (Table 1). The maximum membrane injury rate was recorded in CO8 (71.16%). The minimum rate was recorded in (13.06%) in CO6 genotype under stress. It is well known that drought stress induced production of ROS which leads to lipid peroxidation of membrane lipids reflecting the stress induced damage in leaves. This seems as a parameters for drought screening.

Chlorophyll stability index (CSI)

The chlorophyll stability index was increased under stress in all varieties (0.27 in CO6, 0.12 in CO7, 0.05 in CO8 and 0.39 in VBN2) compared to control plants. The chlorophyll stability index varied between 0.11 (CO7) to 0.21 (CO6) in control plants. Where as in stressed plants it varied from 0.12 (CO7) to 0.39 (VBN2). The tolerant varieties generally possessed high pigment concentration and a higher chlorophyll stability index, which was contrary in case of susceptible types. Hence pigments and chlorophyll stability index may be used for screening drought tolerance. Reduced chlorophyll stability index under drought has been reported in maize in Meenakumari *et al.* (2002). A decrease in CSI was observed in all varieties invariably this reduction in the CSI may be directly correlated to the distraction of the chlorophyll pigments under stress. Similar reports have been obtained in various other crops and observing the CSI will help to understand the tolerance of crops for drought stress (Table 1).

Proteins

The total proteins estimated and the quantity was measured as 6.70, 3.83, 4.83 and 3.93 $mg^{-1000gm}$ in CO6, CO7, CO8 and VBN2 respectively in normal plants. Under stressed conditions, proteins were reduced in quantity in all varieties and maximum protein content seen in CO6. The protein content is varied from (CO7) 3.83 mg g^{-1} to (CO6) 6.70 mg g^{-1} in control plants (Fig 3). Where as in stressed plants varied from (CO6) 1.63 mg g^{-1} to (CO7) 3.20 mg g^{-1}. Accumulation of protein is a results on reciprocal regulation of protein synthesizing enzyme which is increased during stress. Tolerance mechanism in water stress may be associated with an accumulation of osmo-protactants like proline, glycine betaine and sugar. Similarly accumulation of protein also an important way to protect plants from stress.

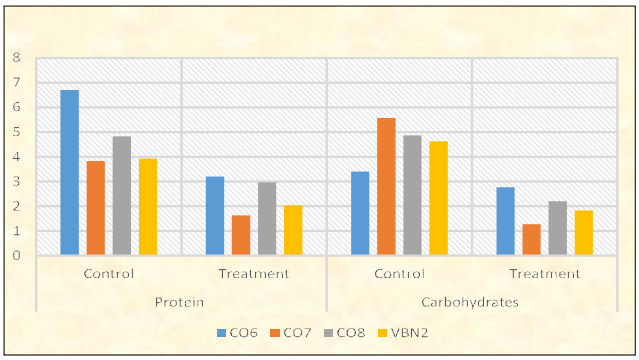

Fig 3: Estimation of total protein and carbohydrates in mungbeans (mg/g^{-1}).

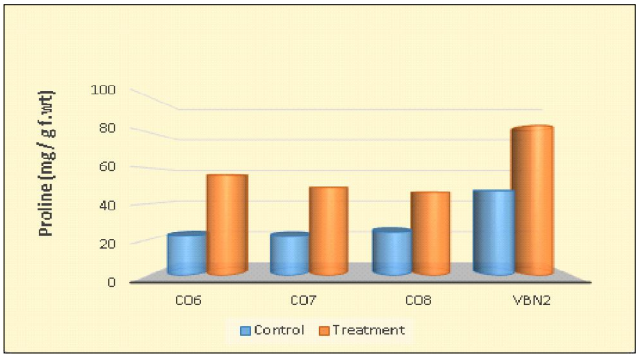

Fig 4: Proline accumulation in mungbeans (µg/g $fr.wt^{-1}$).

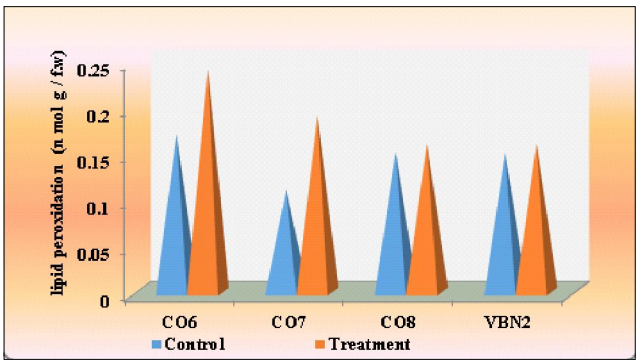

Fig 5: Effect of drought stress on lipid peroxidation.

Carbohydrates

The total carbohydrate estimated and the quantity was measured as 3.40, 5.57, 4.87 and 4.63 mg g^{-1} in CO6, CO7, CO8 and VBN2 respectively in normal plants. Under stressed conditions, carbohydrate were reduced in quantity in all varieties and maximum carbohydrate content seen in CO7. The carbohydrate content is varied from (CO6) 1.27 mg g^{-1} to (CO7) 2.77 mg g^{-1} in control plants. Where as in stressed plants varied from (CO7) 1.63 mg g^{-1} to (CO6) 3.20 mg g^{-1} (Fig 3). Generally there is increase or decrease in total sugar or carbohydrates in order to being about osmotic adjustment a reduction is decreased in stressed plants, which may be an indication of susceptibility of the varieties selected. In general accumulation of sugar is showed which supports osmotic adjustment.

Proline accumulation

After having imposed drought stress for a period of 10 days, the proline content of leaf tissues varied from 21.81 to 47.45µg g^{-1} fr.wt. All the varieties showed an increase level of proline under stress. Proline accumulation varied from 21.81 µg g^{-1} fr.wt (CO7) to 47.45 µg g^{-1} fr.wt in control plants while stressed plants varied from 46.40 µg g^{-1} fr.wt (CO8) to 83.70 µg g^{-1} fr.wt (Fig 4). An overall average increase on proline is two- fold under stress.

Plant accumulates higher concentration of free proline in their leaves and other tissues while exposed to abiotic stress conditions (Errabii *et al*., 2006; Queiroz *et al*., 2011). Zhao *et al*., (2010) suggested that proline was not a sensitive water indicator; whereas Rao and Asokan (1978) found that drought - tolerant varieties of sugarcane accumulated more proline than susceptible ones and suggested that proline accumulation could be used as an index of drought tolerance. Sajitha (2009) observed the 66.6% increase in proline content in stressed plants after 30 days of treatment. Proline accumulation increased to two-fold under the moisture stress in sugarcane.

Lipid peroxidation

During stress, lipid peroxidation activity increased in all the varieties. The activity ranged from (0.11 nmol g^{-1} fw) in CO7 variety to (0.15 nmol g^{-1} fw) VBN2 in non-stressed plants. The maximum lipid peroxidation activity was recorded in CO6 (0.24 nmolg^{-1}fw) variety. The minimum lipid peroxidation activity was recorded control in CO7 (0.11 nmol g^{-1}fw)(Fig 5). It is well known that drought stress induced production of ROS which leads to lipid peroxidation of membrane lipids reflecting the stress induced damage in leaves. This seems as a parameters for drought screening.

Superoxide dismutase activity (SOD)

During stress, SOD activities increased in all varieties. The activity ranged from (CO8) 32.61 units g^{-1} fr.wt to (VBN2) 78.58 units g^{-1} fr.wt in control plants. Two fold increases in SOD activity was observed in stressed plants. Under drought stress the SOD activity ranged from (CO8) 59.50 units g^{-1} fr.wt to (VBN2) 91.53 units g^{-1} fr.wt. In present study, an increase in SOD and POD activities was noticed in the stressed plants (Fig 6). The maintenance or increase in SOD activity under drought stress has been considered an index of tolerance, whilst a reduction in SOD activity could indicate sensitive to drought. Water stress (PEG stress) led to significant ($p \leq 0.05$) increase in the activity of the antioxidant enzymes like CAT, POX, APX and SOD Srinath and Jabeen (2013).

Peroxidase activity (POD)

Peroxidase activity was increased in stressed plant varieties. At maximum stress, a peroxidase activity appreciably increased. In normal plants the POX activity ranged from

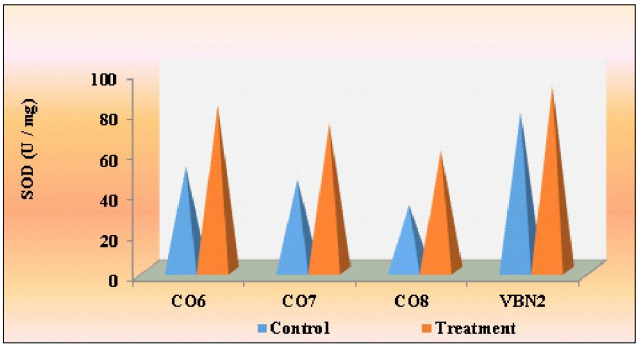

Fig 6: Effect of drought stress on superoxide dismutase (SOD) activity.

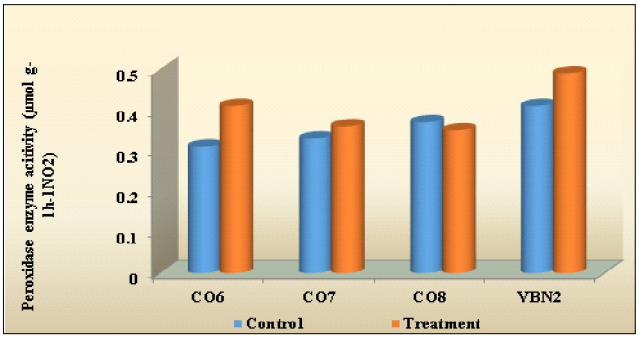

Fig 7: Effect of drought stress on proxidase enzyme activity.

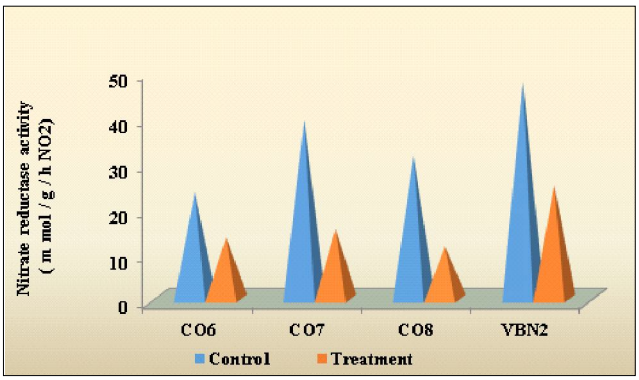

Fig 8: Effect of drought stress on nitrate reductase activity.

(CO6) 0.31 to (VBN2) 0.41units g^{-1}fr.wt, while it varied from (CO8) 0.35 units g^{-1} fr.wt to (VBN2) 0.49 unit/mg under stress. Varieties VBN2 and CO6 peroxidase activity relatively high compared to other varieties (Fig 7). The capacity to maintain high levels of antioxidant is a tolerant mechanism towards drought (Reddy *et al.,* 2014).

Nitrate reductase activity (NRA)

Varieties VBN2 and CO7 which showed high NRA activity under stress resulted in much reduced in control. NRA activity varied from 11.40 µmol $g^{-1}h^{-1}$ NO_2 (CO8) to 24.85 µmol $g^{-1}h^{-1}$ NO_2 (VBN2) in control while in stressed plant it varied from 23.32 µmol $g^{-1}h^{-1}$ NO_2 (CO6) to 47.42 µmol $g^{-1}h^{-1}$ NO_2 (VBN2) (Fig 8). Muhammad *et al.,* (2016) reported that NRA decreased with increasing water stress, maximum NRA were recorded at control level as compared to terminal drought in wheat genotypes.

CONCLUSION

Water stress being a critical constraint to crop growth is recently focused in order to bring about revolutionary changes in the crop production. In this study attempt in being made to add to the knowledge on the response of Mungbean under stress which in turn will be of prime importance, while engineering crops for abiotic stress tolerance.

REFERENCES

Ahmed, Al-Maskri, Waleed Al-Busaidi, Hamed Al-Nadabi, Ahmed Al-Fahdi and M. Mumtaz Khan (2016). Effects of Drought Stress on Wheat (*Triticum aestivum* L.) cv. Coolly. International Conference on Agricultural, Food, Biological and Health Sciences (AFBHS-16) August 22-24, Kuala Lumpur (Malaysia).

Alexieva, V., Sergiev, I., Mapelli, S. and Karanov, E. (2001). The effect of drought and Ultraviolet radiation on growth and stress markers in pea and wheat. Plant Cell Environment. 24: 1337-1344.

Ali, M.A., Abbas, G., Mohy-ud-Din, Q., Ullah, K., Abbas, G. and Aslam, M. (2010). Response of mungbean (*Vigna radiata*) to phosphatic fertilizer under arid climate. Journal of Animal and Plant Sciences. 20(2): 83-86.

Bates L.S., Walden, R.P. and Teare, I.D. (1973). Rapid determination of free proline for water stress studies. Plant and Soil. 39: 205-208.

Beauchamp, C. and Fridovich, J. (1971). Superoxide Dismutase: Improved assays and an assay applicable to acrylamide gels. Annals of Biochemistry. 444: 274-287.

Castillo F.J. (1996). Antioxidative protection in the inducible CAM plant *Sedum album* L. following the imposition of severe water stress and recovery. Oecologia. 107: 469-477.

Chang, Z., Liu, Y., Dong, H., Teng, K., Han, L. and Zhang, X. (2016). Effect of cytokinin and nitrogen on drought tolerance of creeping bentgrass. PLos One. 11(4): e015400.

Choi, S.J. and Park, R.K. (1980). Varietal difference in ecological and morphological characteristics affected tolerance. Korean Journal of Society Crop Science. 25: 10-16.

Errabi, T., Gandonou, C.B., Essalmani, H., Abrini J., Idomar and Senhaji, N.S. (2006). Growth, proline and ion accumulation in sugarcane callus cultures under drought-induced osmotic stress and its subsequent relief. African Journal of Biotechnology. 5: 1148-1193.

Eva Sanchez-Rodriguez, Mar Rhbio-Whilhelmi, M., Cervilla, L.M. and Begona Blasco Leon, M. (2010). Genotypic differences in some physiological parameters symptomatic for oxidative stress under moderate drought in tomato plants. Plant Science. 178(1): 30-40.

Hageman, R.H. and Hucklesby, D.P. (1971). Nitrate Reductase from Higher Plants. In: Methods in Enzymology. San Pietro (ed.), Academic Press, New York. 491-503.

Heath R.L. and Packer, L. (1968). Photoperoxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics. 125: 189-198.

Hsiao, T.C. and Acevedo, E. (1974). Plant responses to water deficits, water-use efficiency and drought resistance. Agricultural Meteorology. 14: 59-84.

Kaloyereas, S.A. (1958). A new method for determining drought resistance. Plant Physiology. 33: 669-672.

Koehler, P.H., Moore, P.H., Jones, C.A., Dela Cruiz, A. and Maretzki, A. (1982). Response of drip-irrigated sugarcane to drought stress. Agronomy Journal. 74: 906-911.

Kumar, R., Singh, C.M., Arya, M., Kumar, R., Mishra, S.B., Singh, U.K. and Paswan, S. (2020). Investigating stress indices to discriminate the physiologically efficient heat tolerant genotypes of mungbean [*Vigna radiate* (L.) Wilczek]. Legume Research. 43(1): 43-49.

Lata Verma and Kushwaha, H.S. (2020). Evaluation of different herbicides against weeds in mungbean (*Vigna radiate* L.) Legume research-An International Journal. 43: 866-871.

Mahdi, Z., Bahman, D., Omid, A. and Arash, A. (2013). The evaluation of various agronomic traits of mungbean (*vinga radiata* L.) genotypes under drought stress and non-stress conditions. International Journal of Farming and Allied Sciences. 2(19): 764-770.

Malik, C.P. and Singh, M.B. (1980). In: Plant Enzymology and Histoenzymology. Kalyani Publishers, New Delhi. 51-53.

Martineuae, J.R., Wiiliams, J.E. and Sullivan, C.Y. (1979). Temperature tolerance in soybeans. Evaluation of a technique for assessing cellular membrane thermostability. Crop Science. 19: 75-78.

Meenakumari, S.D., Vimala, Y. and Pawan, A. (2002). Physiological parameters governing drought tolerance in maize. Indian Journal of Plant Physiology. 9: 203-207.

Muhammad, H.C., Nazir, A.C., Qamaruddin, C., Sheikh M.M., Sadaruddin, C. and Zaid, C. (2016). Physiological characterization of six wheat genotypes for drought tolerance. International Journal of Research-Granthaalayah. 4(2): 184-96.

Parvaze A. Sofi, Rehman K., Asmat Ara and Musharib Gull, (2018). Stress tolerance indices based on yield, phenology and biomass partitioning: A review. Agricultural Reviews. 39(4): 292-299.

Prakash M., Sunilkumar B., Sathyanarayanam G. and Gokulakrishnan, J. (2017). Screening for drought tolerance in mungbean. Legume Research. 40(3): 423-426.

Queriroz, R.J.B., Santos, D.M.M.D., Ferraudo, A.S, Carlin, S.D. and Silva, M.D.A. (2011). Biochemical and physiological responses of sugarcane cultivar to soil water deficiencies. Scientia Agricola. 68(4): 469-476.

Rampino P., Pataleo C., Gerardi C. and Parotta, C. (2006). Drought stress responses in wheat: Physiological and molecular analysis of resistant and sensitive genotypes. Plant Cell Environment. 29: 2143-2152.

Ranawake, A.L., Dahanayaka, N., Amarsinghna, U.G.S., Rodrigo W.D.R.J. and Rodrigo, U.T.D. (2011). Effect of water stress on growth and yield of mung bean (*Vigna radiate* L.). Tropical Agricultural Research and Extention. 14(4): 101-108.

Rao, K. C. and Asokan, S. (1978). Studies of free proline association to drought resistance in sugarcane. Sugar Journal. 40: 23-24.

Reddy, S.K., Liu, S., Rudd, J.C., Xue, Q., Payton, P., Finlayson, S.A., Mahan J., Akhunova A., Holalu, S.V. and Lu, N. (2014). Physiology and transcriptomics of water-deficit stress responses in wheat cultivars TAM 111 and TAM 112. Journal of Plant Physiology. 171: 1289-1298.

Sajitha, B. (2009). Drought responses and osmoregulation in sugarcane: A physiological and biochemical approach towards droght tolerance. Thesis.

Shih, S.F. and Gascho, G.J. (1980). Relationship among stalk elongation, leaf area and dry biomass of sugarcane. Agronomy Journal. 72: 309-313.

Srinath, R. and Jabeen, F.T.Z. (2013). *In vitro* selection and characterization of polyethylene glycol (PEG) tolerant callus lines and regeneration of plantlets from the selected callus lines in sugarcane (*Saccharum officinarum* L.). Physiology and Molecular Biology of Plants. 19(2): 261-268.

Turk, K.J. and Hall, A.E. (1980). Drought adaptation of cowpea III. Influence of drought on plant growth and relations with seed yield. Agronomy Journal. 72: 428-433.

Zhao, X., Huang, Y., Jiang, C., Hu, Y., Shi, C., Yu, Y. and Liu, C. (2007). Photosynthesis of *Rehmannia glutinosa* subjected to drought stress enhanced by choline chloride through alleviating lipid peroxidation and increased proline accumulation. Plant Growth Regulators. 51(3): 255-262.