P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(1): 862-868
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Received: 14-11-2018
Accepted: 18-12-2018

Effect of water stress on biochemical characteristics of summer Mungbean (*Vigna radiata* (L.)Wilczek)

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Abstract
In order to investigate the effect of water stress on mungbean genotypes and its biochemical responses to yield. A field experiment was conducted using split plot design with the four irrigation level and the biochemical traits like chlorophyll, protein, proline, glycine betaine, lipid peroxidation, catalase activity and peroxidase activity were determined. The biochemical parameters like chlorophyll content and protein significantly decreased under the water stress condition. Significantly highest accumulation of other biochemical parameters such as glycine-betaine, proline, lipid peroxidation, catalase and peroxidase increased under the stress condition compared to irrigation condition. Among all the six genotypes, NKM-15-08 showed higher seed yield which was due to accumulation of glycine-betaine, proline, catalase and peroxidase was higher under the water stress condition. Thus it enabled the genotype to thrive better in the water stress condition and produce higher yield under water deficient.

Keywords: strass, glycine-betaine, proline, LPID peroxidation, catalase, peroxidase, protein, DMSO, DAS

Introduction
Mungbean [*Vigna radiata* (L.) Wilczek] it belongs to the family *Fabaceae*, genus *Vigna* has been broadened to include about 150 species but only twenty two species are native to India (Polhill and Van der Maesen, 1985) [16]. Mungbean is one of the most important pulse crops in India, ranks third after gram and arhar (Anonymous, 1999) [1]. Mungbean has been grown in India since ancient times. Mungbean is an important short duration legume crop with wide adaptability, low input requirement and has ability to improve the soil fertility by fixing atmospheric nitrogen. It also plays a vital role in sustainable agriculture, while it is cultivated as mixed crop, inter crop, rotational crop to improve nitrogen status of soil or to break the disease or pest cycles (Jaiwal *et al*. 2001) [9]. Mungbean is an excellent source of easily digestible protein, which complements the stable rice diet in the country. The major portion of mungbean is utilized in making dal, curries, soup, sweets, fried foods, desserts, noodles, snacks etc. Mungbean seed contains 51% carbohydrate, 26% protein, 4% minerals and 3% vitamins (Kaul, 1982) [10]. India is the largest producer of mungbean and contributes about 75% of the world production of mungbean (Taunk *et al.,* 2012) [20]. Mungbean is cultivated in three different seasons in India, *viz*., *kharif*, *rabi* and summer. Short maturity duration (<60 days) make the crop ideal for catch cropping, intercropping and relay cropping. Despite holding such great promise, mungbean is often grown in marginal lands with limited inputs making it prone to a number of abiotic stresses causing tremendous yield loss (Biyan *et al.* 2012) [4]. The summer season crop requires assured pre-sowing irrigation for germination and early establishment of seedlings. Thereafter, the crop meets its water requirement from monsoon rains. However, due to the erratic nature of monsoon rains and high vapour pressure deficit in hot and windy summers, stored soil moisture depletes rapidly resulting in severe water deficits and drastic reductions in Mung bean yields. Studies relating to biochemical aspects of growth, especially the water stress effect on biochemical characters in different stages of crop growth are limited. The present investigation was taken up to the effect of water stress on biochemical parameter in different crop growth stages.

Materials and Methods
The study was carried out on “Effect of water stress on physiological, biochemical and yield attributing parameters in summer mungbean (*Vigna radiate* (L). Wilczek)” in season of 2017 at College Farm, N. M. College of Agriculture, Navsari Agricultural University, Navsari.

The weather during the growing season was normal and favourable for crop growth. The seed of six genotypes of mungbean i.e.GAM-5, NKM-15-06, NKM-15-08, NKM-15-09, NKM-15-10 and NKM-15-12 were studied under four different irrigation levels (T_1- 15 DAS Stress (Initiation vegetative stage), T_2- 30 DAS Stress (Grand growth stage), T_3- 45 DAS Stress (Flowering/pod formation stage) and T_4-Irrigation as per recommendation (control).

Estimation of chlorophyll content

Total chlorophyll in leaves was determined by DMSO (Dimethylsulphoxide) method described by Arnon, 1949 [2]. Finely chopped 50 mg leaves were weighed in graduated test tube. Ten ml of DMSO was added to each and the tube incubated at 65ºC for 3 hours. After incubation the tube were allowed to cool at room temperature and the volume made up to a total of 10 ml by adding DMSO. The optical density (OD) was recorded at 663 and 645 nm by taking DMSO as blank. The amount of chlorophyll present in the sample was calculated using standard formula:

$$\text{Total chlorophyll (mg g}^{-1}) = \frac{22.2\ (\text{OD at 663 nm}) + 8.02\ (\text{OD at 645 nm})}{1000 \times W}$$

Estimation of proline

Free proline content was estimated by following the method of Bates *et al*., (1973) [3]. A known weight (0.5 g) of fresh leaf sample was macerated in a mortar using 10 ml of 3 percent sulphosalicylic acid. The extract was filtered and 2.0 ml of the filtrate was used for proline estimation. To this 2.0 ml of filtrate, 2.0 ml of acid ninhydrin reagent (2.5 g of ninhydrin dissolved in 40 ml of 6.0 M Ortho phosphoric acid and 60 ml of glacial acetic acid), 2.0 ml of glacial acetic acid were added and placed in boiling water bath for one hour. Following this, test tubes containing the samples were transferred to an ice bath for cooling. The contents of each test tube were transferred to a separatory funnel and 6.0 ml of toulene was added, shaken thoroughly and allowed for few minutes for separation of two layers. The lower layer was discarded and the upper toulene layer containing the colour complex was taken into a test tube. The optical density was read at 520 nm using spectrophotometer (Model No: UV-1800, Shimadzu, UV spectrophotometer) and the proline content was calculated as on fresh-weight-basis as follows:

$$\mu\text{moles per g tissue} = \frac{\frac{\mu\text{g proline}}{\text{mL}} \times \text{mL toluene}}{115.5} \times \frac{5}{\text{g sample}}$$

Estimation of seed protein

The seed protein estimated on the basis of nitrogen content which was determine by the kjeldahl method (developed in 1883 by a brewer called Johann kjeldahl) and subsequently multiplied by 6.25 factor.

(A) Digestion: Transfer 0.5 g powdered leaf sample wrapped in piece of filter paper to 100 ml kjeldahl flask. Add to it 50 ml of the sulphuric salicylic acid mixture and shake to get intimate contact of the sample with the reagent. Add 5 g sodium thiosulphate and heat gently for about 5 minutes, taking care to avoid frothing. Cool, add 10 g catalyst mixture, and then digest in kjeldahal flask at full heat till the solution become clear. Cool and add 100 ml distill water. Now distillation is carried out as follows.

(B) Distillation: 25 ml of 4% boric acid solution containing mix indicator is taken in a conical flask and flask is placed in such a way that condenser outlet of distillation apparatus is dipped into this boric acid solution. Transfer the content of kjeldahl flask to distillation flask. Wash 2 to 3 time with distilled water to ensure that whole content of kjeldahl flask is transferred. Add 100 ml 40% sodium hydroxide along the sides of the flask and 5 to 10 glass beads (5 mm in diameter). Connect to distillation head, and distil off 150 ml into 25 ml of 4% boric acid solution. Titrate it to the first faint pink colour with standard (0.1N) sulphuric acid solution. Blank should be run and the titration carried to the same end point in exactly the same manner.

$$\text{For 100 g plant sample: } \frac{(S-B) \times 0.0014\ g\ N \times 100}{0.5}$$

$$\text{Protein (\%)} = \text{Nitrogen (\%)} \times 6.25$$

Estimation of glycine betaine content

Estimation of Glycine Betaine was done by following the method of Grieve and Grattam, (1983) [6]. Leaf tissues (2.0g) were finely- ground using liquid nitrogen in mortar-pestle. A finely ground plant sample 0.5g of each genotype was mechanically shaken with 20 ml of deionized H2O for 24 hours at 250 C. The samples were then filtered. The extracts were diluted 1:1 with 2N H_2SO_4. Aliquots (050 ml) were measured into 2.0 ml eppendrof tubes and cooled in ice-water for 1 hr. Cold KI- I2 reagent (0.20 ml) was added to each tube and reactants gently stirred on a vortex mixer. The tubes were stored at 0-4 c for 16 hrs. and then centrifuged at 10000 rpm for 15 mins at 0 C. The supernatents were carefully aspirated. The per-iodide crystals were dissolved in 9.0 ml of 1, 2-dichloroethane. Vigorous vortex mixing was frequently required to effect complete solubilisation in the developing solvent. After 2-2.5 hrs, the absorbance was measured at 365 nm on a Spectrophotometer (UV-1800). Reference standard of GB (50-200 microgram/ml) were prepared in 1N H_2SO_4. Standard curve were prepared and the GB content of sample calculated.

$$\text{Glycine betaine} = \text{Sample O.D.} \times \text{Graph factor} \times \text{Dilution factor}$$

Estimation of Lipid peroxidation product (MDA)

Estimation of lipid peroxidation was done by following the method of Heath and Packer (1968) [7]. Leaf sample (0.5 g) was homogenized in 10 mL of 0.1% TCA. The homogenate was centrifuge at 15000× g for 5 min. to 1.0 mL aliquot of the supernatant, 4.0 mL of 0.5% thiobarbituric acid (TBA) in 20% TCA was added. The mixture was heated at 95ºC for 30 min. and then quickly cooled in an ice bath. After centrifuge at 10000× g for 10 min, the absorbance of supernatant was recorded at 532 nm. The value for nonspecific absorption at 600 nm was subtracted. The MDA equivalent was calculated as follows:

$$\text{MDA (nmol ml}^{-1}\text{ FW)} = \frac{A532 - A600}{155000} \times 10$$

Estimation of Catalase

Catalase was estimated by grinding 1.0 g of leaf tissue with 0.1M phosphate buffer, in a prechilled mortar and pestle. Centrifuge at 15000g for 30 min at 4ºC. Use the supernatant as enzyme. Pipette out 3ml of phosphate buffer, 2ml of H_2O_2 and 1ml of enzyme extract into a test tube. Incubate at 20ºC for 1min. after 1min stop the reaction by adding 10 ml of 0.7

H_2SO_4. Titrate the reaction by adding 0.01N $KMNO_4$ to find out the residual H_2O_2 until a faint purple colour persists for at least 15sec. prepare the blank by adding the enzyme extract to an acidified solution of reaction mixture at zero time. Express the enzyme activity as units/min and specific activity as units/min/mg protein or per g weight of sample.

Enzyme activity $= \frac{X}{1\times0.5} \times 10 \times\times 1 \times 0.85$

Estimation of Peroxidise (POD)
Estimation of peroxidise (POD) activity on was done by following the method of perur (1962). Homogenize the material in ice-cold 0.1M phosphate buffer, pH 6.0 in a chilled pestle and mortar using white sand as abrasive. Stain through two folds of muslin cloth and centrifuge the homogenate at 16000g for 20min at 4ºC. Use the supernatant as enzyme source. Pipette out of o-dianisidine, 0.5ml of H_2O_2, 1 ml of phosphate buffer and 2.4 ml of distilled water into a test tube. For blank exclude H_2O_2 but add additional volume of water. Incubate at 30ºC and start the reaction by adding 0.2 ml of enzyme. After 5min, stop the reaction by adding 1ml of 2N H_2SO_4. Read the absorbance at 430nm. Express the enzyme activity as units/min and specific activity as units/min/mg protein or per g weight of sample.

Enzyme activity $= \frac{X\times60\times10\times1000}{1\times30\times500}$

Result
The experimental results of the water stress on the biochemical parameters are resulted below of the six different mungbean genotypes. The accumulation of the chlorophyll content, protein, proline, glycine betaine, lipid peroxidation, catalase and peroxidase were recorded at different days after sowing of the mungbean genotypes.

Chlorophyll content
Significant results were observed in the chlorophyll content among the six mungbean genotypes in the given irrigation conditions. At 15 DAS it was observed that the mean chlorophyll content was found maximum in the genotype NKM-15-08 (1.19 mg g^{-1} FW) while the minimum mean chlorophyll content observed was in the genotype NKM-15-10 (1.13 mg g^{-1} FW). The irrigation in which the chlorophyll content was found highest was T_4 condition (1.26 mg g^{-1} FW) while the minimum was recorded in the irrigation condition T_1 (0.88 mg g^{-1} FW). At 30 DAS it was observed that the mean chlorophyll content was found maximum in the genotype NKM-15-0.8 (1.72 mg g^{-1} FW) which was at par with NKM-15-12 (1.70 mg g^{-1} FW), NKM-15-09 (1.70 mg g^{-1} FW) and NKM-15-06 (1.69 mg g^{-1} FW) while the minimum mean chlorophyll content observed was in the genotype NKM-15-10 (1.66 mg g^{-1} FW).The irrigation in which the chlorophyll content was found highest was T_4 condition (1.92 mg g^{-1} FW) which similar with T_3 (1.92 mg g^{-1} FW) while the minimum was recorded in the irrigation condition T_2 (1.28 mg g^{-1} FW). At 45 DAS it was observed that the mean chlorophyll content was found maximum in the genotype NKM-15-08 (1.81 mg g^{-1} FW) which was at par with NKM-5-12 (1.8 mg g^{-1} FW), NKM-15-09 and NKM-15-06 (1.77 mg g^{-1} FW) while the minimum mean chlorophyll content observed was in the genotype NKM-15-10 (1.75 mg g^{-1} FW).The irrigation in which the chlorophyll content was found highest was T_4 condition (1.97 mg g^{-1} FW) which followed by T_1 (1.96 mg g^{-1} FW) while the minimum was recorded in the irrigation condition T_3 (1.32 mg g^{-1} FW). The interaction effect was found non-significant for chlorophyll content at 15, 30 and 45 DAS.

Protein content (mg g^{-1})
Table. 1 indicated that protein content was found significantly different among different genotypes at different irrigation levels. The protein content significantly decreased with the increasing stress as compared to the irrigation conditions. Under T3 irrigation condition the protein content significantly decreased over all the given irrigated condition. Under irrigated condition, the mean protein content was in T_4 (22.41 mg g^{-1}), T_1 (20.63 mg g^{-1}), T_2 (18.32 mg g^{-1}) and T_3 (15.74 mg g^{-1}). The maximum protein content was recorded in genotype NKM-15-12 (21.49 mg g^{-1}). The minimum protein content was observed in the genotype GAM-5 (20.06 mg g^{-1}), NKM-15-08 (19.44 mg g^{-1}), NKM-15-06 (18.68 mg g^{-1}), NKM-15-09 (18.34 mg g^{-1}) and NKM-15-10 (17.65 mg g^{-1}) respectively. The interaction effect was found non-significant for protein content.

Proline content (mg g^{-1} FW)
Under irrigated conditions at 15 DAS the mean proline content was (1.45 mg g^{-1} FW) at T_2 level, (1.47 mg g^{-1} FW) at T_3 irrigation, (1.49 mg g^{-1} FW) at T4 irrigation and (2.50 mg g^{-1} FW) at T1 irrigation. Maximum proline content was observed in the irrigation level T_1 (2.50 mg g^{-1} FW) at 15 DAS, while the minimum proline content was observed in the irrigation level T2 (1.45 mg g^{-1} FW) and similar in T_3 (1.45 mg g^{-1} FW). Genotype NKM-15-08 proved the best at 15 DAS with the highest proline accumulation (1.87 mg g^{-1} FW). The interaction of the irrigation and the genotype was found significant at 15 DAS. Maximum proline content in T_3G_2 while, minimum in T_3G_3. At 30 DAS the irrigation condition T4 was found with least proline accumulation (2.35 mg g^{-1} FW) which resulted better while the maximum proline accumulation was observed at the irrigation level T_2 (4.21 mg g^{-1} FW).The genotype NKM-15-08 showed the highest proline accumulation (2.97 mg g^{-1} FW) and minimum proline accumulation showed in NKM-15-10 (2.70 mg g^{-1} FW). At 45 DAS the mean proline content was (4.10 mg g^{-1} FW) at T4 level, (4.14 mg g^{-1} FW) at T1 irrigation, (4.20 mg g^{-1}FW) at T_2 irrigation and (5.45 mg g^{-1} FW) at T3 irrigation. Maximum proline content was observed in the irrigation level T_3 (5.45 mg g^{-1} FW) at 45 DAS, while the minimum proline content was observed in the irrigation level T_4 (4.10 mg g^{-1} FW). Genotype NKM-15-08 proved the best at 45 DAS with the highest proline accumulation (4.62 mg g^{-1} FW) and low in NKM-15-10 (4.32 mg g^{-1} FW).

Glycine betaine (µg g^{-1} DW)
The mungbean genotypes at 15 DAS ranged from (0.68 µg g^{-1} DW) to (0.59 µg g^{-1} DW). The maximum glycine betaine content at 15 DAS was observed in the genotype NKM-15-08 (0.68 µg g^{-1} DW) remained at par with NKM-15-12 (0.66 µg g^{-1} DW), while the least glycine betaine content was observed in the genotype NKM-15-10 (0.59 µg g^{-1} DW). The accumulation of glycine betaine was found maximum in T1 (0.94 µg g^{-1} DW) while the minimum glycine betaine content was observed in T_2 (0.52µg g^{-1} DW). At 30 DAS the maximum glycine betaine content was observed in the genotype NKM-15-08 (1.18 µg g^{-1} DW) while the minimum of the glycine betaine content was observed in the genotype NKM-15-10 (1.04 µg g^{-1} DW). The glycine betaine content was found maximum in the irrigation level T_2 (1.82 µg g^{-1}

DW), while the minimum of the glycine betaine content was observed in the irrigation T_3 (0.84 µg g^{-1} DW). The mungbean genotypes at 45 DAS the maximum glycine betaine content was observed in the genotype NKM-15-08 (1.95 µg g^{-1} DW), while the least glycine betaine content was observed in the genotype NKM-15-10 (1.49 µg g^{-1} DW). The accumulation of glycine betaine was found maximum in T3 (2.56 µg g^{-1} DW) while the minimum glycine betaine content was observed in T_4 (1.49 µg g^{-1} DW).

Lipid peroxidation (µmol g^{-1} FW)

The mungbean genotypes at 15 DAS minimum lipid peroxidation content at 15 DAS was observed in the genotype NKM-15-08 (0.27 µmol g^{-1} FW) remained at par with NKM-15-12 (0.29 µmol g^{-1} FW), while the maximum lipid peroxidation content was observed in the genotype NKM-15-10 (0.36 µmol g^{-1} FW). In irrigation level of lipid peroxidation was found maximum in T_1 (0.39 µmol g^{-1} FW) while the minimum lipid peroxidation content was observed in T_4 (0.28 µmol g^{-1} FW). The lipid peroxidation content at 30 DAS also increased as the stress condition increased. The minimum lipid peroxidation content was observed in the genotype NKM-15-08 (0.46 µmol g^{-1} FW) while the maximum of the lipid peroxidation content was observed in the genotype NKM-15-10 (0.57 µmol g^{-1} FW). The lipid peroxidation content was found maximum in the irrigation level T_2 (0.60 µmol g^{-1} FW), while the minimum of the lipid peroxidation content was observed in the irrigation T_4 (0.49 µmol g^{-1} FW). At 45 DAS the maximum lipid peroxidation content was observed in the genotype NKM-15-10 (0.67 µmol g^{-1} FW), while the least lipid peroxidation content was observed in the genotype NKM-15-08 (0.55 µmol g^{-1} FW). The accumulation of lipid peroxidation was found maximum in T_3 (0.75 µmol g^{-1} FW) while the minimum lipid peroxidation content was observed in T_4 (0.56 µmol g^{-1} FW).

Estimation of catalase activity (µmol H_2O_2 mg^{-1} m^{-1})

The data on catalase product are presented in the Table 4.1 it indicated significant differences in catalase product amongst the six mungbean Genotypes. The mungbean genotypes at 15 DAS the maximum catalase product was observed in the genotype NKM-15-08 (0.55 µmol H_2O_2 mg^{-1} m^{-1}), while the least catalase product content was observed in the genotype NKM-15-10 (0.47 µmol H_2O_2 mg^{-1} m^{-1}).The accumulation of catalase product was found maximum in T1 (0.73 µmol H_2O_2 mg^{-1} m^{-1}) while the minimum catalase product was observed in T_4 (0.42 µmol H_2O_2 mg^{-1} m^{-1}). At 30 DAS was observed that the maximum catalase product in the genotype NKM-15-08 (µmol H_2O_2 mg^{-1} m^{-1}) which at par with NKM-15-12 (0.97 µmol H_2O_2 mg^{-1} m^{-1}), while the least catalase product content was observed in the genotype NKM-15-10 (0.91 µmol H_2O_2 mg^{-1} m^{-1}). Catalase product was found maximum in T_2 (1.54 µmol H_2O_2 mg^{-1}) while the minimum catalase product was observed in T_3 (0.68 µmol H_2O_2 mg^{-1} m^{-1}). The maximum catalase product at 45 DAS was observed in the genotype NKM-15-08 (0.91 µmol H_2O_2 mg^{-1} m^{-1}) which at par with NKM-15-12 (0.89 µmol H_2O_2 mg^{-1} m^{-1}), while the least catalase product content was observed in the genotype NKM-15-10 (0.83 µmol H_2O_2 mg^{-1} m^{-1}). Catalase product was found maximum in T_3 (1.04 µmol H_2O_2 mg^{-1} m^{-1}) while the minimum catalase product was observed in T_4 (0.75 µmol H_2O_2 mg^{-1} m^{-1}).

Estimation of peroxidase activity (POD) (µmol mg^{-1})

Under irrigated conditions at 15 DAS accumulation of peroxidase product was found maximum in T_1 (2.70 µmol mg^{-1}) while the minimum peroxidase product was observed in T_4 (1.47 µmol mg^{-1}). The maximum peroxidase product was observed in the genotype NKM-15-08 (1.85 µmol mg^{-1}), while the least peroxidase product content was observed in the genotype NKM-15-10 (1.71 µmol mg^{-1}). At 30 DAS the irrigation condition T_4 was found with least peroxidase product accumulation (1.78 µmol mg^{-1}) which resulted better while maximum peroxidase product was observed in T_2 (3.34 µmol mg^{-1}). Genotype NKM-15-08 was maximum peroxidase product (2.30 µmol mg^{-1}), while the least peroxidase product content was observed in the genotype NKM-15-10 (2.15 µmol mg^{-1}). The mungbean genotypes at 45 DAS significantly different. It was observed in the genotype NKM-15-08 (2.73 µmol mg^{-1}), while the least peroxidase product content was observed in the genotype NKM-15-10 (2.58 µmol mg^{-1}).The accumulation of peroxidase product was found maximum in T3 (3.67 µmol mg^{-1}) while the minimum peroxidase product was observed in T_4 (2.16 µmol mg^{-1}).

Seed yield per plot (g plot^{-1})

The maximum seed yield per plot was observed in the genotype NKM-15-08 with the yield (369.99 g plot^{-1}) and the minimum was observed in the genotype NKM-15-10 (250.19 g plot^{-1}). The irrigation in which the seed yield found highest was T_4 with the mean seed yield (401.66 g plot^{-1}) and the lowest yield in the irrigation T_3 with the mean seed yield (212.83 g plot^{-1}). Statistically the result of the irrigation and genotype was found to be significant and the treatments combination was found to be non-significant.

Table 1: Effect of water stress on chlorophyll content of mungbean genotypes

Genotypes	Chlorophyll (mg g^{-1}FW)														
	15 DAS					30 DAS					45 DAS				
	T_1	T_2	T_3	T_4	Mean	T_1	T_2	T_3	T_4	Mean	T_1	T_2	T_3	T_4	Mean
GAM-5	0.82	1.25	1.25	1.26	1.14	1.63	1.26	1.90	1.91	1.67	1.95	1.85	1.28	1.96	1.76
NKM-15-08	0.92	1.27	1.27	1.28	1.19	1.67	1.30	1.95	1.95	1.72	1.98	1.87	1.41	2.00	1.81
NKM-15-10	0.78	1.25	1.24	1.25	1.13	1.62	1.25	1.90	1.89	1.66	1.94	1.83	1.25	1.95	1.75
NKM-15-06	0.86	1.26	1.26	1.26	1.16	1.63	1.28	1.91	1.92	1.69	1.96	1.85	1.30	1.97	1.77
NKM-15-09	0.88	1.26	1.27	1.27	1.17	1.64	1.29	1.93	1.93	1.70	1.96	1.86	1.31	1.98	1.78
NKM-15-12	0.91	1.27	1.27	1.28	1.18	1.65	1.29	1.94	1.94	1.70	1.98	1.86	1.40	1.98	1.80
Mean	0.86	1.26	1.26	1.26		1.64	1.28	1.92	1.92		1.96	1.85	1.33	1.97	
	T	G	T×G			T	G	T×G			T	G	T×G		
S.E.m±	0.007	0.006	0.013			0.012	0.010	0.020			0.016	0.012	0.023		
CD 0.05	0.022	0.018	0.036			0.039	0.029	NS			0.050	0.033	NS		
CV %	2.85	2.16				3.49	2.40				4.29	2.60			

Table 2: Effect of water stress on seed protein of mungbean genotypes

Genotypes	Seed protein (%)				
	T_1	T_2	T_3	T_4	Mean
GAM-5	22.10	18.85	16.18	23.11	20.06
NKM-15-08	20.86	18.45	15.69	22.76	19.44
NKM-15-10	18.73	17.16	14.48	20.24	17.65
NKM-15-06	19.59	17.74	15.50	21.91	18.68
NKM-15-09	19.29	17.42	15.10	21.54	18.34
NKM-15-12	23.25	20.33	17.49	24.88	21.49
Mean	20.63	18.32	15.74	22.41	
	T	G	T×G		
S.E.m±	0.22	0.23	0.66		
CD 0.05	0.72	0.66	NS		
CV %	5.71	4.79			

Table 3: Effect of water stress on proline of mungbean genotypes

Genotypes	proline (μg g^{-1})														
	15 DAS					30 DAS					45 DAS				
	T_1	T_2	T_3	T_4	Mean	T_1	T_2	T_3	T_4	Mean	T_1	T_2	T_3	T_4	Mean
GAM-5	2.37	1.36	1.39	1.41	1.63	1.89	4.12	1.81	1.84	2.41	2.44	3.28	5.35	2.41	3.37
NKM-15-08	2.71	1.58	1.59	1.61	1.87	2.05	4.36	1.96	1.98	2.59	2.57	3.47	5.63	2.54	3.55
NKM-15-10	2.29	1.33	1.35	1.37	1.59	1.84	4.06	1.78	1.78	2.36	2.39	3.22	5.28	2.38	3.32
NKM-15-06	2.46	1.42	1.45	1.47	1.70	1.94	4.19	1.85	1.86	2.46	2.46	3.35	5.42	2.46	3.42
NKM-15-09	2.54	1.48	1.52	1.52	1.77	1.98	4.23	1.89	1.91	2.50	2.51	3.40	5.49	2.49	3.47
NKM-15-12	2.60	1.54	1.56	1.57	1.82	2.02	4.27	1.93	1.95	2.54	2.54	3.44	5.55	2.52	3.51
Mean	2.50	1.45	1.47	1.49		1.95	4.21	1.87	1.89		2.49	3.36	5.45	2.46	
	T	G	T×G			T	G	T×G			T	G	T×G		
S.E.m±	0.016	0.009	0.019			0.024	0.0121	0.023			0.028	0.022	0.043		
CD 0.05	0.050	0.027	0.054			0.077	0.033	NS			0.091	0.062	NS		
CV %	4.45	2.19				4.78	1.84				4.05	2.51			

Table 4: Effect of water stress on glycine betaine content of mungbean genotypes

Genotypes	Glycine betaine content (μ mole g^{-1})														
	15 DAS					30 DAS					45 DAS				
	T_1	T_2	T_3	T_4	Mean	T_1	T_2	T_3	T_4	Mean	T_1	T_2	T_3	T_4	Mean
GAM-5	0.90	0.50	0.50	0.52	0.61	0.89	1.77	0.80	0.83	1.07	1.46	1.65	2.64	1.40	1.79
NKM-15-08	1.01	0.56	0.57	0.58	0.68	0.99	1.90	0.91	0.93	1.18	1.59	1.81	2.85	1.56	1.95
NKM-15-10	0.87	0.49	0.49	0.51	0.59	0.86	1.72	0.77	0.80	1.04	1.40	1.60	1.60	1.36	1.49
NKM-15-06	0.93	0.52	0.51	0.54	0.62	0.92	1.81	0.83	0.85	1.10	1.49	1.69	2.70	1.45	1.83
NKM-15-09	0.96	0.53	0.53	0.55	0.64	0.94	1.85	0.86	0.88	1.13	1.52	1.74	2.76	1.49	1.88
NKM-15-12	0.98	0.55	0.55	0.57	0.66	0.96	1.87	0.89	0.90	1.15	1.56	1.78	2.80	1.52	1.91
Mean	0.94	0.52	0.53	0.54		0.93	1.82	0.84	0.87		1.50	1.71	2.56	1.46	
	T	G	T×G			T	G	T×G			T	G	T×G		
S.E.m±	0.006	0.005	0.010			0.006	0.006	0.012			0.017	0.009	0.018		
CD 0.05	0.021	0.015	NS			0.019	0.017	NS			0.055	0.026	0.052		
CV %	4.98	3.25				2.67	2.13				4.66	2.03			

Table 5: Effect of water stress on lipid peroxidation of mungbean genotypes

Genotypes	lipid peroxidation (μ mole g^{-1})														
	15 DAS					30 DAS					45 DAS				
	T_1	T_2	T_3	T_4	Mean	T_1	T_2	T_3	T_4	Mean	T_1	T_2	T_3	T_4	Mean
GAM-5	0.41	0.30	0.51	0.31	0.33	0.54	0.63	0.53	0.53	0.55	0.61	0.62	0.79	0.59	0.65
NKM-15-08	0.35	0.24	0.25	0.25	0.27	0.45	0.54	0.43	0.44	0.46	0.51	0.52	0.65	0.51	0.55
NKM-15-10	0.85	0.34	0.33	0.33	0.36	0.55	0.65	0.54	0.55	0.57	0.63	0.64	0.81	0.90	0.67
NKM-15-06	0.40	0.30	0.30	0.30	0.32	0.52	0.61	0.50	0.50	0.53	0.58	0.59	0.75	0.57	0.62
NKM-15-09	0.38	0.28	0.28	0.28	0.30	0.49	0.59	0.48	0.48	0.51	0.56	0.57	0.72	0.56	0.60
NKM-15-12	0.36	0.26	0.26	0.27	0.29	0.47	0.56	0.46	0.46	0.48	0.53	0.55	0.68	0.54	0.57
Mean	0.39	0.29	0.29	0.28		0.50	0.60	0.49	0.49		0.57	0.58	0.73	0.56	
	T	G	T×G			T	G	T×G			T	G	T×G		
S.E.m±	0.003	0.004	0.007			0.005	0.005	0.010			0.006	0.006	0.011		
CD 0.05	0.011	0.010	NS			0.016	0.014	NS			0.019	0.016	NS		
CV %	5.32	4.52				4.78	3.88				4.81	3.63			

Table 6: Effect of water stress on catalase activity of mungbean genotypes

Genotypes	catalase activity (µmol H_2O_2 mg^{-1} m^{-1})														
	15 DAS					30 DAS					45 DAS				
	T_1	T_2	T_3	T_4	Mean	T_1	T_2	T_3	T_4	Mean	T_1	T_2	T_3	T_4	Mean
GAM-5	0.71	0.41	0.41	0.40	0.48	0.86	1.51	0.67	0.68	0.93	0.79	0.85	1.02	0.74	0.85
NKM-15-08	0.79	0.46	0.47	0.47	0.55	0.92	1.58	0.72	0.73	0.99	0.85	0.90	1.10	0.79	0.91
NKM-15-10	0.70	0.39	0.41	0.39	0.47	0.84	1.50	0.66	0.67	0.91	0.77	0.83	1.00	0.72	0.83
NKM-15-06	0.72	0.43	0.42	0.42	0.50	0.87	1.53	0.68	0.69	0.94	0.80	0.86	1.04	0.75	0.86
NKM-15-09	0.73	0.44	0.44	0.43	0.51	0.88	1.54	0.70	0.70	0.96	0.82	0.88	1.07	0.76	0.88
NKM-15-12	0.75	0.45	0.45	0.45	0.53	0.90	1.56	0.71	0.72	0.97	0.83	0.89	1.08	0.77	0.89
Mean	0.73	0.43	0.43	0.42		0.88	1.54	0.69	0.70		0.81	0.87	1.05	0.75	
	T	G	T×G			T	G	T×G			T	G	T×G		
S.E.m±	0.007	0.007	0.013			0.007	0.007	0.015			0.008	0.008	0.015		
CD 0.05	0.021	0.019	NS			0.025	0.022	NS			0.026	0.022	NS		
CV %	6.37	5.27				4.03	3.02				4.64	3.54			

Table 7: Effect of water stress on peroxidase (POD) activity of mungbean genotypes

Genotypes	peroxidase (POD) activity (µ mol mg^{-1})														
	15 DAS					30 DAS					45 DAS				
	T_1	T_2	T_3	T_4	Mean	T_1	T_2	T_3	T_4	Mean	T_1	T_2	T_3	T_4	Mean
GAM-5	2.66	1.43	1.43	1.43	1.74	1.97	3.29	1.73	1.74	2.18	2.20	2.51	3.63	2.12	2.61
NKM-15-08	2.77	1.55	1.54	1.55	1.85	2.08	3.41	1.85	1.85	2.30	2.32	2.62	3.77	2.21	2.73
NKM-15-10	2.64	1.40	1.41	1.41	1.71	1.93	3.26	1.70	1.71	2.15	2.16	2.48	3.60	2.08	2.58
NKM-15-06	2.69	1.46	1.45	1.46	1.76	2.00	3.32	1.77	1.77	2.21	2.23	2.54	3.67	2.15	2.64
NKM-15-09	2.71	1.48	1.48	1.49	1.79	2.04	3.35	1.78	1.79	2.24	2.26	2.56	3.70	2.18	2.67
NKM-15-12	2.74	1.51	1.51	1.52	1.82	2.06	3.38	1.82	1.82	2.27	2.29	2.60	3.73	2.21	2.71
Mean	2.70	1.47	1.47	1.47		2.01	3.34	1.77	1.78		2.24	2.55	3.68	2.16	
	T	G	T×G			T	G	T×G			T	G	T×G		
S.E.m±	0.013	0.012	0.024			0.019	0.016	0.033			0.024	0.014	0.028		
CD 0.05	0.040	0.034	NS			0.061	0.047	NS			0.075	0.040	NS		
CV %	3.44	2.67				4.18	2.94				4.34	2.11			

Discussion

Oxidative stress is the major symptom of decrease in chlorophyll content results under water stress and may be the consequence of pigment photo-oxidation and chlorophyll degradation. Photosynthetic pigments are concerned with capturing sun light and production of reducing powers in the form of NADPH. Photosynthetic pigments decreased with water stress (Sanadhya *et al.* 2012) [19]. Rambabu *et al.* (2016) [17] reported that water stress at maximum vegetative stage decreased the total chlorophyll content by 52 percent, whereas at the flowering stage, decreased by 14.50 percent compared to irrigated control. Water stress affected the chlorophyll content, which ultimately affects the seed yield.

Hossain *et al.* (2013) [8] reported that irrigation cutting causes protein density reduction in a seed of plant, also those cultivars that have got more protein are more resistance to irrigation cutting, in whole they gained results by physiological characters evaluation shows that physiological indexes had got the least damage in seed formation stage and the most significance difference in flowering stage.

The proline content significantly increased with the increase in the stress as compared to the irrigation conditions. The results were found homologous with Kumar *et al.* (2014) [13] and Hossain *et al.* (2013) [8].

It was indicated significant differences in glycine betaine content amongst the six mungbean genotypes. The data also indicated that glycine betaine content significantly increased due to stress. The accumulation of the glycine betaine increased with the increase in the stress. The results are homologous to the findings of Sadeghipour (2015) [18], Tawfiket *et al*. (2008) [21] and Dutta and Bera (2007) [5].

Lipid peroxidation product (MDA) which accumulates more in mungbean under water stress condition as compared to irrigated. Function of plasmas membrane are adversely affected by environmental stress that can be measured as level of membrane lipid peroxidation (Sadeghipour, 2015) [18].

Free radicals (OH^+), single oxygen and superoxide oxygen (O_2^{-}) were destroyed by SOD, catalase and peroxidase so as to reduce the damage to lipid peroxidation during period of stress. Peroxidase and catalase scavenge the hydrogen peroxide, which accumulates in the plants under water stress. The increase in peroxidase activity might be due to formation of large amount of H_2O_2 during water stress whereas the decrease in catalase activity under water stress could be due to inhibition of protein synthesis reported by Rambabu *et al.* (2016) [17], Kumar *et al*. (2014) [13] and Dutta and Bera (2007) [5].

The biochemical parameters i.e. proline, glycine-betaine, catalase and peroxidase was significantly increased under water stress which leads to better maintenance of turgidity which helps the crop to thrive well under water stress and minimum reduction in seed yield was observed in some genotypes.

Conclusion

The present study indicated that water stress decrease chlorophyll and protein. Whereas, increased osmoregulator (proline and glycine betaine) oxidative damage, membrane lipid peroxidation as well as antioxidant enzymes (CAT and POX) activity and proline level in mungbean leaves. Among the five genotypes under this study, NKM-15-08 and NKM-15-12 cultivars had higher total chlorophyll content, protein, proline, glycine betaine, catalase and peroxidase activity under drought stress conditions and recorded high yield compared to other genotypes and were less affected by drought stress.

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