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Comparative analysis of biochemical parameters in response to stress in horsegram (*Macrotyloma uniflorum* L.) germplasm

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Abstract

Plants are continuously exposed to number of biotic and abiotic stresses during its life span. Amongst all, drought is one of the major abiotic stresses affecting the agricultural production worldwide. Horsegram (*Macrotyoma uniflorum* L.) is considered to be drought tolerant legume, which was chosen to compare the various biochemical parameters in response to drought stress. Plants of mapping population consisting of 86 germplasm lines were subjected to wilting under drought stress and compared with the plants of control for various biochemical parameters. The proline content was significantly higher in tolerant lines as compared to sensitive genotypes under drought stress. The maximum variation within the panel was observed for proline contents among all the five biochemical parameters used for estimation. The results suggested that higher levels of proline in horsegram lines could be used for identifying drought tolerant lines for the development of drought tolerant and high yielding varieties.

Key words: Macrotyloma uniflorum L., proline, MDA, chlorophyll A, chlorophyll B, carotenoids, drought stress.

Plant growth and development is regulated by number of environmental factors and many a times plant experiences different types of stresses affecting agricultural productivity worldwide. Drought, being the most important environmental stress, severely affects the plant growth and development and limits plant production and performance of crop more than any other environmental factor (Shao et al. 2009). Plant tends to protect itself in response to stresses by altering its pathway by producing certain biochemicals and modifying its physiological processes to reduce water loss. Despite being major producer and consumer of pulses India has been reported to have losses of 2.3 billion dollar foreign exchange every year (Lokeshwer 1997). This may be due the fact that major pulses in India are grown during the rainy (Kharif) season (June-october) and few of them like chickpea, lentil and peas are grown only during winter season. Due to lesser rainfall and drought like situation there has been seen a considerable reduction in production of pulses. (Chaudhary, 2013). Horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.) is a legume, which can withstand in adverse environmental conditions such as drought, salinity and heavy metal contamination (Reddy *et al*. 2008). With appreciable amount of valuable nutritional composition like proteins, minerals, vitamins, it is considered as a poor man crop. Though it is considered as underutilized and unexplored legume, horsegram ranks remarkably well among the most important pulse crop of India (Lokeshwar 1997).

Plants in general respond to the abiotic stresses differently at different level of growth such as the accumulation of osmolytes and proline specifically in response to drought stress (Shinozaki and Yamaguchi 2007). Keeping in view that the accumulation of various biochemical molecules protects plants from water stress, elusive studies are required on accumulation and involvement of biochemicals in drought stress tolerance. Furthermore, identification of germplasm that carry genes for drought tolerance and higher water use efficiency in horsegram is urgently required to be includes in cultivation system. Therefore, the present study was undertaken with objectives to estimate the levels of various biochemical parameters like Chlorophyll levels, Proline content, carotenoids and lipid peroxidation in 88 horsegram lines in response to drought stress and to identify drought tolerant lines (Table 1).

Materials and Methods

Plant material: Eighty eight accessions of horsegram germplasm (Table 1) were planted under glasshouse condition for the biochemical studies. These lines were sown in the month of August, 2020 and drought stress was induced by withholding irrigation to plants in order to study the various biochemical parameters under controlled and stressed conditions. Water stress was given to plants at the time of 50% of flowering in each line in order to cause wilting to the plant. Leaf samples were collected from all the plants at the time of wilting along with control, which were further subjected to various biochemical assays.

Chlorophyll and carotenoids content estimation: One gram of leaf sample was homogenized in 10ml of 80 % acetone until the residue become colourless (Yoshida *et al.* 1976). This colourless residue is used to measure the absorbance at 663nm for chlorophyll A, 645nm for chlorophyll B and 450nm for carotenoids by using the Eppendorf biospectrometer D30 spectophotometer. The optical densities are further used to measure the content of chlorophyll present in each sample under both stressed and control conditions by using the formulae,

ChlA=12.7 x A663-2.69 x A645x vol. made up/1000x wt of sample.

ChlB=22.9 x A645-4.68 xA663x vol. made up/1000x wt of sample.

Carotenoid =A480+ 0.114 x A663-0.638 x A645x vol. made up/wt of sample.

Lipid peroxidation was estimated as described by (Health and Packer 1968) by measuring the level of **Malondialdehyde (MDA).** Similarly the proline content by using the ninhydrin reagent was estimated by following the method described by (Bates *et al.* 1973). For calculating the proline and MDA content following formulae were used.

MDA=Concentration of MDA (mM) = (A532 - A600)/155

Proline= [(µg proline/ml) x ml toluene)/115.5 µg/µmole] / [(g sample)/5].

Statistical analysis: The results presented are the standard deviations of each biochemical content and correlation of the biochemical contents estimated by using PAST4.03 software.

Results and Discussion

Stress is a multidimensional phenomenon affecting plant growth at various stages (Yordanov *et al.* 2003) such as affecting photosynthetic activity, osmotic adjustment and various phenomenon in plant that impacted the plant parameters like yield, pigment content (Praba *et al.* 2009). The impact of drought on plants has been studied by understanding the mechanism of drought stress is a challenging task as it affects various physiological and biochemical processes in the plant. Keeping in view various climatic change models in many regions of the world (Anjum *et al.* 2011). The Present investigation reveals that the biochemical content for chlorophyll, malonaldehyde, proline, and carotenoids observed to be varied in two different environments i.e. normal and stressed conditions.

1. Accumulation of proline content in response to drought stress : Screening for drought tolerant genotypes can be done by examining genotypic efficiency to accumulate proline under stress, as its accumulation is a heritable trait. Proline is acting as an osmolyte for osmotic adjustments its accumulation normally occur in cytoplasm, where by functioning as a chaperon stabilizes the structure of proteins and maintain cell redox status. Keeping in view the diverse roles of proline in plants, it is important to understand the role of proline in water stress response of different genotypes of horsegram. Proline content was observed to be significantly higher under the stressed conditions in general. In the present investigation a range of 0.014 to 1.99 µ mole per gram with SD $\pm$ 0.124 was observed under the controlled condition, whereas the proline ranged from 0.5 to 2.98 µ mole per gram having SD $\pm$ 0.323 (Table 2). Within the panel of diverse genotype

Table 1. Details of horsegram Accessions used in the study along with their source

S. No.	Accession	State
1	IC56145	Punjab
2	IC22801	Bihar
3	IC19432	Madhya Pradesh
4	IC-120841	Madhya Pradesh
5	IC47119	Madhya Pradesh
6	IC 53641	Madhya Pradesh
7	IC-139503	Madhya Pradesh
8	IC49289	Madhya Pradesh
9	IC47461	Sikkim
10	IC110286	Himachal Pradesh
11	IC105785	Himachal Pradesh
12	IC120825	Madhya Pradesh
13	IC120806	Madhya Pradesh
14	IC120837	Punjab
15	IC139393	Himachal Pradesh
16	IC139360	Punjab
17	IC1301431	Madhya Pradesh
18	IC11394401	Sikkim
19	M AXIALLARE	Australia
20	IC544228	Andhra Pradesh
21	IC1074446	Madhya Pradesh
22	IC469266	Punjab
23	IC469259	Andhra Pradesh
24	IC107344	Punjab
25	IC547543	Tamil Nadu
26	IC391770	Tamil Nadu
27	HPKM-11-45	CSKHPKV Palampur
28	HPKM-11-56	CSKHPKV Palampur
29	HIMGANGA	Himachal Pradesh
30	HPKM-151	CSKHPKV Palampur
31	HPKM-317	CSKHPKV Palampur
32	HPKM-150	CSKHPKV Palampur
33	IC416486	Tamil Nadu
34	IC212722	Punjab
35	IC278828	Sikkim
36	IC280030	Madhya Pradesh
37	IC426526	Madhya Pradesh
38	IC426531	Sikkim
39	IC426532	Punjab
40	IC426538	Madhya Pradesh
41	IC426546	Punjab
42	IC926553	Madhya Pradesh
43	IC426557	Punjab
44	IC426577	Madhya Pradesh
45	IC426572	Madhya Pradesh
46	IC476573	Madhya Pradesh
47	IC426574	Tamil Nadu
48	IC426576	Punjab
49	IC-278826	Madhya Pradesh
50	IC-120821	Madhya Pradesh
51	IC426579	Sikkim
52	IC46785	Punjab
53	IC426457	Andhra Pradesh
54	IC426464	Tamil Nadu
55	IC313369	Tamil Nadu
56	IC426504	Andhra Pradesh
57	IC426513	Tamil Nadu
58	IC426516	Madhya Pradesh
59	IC426524	Sikkim
60	TCR253	Andhra Pradesh
61	TCR475	Andhra Pradesh
62	TCR467	Andhra Pradesh
63	TCR-628	Andhra Pradesh
64	TCR475	Andhra Pradesh
65	TCR491	Andhra Pradesh
66	TCR584	Andhra Pradesh
67	TCR591	Andhra Pradesh
68	TCR606	Andhra Pradesh
69	TCR615	Andhra Pradesh
70	TCR637	Andhra Pradesh
71	TCR658	Andhra Pradesh
72	TCR660	Andhra Pradesh
73	TCR663	Andhra Pradesh
74	TRC814	Andhra Pradesh
75	TCR814	Andhra Pradesh
76	TCR1081	Andhra Pradesh
77	TCR1088	Andhra Pradesh
78	TCR1103	Andhra Pradesh
79	TCR1200	Andhra Pradesh
80	TCR1351	Andhra Pradesh
81	TCR1378	Andhra Pradesh
82	TCR1436	Andhra Pradesh
83	TCR1441	Andhra Pradesh
84	TCR1451	Andhra Pradesh
85	HPKM201	CSKHPKV Palampur
86	IC49550	Tamil Nadu
87	IC-313365	Tamil Nadu
88	IC-139442	Himachal Pradesh

the highest proline content was recorded in the accession TCR-491; whereas it was minimum in IC-110286. Similar studies have been performed on chickpea where appreciable increase in proline content was observed under drought in chickpea varieties that grow well compared to varieties with retarded growth (Salma *et al.* 2016). Rapid shoot in free amino acid particularly proline was observed as an effect of water stress to plant. This accumulation of proline was envisaged as adoption mechanism in common bean (Jager and Meyer 1977). In chickpea genes involved in carotenoid biosynthesis and proline were found to be positively correlated (Rezaei *et al.* 2019).

2. Accumulation of Carotenoids in response to drought stress: Carotenoids is another biochemical, which got accumulated in plant leaves to stabilize turgor pressure in response to water stress. In the present investigation a range of carotenoid concentration was observed from 1.19 to 3.9 μ mole per gram under the stressed conditions. Lowest concentration of carotenoids was observed in IC-120806 followed by IC-22801, whereas it is recorded highest in the lines IC-139440 followed by IC-549228. A positive correlation between carotenoids and proline was observed indicating that measurement of one parameter is enough to identify drought tolerant plants. Similar observation in the plants producing these biochemicals content under drought has been recorded in soybean where PEG (Polyethylene glycol) treatment was given to plants to induce drought (Basal and Szabó 2020).

3. Accumulation of chlorophyll content under drought stress: The decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation (Anjum *et al.* 2011). In the present investigation drought imposed to the plants was observed with decreased chlorophyll content. The estimation of chlorophyll revealed that the range of concentration 0.083 - 2.86μ mole/gm of chlorophyll was observed. The lines with lowest chlorophyll content among the panel were TCR-568 followed by IC-31369, and the highest chlorophyll content was observed in the lines IC-426526 followed by IC-426576. No significant positive correlation of chlorophyll has been observed with any of the biochemical. Decreased chlorophyll level during drought stress has been reported in chickpea (Mafakheri *et al.* 2010).

4) Accumulation of Melonaldehyde during drought stress: Melonaldehyde is produced due to peroxidation of unsaturated fatty acids in phospholipids, which is used as indicator to free radical damage to cell membrane under stressful conditions (Wang *et al.* 2014). The MDA content does not increase much from controlled condition unlike proline but it increases more than the chlorophyll and carotenoids content respectively. MDA was observed in range of 0.11- 2.38 μ mole per gram in controlled conditions and it was 0.864 - 3.1μ mole/gm in the stressed conditions. The lines with lowest MDA content were IC-426577followed by IC-47119, IC-469266 and MDA was highest in the lines IC-56145 followed by IC-53641, IC-19432. MDA was not positively correlated with any of the biochemical parameters recorded in the present study. Similar observations in horsegram have been recorded where slight increase in MDA concentration has been observed under stressed condition (Bhardwaj and yadav, 2012) the details of all the correlations is provided in table 3.

Table 2. Mean and standard deviation of biochemicals in controlled and standard conditions

	Chlorophyll A		Chlorophyll B		Proline		Carotenoids		MDA	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Mean	2.419*	0.681	0.577	1.731*	0.915	1.960*	0.479	2.581*	1.318	1.941
SD+	0.053	0.081	0.015	0.079	0.164	0.323	0.015	0.247	0.387	0.296

Table 3. Represents correlation values of biochemical parameters

	Chlorophyll A stress	Chlorophyll B stress	Proline stress	Carotenoids stress	MDA stress
Chlorophyll A stresss		0.6342*	0.0093	-0.0420	0.1688
Chlorophyll B stress	0.6342		-0.1696	-0.1449	0.0504
Proline stress	0.0093	-0.1696		0.6731*	-0.0124
Carotenoids stress	-0.0420	-0.1449	0.6731		-0.0292
MDA stress	0.1688	0.0504	-0.0124	-0.0292	

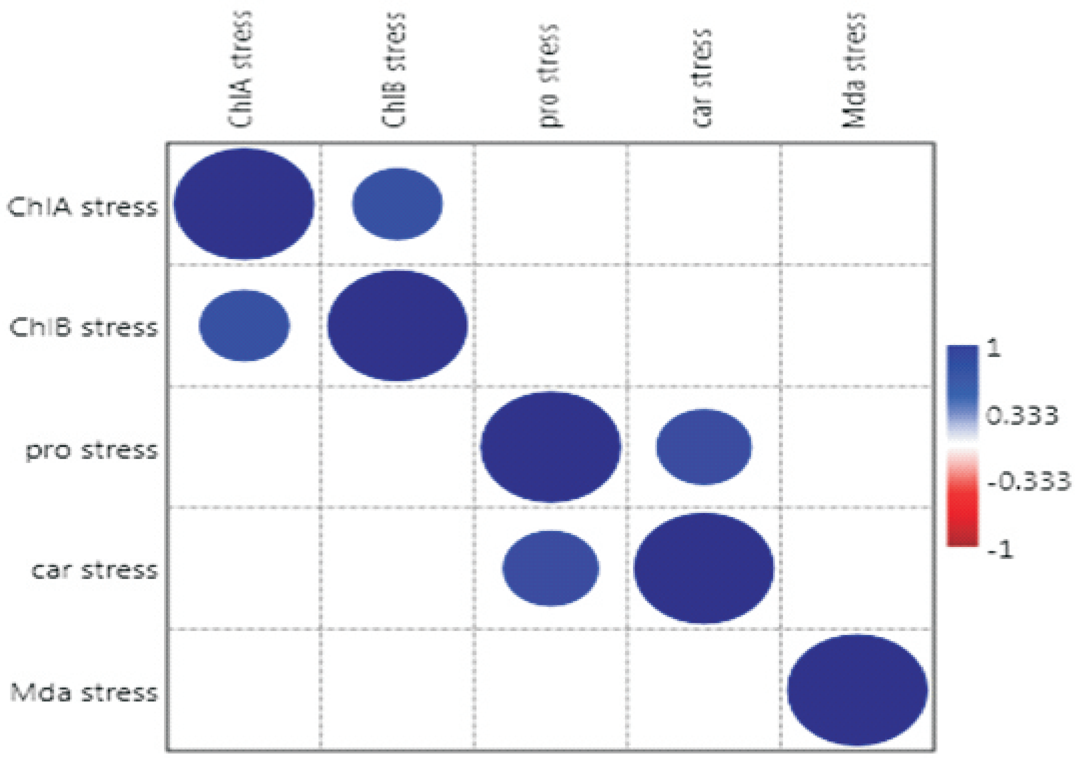

Fig 1. Pictorial representation of correlation among various biochemical and physiological parameters

Conclusion

The present study concludes that change in concentration of biochemical parameters in horsegram germplasm under the drought stress plays an important role in adopting plants against stressful conditions. It can be concluded that under the stressed condition there is increase in accumulation of proline and MDA, which suggest their important role in protecting the plant in response to drought stress. On the basis of biochemical parameters some of lines in horsegram germplasm namely TCR-491, IC-110286, IC-56145 and IC-53641 have been identified. These promising lines can be grown in drought prone area and are important in these changing water deficit conditions.

Conflict of interest: There is no conflict of interest among the authors for the present study.

References

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

Basal O and Szabo A. 2020. Physiomorphology of soybean as affected by drought stress and nitrogen application. Scientifica:1-7.

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

Bhardwaj J and Yadav SK. 2012. Comparative study on biochemical parameters and antioxidant enzymes in a drought tolerant and a sensitive variety of horsegram (*Macrotyloma umiflorum*) under drought stress. American Journal of Plant Physiology **7** (1): 17-29.

Chaudhary AK. 2013. Technological and extension yield gaps in pulse crops in Mandi district of Himachal Pradesh, India. Indian Journal of Soil Conservation **41** (1): 88-97.

Heath RL and Packer L. 1968. Photoperoxidation in isolated chloroplasts. Archives of Biochemistry and Biophysics **125** (1): 189-198.

Lokeshwar RR. 1997. Handbook of Agriculture. Indian Council of Agricultural Research, New Delhi.

Mafakheri A, Siosamerdeh A, Bahramnejad B, Strick PC and Sohrabi E. 2010. Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Australian Journal of Crop Science **4** (8):580-585.

Praba ML, Cairns J.E, Babu RC and Lafitte HR. 2009. Identification of physiological traits underlying cultivar differences in drought tolerance in rice and wheat. Journal of Agronomy and Crop Science **195** (1): 30 - 46.

Reddy PCO, Sairanganayakulu G, Thippeswamy M, Reddy PS Reddy MK and Sudhakar C. 2008. Identification of stress induced genes from the drought tolerant semi-arid legume crop horsegram (*Macrotyloma uniflorum Lam.*) Verdc. through analysis of subtracted expressed sequence tags. Plant Science **175** (3): 372-384.

Rezaei MK, Deokar AA, Arganosa G, Roorkiwal M, Pandey SK, Warkentin TD, Varshney RK and Taran B. 2019. Mapping quantitative trait loci for carotenoid concentration in three F2 populations of chickpea. The Plant Genome **12** (3): 1-12.

Salma UK, Khatun F, Bhuiyan MJH, Yasmin S, Khan TH. 2016. In vitro screening for drought tolerance of some chickpea varieties in Bangladesh. Progressive Agriculture **27** (2): 110-118.

Shao HB, Chu LY, Jaleel CA, Manivannan P, Panneerselvam R and Shao MA. 2009. Understanding water deficit stress-induced changes in the basic metabolism of higher plants-biotechnologically and sustainably improving agriculture and the co-environment in arid regions of the globe. Critical Reviews of Biotechnology **29** (2): 131-151.

Shinozaki K and Shinozaki KY. 2007. Gene networks involved in drought stress response and tolerance. Journal of Experimental Botany **58** (2): 221-227.

Wang X, Lei XG and Wang J. 2014. Malondialdehyde regulates glucose-stimulated insulin secretion in murine islets via TCF7L2-dependent Wnt signaling pathway. Molecular and Cellular Endocrinology **382** (1):8-16.

Yordanov I, Velikova V and Tsonev T. 2003. Plant responses to drought and stress tolerance. Bulgarian Journal of Plant Physiology (Special issue): **187**-206.