Research Journal of Biological Science
MEDWELL PUBLICATIONS SINCE 2000

Influence of Exogenous Application of Proline on Some Physio-Biochemical Parameters of Maize (*Zea mays* L.) Under Drought Stress

[1]Rukhsana Qamar, [2]Sibgha Noreen, [3]Muhammad Safdar and [4]Masroor Ellahi Babar
[1]*Department of Molecular Biology, Faculty of Science and Technology, Virtual University of Pakistan, Lahore, Pakistan*
[2]*Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan*
[3]*Department of Breeding and Genetics, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan*
[4]*Virtual University of Pakistan, Lahore, Pakistan*

Key words: Abiotic stress, drought, free amino acid, proline, proteins, maize

Corresponding Author:
Sibgha Noreen
Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan

Page No.: 53-59
Volume: 15, Issue 2, 2020
ISSN: 1815-8846
Research Journal of Biological Sciences

Abstract: Among abiotic stresses drought is a major detrimental factor all over the world. The present study was designed to investigate the influence of seed priming with proline under drought stress. The seeds of two varieties of maize (---------) were grown in control and drought stress condition under the exogenous application of proline as seed priming with varying regimes (0, 200, 400 ppm). Both varieties differ in terms of drought tolerance. The biomass production was reduced due to drought stress but proline enhanced the biomass production in terms of shoot and root fresh and dry organs at the 400 ppm as compared to other regimes. The cholorophyll “a”, “b”, “a/b” and “a+b” content was reduced under drought stress while 400 ppm pre-soaked proline enhanced the cholorophyll “a”, “b”, “a/b” and “a+b” content in shoot organ of both varieties. Total protein and aminoacid content in leaf was greatly affected due to drought stress but seed primed with proline @400 ppm enhanced the production of total protein and amino acids in leaf organ of both varieties. SDS-PAGE protein profiling showed that 200 ppm concentration of proline was most effective in both varieties. Among both varieties the variety “Hay Corn” is most tolerant toward the drought stress.

INTRODUCTION

In nature, the plants are exposed to various biotic and abiotic stress factors. In abiotic factor soil salinity, water deficit, heavy metal and extreme temperature are included are the major factors which directly inhibit growth and development of crop plants[1]. In these abiotic stresses, the drought/water deficit is major abiotic stress that limits biological yield[2]. Drought stress causes the changes at anatomical, physiological, biochemical and molecular level in plants at all stages of life cycle[3]. All these metabolic processes are determined the plant health.

Disturbance in one of these can affect the plant growth and development. Drought or water deficit condition severely effects on seed germination and cell growth of plants[4]. The activity of meristematic cell division and other expansion of newly develop cell increases the growth of plant depends upon turgor pressure of plant cell. Under drought stress, growth is retarded in higher plant due to disturbance in water movement from xylem to the make longer cell[5].

Drought stress reduced the shoot and root fresh weight[6] (), reduced ion uptake like K and Ca[7] (), enhanced the uptake of Na[8] (-----), decreased the accumulation of protein and amino acids[9] (-------), decrease the cholorophyll content (-----) and causes the photo-oxidation of chlorophyll. Water deficit decrease the chlorophyll a, b, a/b and over-all chlorophyll contents[10]. Photosynthetic efficiency of plant decreases chlorophyll contents in water deficit condition. Drought change a variety of plant responses that ranges from cellular metabolism as result of this growth rates and crop yields are reduced.

To save the plant from the harms of drought or other stresses plant accumulate the low molecular weight enzymatic or non-enzymatic antioxidants. Superoxide dismutase, Peroxides, Catalase (CAT) and Ascorbate Peroxides (APX) are enzymatic antioxidants[11]. While glutathione, ascorbate and carotenoids are non-enzymatic antioxidants. These both enzymatic and non-enzymatic antioxidants work together in scavenger mechanism to ROS[[12]. The osmolyte may be sucrose, soluble carbohydrates, glycine betaine and other solutes. The drought tolerant plant may have increased level of these osmolytes. While their level decreases in sensitive plants[13].

Among the seosmolytes the proline which is water soluble amino acid is the most important in the protection of plant facing drought stress. Under drought stress, the proline is first osmolytes that protect the plant from the injury to cell[4]. So, to overcome the drought stress condition, the exogenous application of proline is a smart tool.

Proline could be turned as a signaling molecule to modify plant physiological functions in termns of osmotic adjustment[14] (----), upgrade photosynthreticrate[15] (-----------), enhanced ion uptake[16] (--------), enhanced antioxidant activity and reducec ROS production[10] (-------), improve biological yield[17] (------) and also effects on cell explosion or cell death and cause gene expression, that can be vital for plant rescue from osmotic stress. Proline accumulation was observed in many stress tolerant plants like maize (), rice (), wheat ()[17, 18].

Maize (*Zea mays* L.) is cereal crop belong to family Poaceae[19]. Maize is 3rd significant crop among cereals and it is consumed by man as food also forage for cattle and poultry[20]. Due to over population food demand is increasing with the passage of time and this crop is gaining an imperative position in crop cultivation/farming due to its high yield prospective, high nutritious worth, short growth period, consumption in industry to make corn silk, corn sugar and corn flask[21].

The seed of maize is much importance in nutrition. Its seeds contain about 72% starch, 10% protein, 4.8% oil, 8.5% fiber and 1.7% ash. It is also used for the forage of livestock and for making the foodstuff such as starch, dextrose, glucose and other particular commodities. It is also used for producing biofuel[8, 12, 16].

Thus, the present study was designed to find out the physiological, biochemical and molecular aspects of maize under drought stress treated with exogenous application of proline.

MATERIALS AND METHODS

The experiment was carried out in Botanical Garden of Bahauddin Zakariya University Multan. The experiment was arranged in Complete Randomized Design with four replicates. The pots were filled with soil. The seeds of two varieties of maize SG 2002 F-Goi and Hay corn were pre-soaked in 0, 200 and 400 ppm proline. Plants of three weeks were treated with drought stress by skipping the irrigation. After a week of stress, the plants were harvested for further morphometric, physio-biochemical and proteomic profile.

Morphometric attributes

Shoot and root length: The shoot and root length was measured by using the tape-meter.

Fresh and dry weight of root and shoot: The fresh weight of root and shoot weight was calculated by using the electric balance. Then the plants root and shoot were kept in oven at 50°C for a week. After 1 week, the plants were completely dried. Then the weight of samples was done.

Biochemical assays

Chlorophyll estimation: The chlorophyll was measured by the method of Arnon *et al*.[6]. The leaf sample about 1 g was ground into 3 mL acetone, filtered and raised up to 10 mL with acetone. The values were taken by using double beam spectrophotometer (U-Hiteach 2900) at 663, 652 and 645 nm.

Proteins quantification: Proteins quantification was done following the procedure of Bradford[9].

Total soluble proteins: The leaf samples about 200 g was ground into sodium phosphate buffer having pH 6.8 in chill condition and centrifuged at 15000 rpm. Supernatant was kept for further quantification of proteins. The 30 µL supernant was mixed in 1500 µL Bradford reagent in dark condition. Then the reading was taken by using double beam spectrophotometer (U-Hitachi) at 595 nm.

Total free amino acids: The 1 mL supernant was mixed with 1 mL ninhydrin and 1 mL pyridine. Then the mixture was heated in water bath for 30 min at 100°C and raised up to 100 mL by using distal water. Then, the read was taken in double beam spectrophotometer at 570 nm.

Molecular investigations

Proteins profiling: Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) was performed to resolve the proteins. It was performed by using the protocol of Laemmli[19].

Statistical analysis: The Analysis of Variance (ANOVA) was done by using the SPSS Software.

RESULTS AND DISCUSSION

The analysis of variance for the shoot length had been shown in Table 1. The significance results were observed when maize is subjected to pre-soaked proline under drought stress. The maximum shoot length was recorded in SG 2002 F-Goi varieties in control condition without the pre-soaked with proline. While the minimum shoot length was recorded in Hay corn in control condition as shown in Fig. 1a. Under control condition 0 ppm proline pre-soaked, the variety Hay corn showed minimum shoot length than other variety. The analysis of variance of root length was shown in Table 1. The analysis showed non-significance interaction between the drought stresses in different pre-soaked level of proline. While the both two varieties showed the significance results with pre-soaked proline and without pre-soaked proline in control condition. The Hay Corn in 0 ppm proline was showed highest root length.

The analysis of variance for fresh weight of shoot has been shown in Table 1. The maximum fresh weight shoot was seen in Hay corn with 200 and 400 ppm proline treatment in control condition and minimum with proline 200 ppm under drought (Fig. 2a). The analysis of variance for the fresh weight of root has been shown in Table 1. The fresh weight of root was decreased in both varieties in drought condition without and with pre-soaked proline. The variety SG 2002 F-Goi was showed highest fresh weight of root in control condition with pre-soaked 400 ppm proline as shown in Fig. 2b. The analysis of variance for the dry weight of shoot had been presented in Table 1. From Table 1 it has shown application of

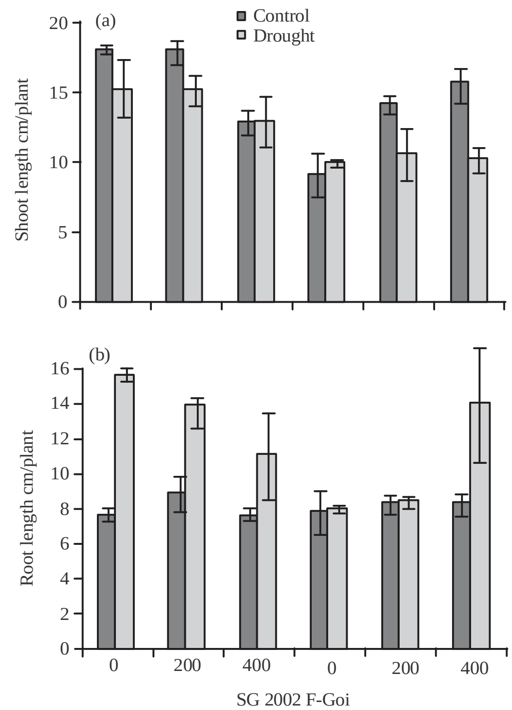

Fig. 1(a, b): (a) Shoot and (b) Root length of maize varieties pre-soaked with 0, 200 and 400 ppm proline grown under control and drought condition

proline shown significance enhancement of dry weight of shoot in 0 and 200 ppm in SG 2002 F-Gounder drought stress. The dry weight of shoot was decreased in pre-soaked in Hay corn variety under drought condition as shown in Fig. 2c.

From Table 1 and Fig. 2 it has been cleared that the drought has negative effect on the dry weight of root. The variety SG 2002 F-Goi was showed better performance under drought and control condition with and without pre-soaked proline. In control condition with 200 ppm pre-soaked proline. The variety SG 2002 F-Goi was showed maximum dry weight of root in control condition with pre-soaked 200 ppm proline as shown in Fig. 2d.

The chlorophyll a content was shown in Table 1 and Fig. 3a. From the Fig. 3, it is cleared that chlorophyll a contents were reduce in drought stress as compared to control except 0 ppm. Under control condition with pre-soaked with 400 ppm showed highest chlorophyll contents. While under drought stress all pre-soaked with 0, 200 and 400 ppm was showed equal chlorophyll a contents. The chlorophyll b contents are presented in Fig. 3b. From Fig. 3, it is cleared that the chlorophyll b contents were reduced in drought stress as compared to control condition in variety SG 2002 F-Goi. Under drought condition, the pre-soaked 400 ppm was highest chlorophyll b contents in Hay corn variety. In variety SG

Table 1: Analysis of variance for shoot length, root length, fresh weight of shoot and root, dry weight of shoot and root, chlorophyll contents, total soluble proteins and total free amino acids

Source of Variance (SOV)	Shoot length (cm/plant)	Root length (cm/plant)	Shoot fresh weight (g/plant)	Root fresh weight (g/plant)	Shoot dry wight (g/plant)	Root dry weight (g/plant)	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Chlorophyll a/b (mg g^{-1})	Total chlorophyll (mg g^{-1})	Total soluble proteins (mg g^{-1})	Total free amino acids (mg g^{-1})
Varieties	25.729***	23.761***	0.446 ns	1.695 ns	57.517***	32.420***	6.917*	14.730***	14.474***	0.012 ns	0.350 ns	0.918 ns
Drought	9.357 **	4.614*	42.34***	32.789***	98.193***	18.525***	32.931***	7.736 *	1.110 ns	45.274***	16.332***	19.085***
Proline priming	1.673 ns	0.138ns	6.991**	0.713ns	9.647 ***	1.1576 ns	5.351 *	19.303 ***	3.235 ns	21.441***	1.466 ns	9.138**
Varieties *Drought	0.433 ns	4.976*	5.559*	1.555ns	4.737*	9.461**	7.062*	7.410 *	3.980 ns	0.374 ns	2.324 ns	0.749 ns
Varieties *Proline priming	8.030**	0.616 ns	4.281*	1.360 ns	16.360***	3.389 ns	2.329 ns	0.827 ns	0.516 ns	3.542*	0.086 ns	6.043**
Drought *Proline priming	0.833 ns	4.875*	8.633**	1.474 ns	67.406***	6.921**	3.246 ns	0.149 ns	0.802 ns	2.162 ns	0.125 ns	1.756 ns
Varieties *Drought *Proline priming	3.280 ns	3.783*	5.915**	2.853 ns	27.528***	15.46***	1.848ns	0.273 ns	0.421 ns	0.734 ns	0.060 ns	5.35*
Error	4.632	4.069	0.033	0.0350	6.080	9.573	0.018	0.009	0.461	0.023	701.354	2632026.9

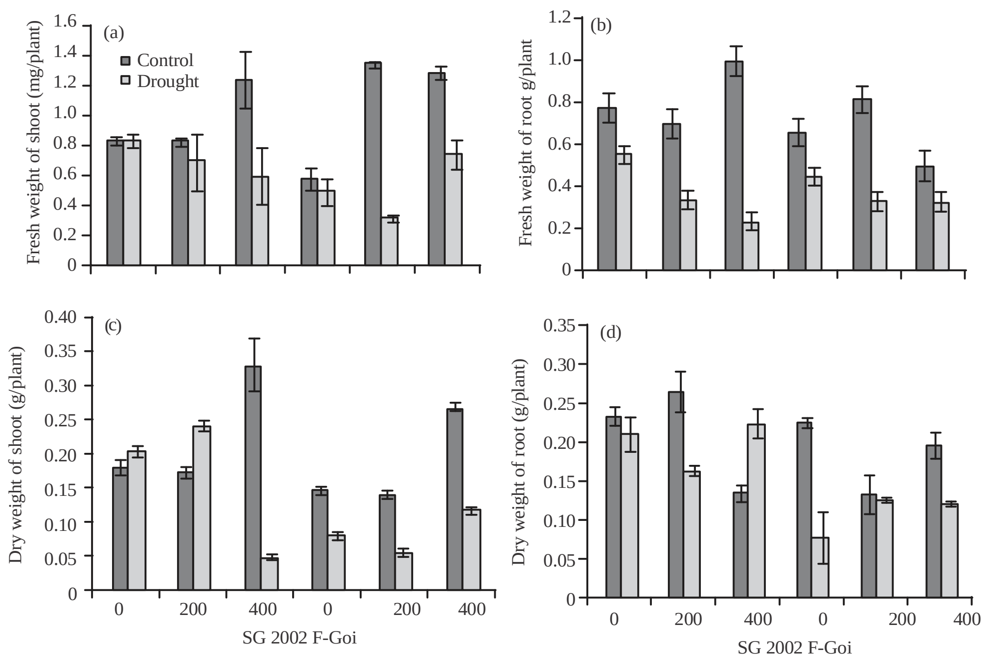

Fig. 2(a-d): (a) Fresh weight of shoot (b), Fresh weight of root (c), Dry weight of shoot and (d) Dry weight of root of maize varieties pre-Soaked with 0, 200 and 400 ppm proline grown under control and drought condition

2002 F-Goi, the a/b was increased 0 ppm under drought stress. Under drought stress, the a/b of chlorophyll was decreased in all treatment of proline The total chlorophyll contents were shown in Fig. 3d and Table 1. Under control and drought condition pre-soaked 400 ppm proline was showed highest chlorophyll contents. While under control and drought condition 0 ppm was showed lowest chlorophyll contents.

The analysis of variance for the total soluble proteins has been presented in Table 1. The both varieties showed non-significance results in control with and without pre-soaked proline. These result also non-significance between two varieties under drought stress as shown in Fig. 4a and Table 1. The analysis of variance for total free amino acids has been presented in Table 1. From Table 1 and Fig. 4, it has been shown that the both varieties in 0 and 200 ppm showed non-significance results in control condition. The maximum value of total free amino acids has been recorded in SG 2002 F-Goi variety under drought stress pre-soaked with 400 ppm proline as shown in Fig. 4b.

Figure 5a-d showed the different banding pattern under reducing and non-reducing condition of maize varieties pre-soaked with proline 0, 200 and 400 ppm concentration. Under reducing condition in both varieties, the bands are more clear and reflect the expression of

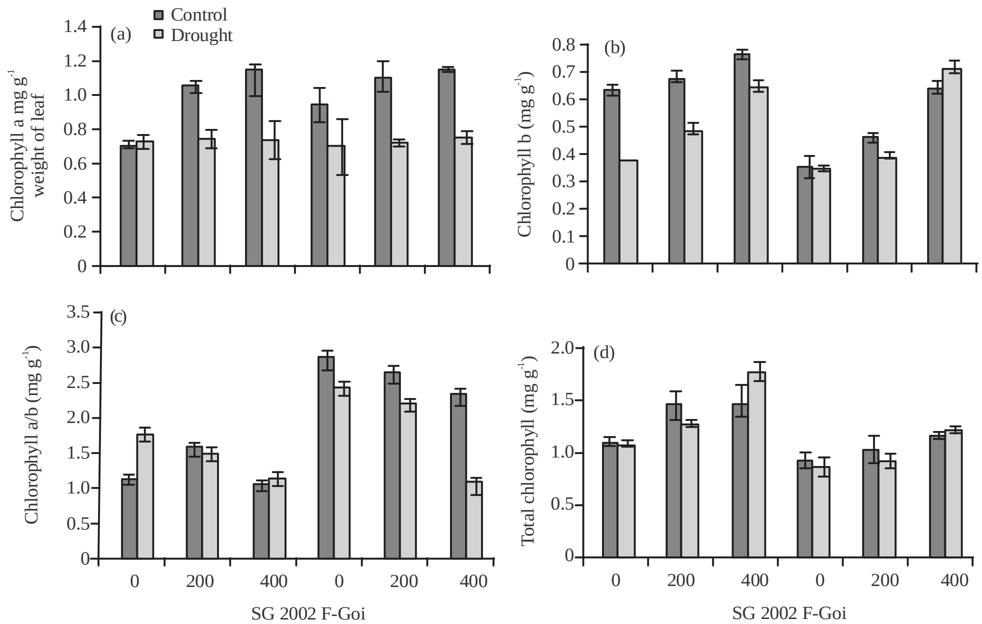

Fig. 3(a-d): Chlorophyll a (a), b (b), a/b (c) and Total chlorophyll and (d) Contents of maize varieties pre-soaked with 0, 200 and 400 ppm proline grown under control and drought condition

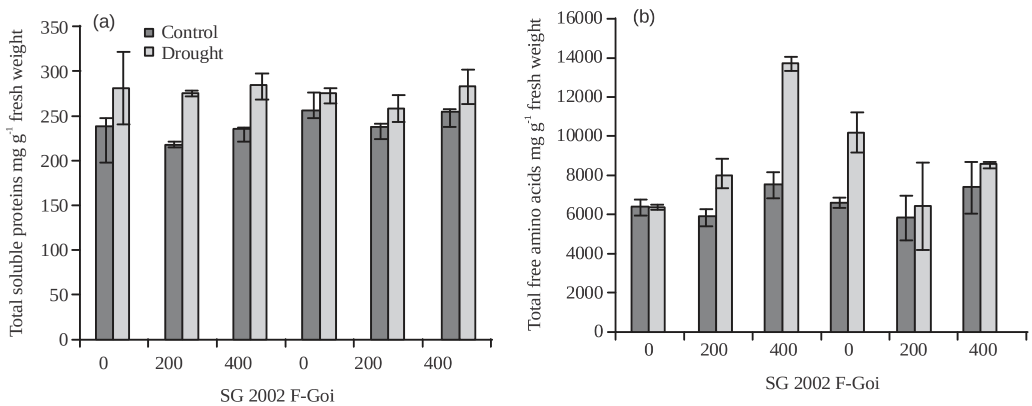

Fig. 4(a, b): (a) Total soluble proteins and (b) Total free amino acids of maize varieties pre-soaked with 0, 200 and 400 ppm proline grown under control and drought condition

proteins under drought and pre-soaked condition. Drought is major abiotic stress that decreased the plant growth, development and yield of crop plant. Plant accumulates different osmolytes such as proline. The exogenous application of proline relief the plant under stress condition[22].

Biomass production of Maize was decreased when it is subjected to drought in comparison to control condition and increased while in the plants treated with proline concentrations. Same happened with root and shoot length. The reason for reduction in shoot and root length as well as reduction in their biomass like may be due to enhance in osmotic potential by cumulative salts which centrals to dehydration, ionic imbalance in developing leaves that caused reduction in meristem activity and cell elongation. These results are similar to previous studies by Kausar *et al*.[18]. Harris *et al*.[14] and Noreen *et al*.[23] exhibited reduction in biomass production by the imposition of salt in barley, wheat, pigeon pea and cotton.

These results are similar to the findings of Aldesuquy *et al*.[2]. In the present study, the chlorophyll contents were decreased under drought stress and showed high graph in treatment with proline. Stress avoids the plant to set off working of pigments like chlorophyll and causes the production of protein[13]. Similar findings were observed in the chlorophyll contents of maize[24].

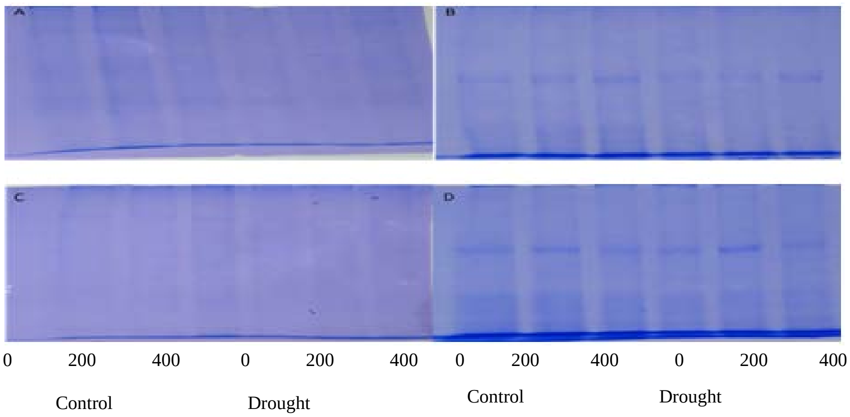

Fig. 5(a-d): SDS-PAGE gel stain with Coomassie brilliant blue dye G-250 in non-reducing condition (a) and Reducing (b) of maize variety SG 2002 F-Goi while (c) and (d) Non-reducing and reducing respectively for Hay corn maize variety with 0, 200 and 400 ppm proline grown under control and drought condition

Fig. 6: SG 2002 F-Goi maize variety pre-soaked with 0, 200 and 400 ppm proline grown under control and drought condition

Fig. 7: Hay corn maize variety pre-soaked with 0, 200 and 400 ppm proline grown under control and drought condition

CONCLUSION

Drought stress has significance effect on proteins and amino acids. The current study indicates that the drought stress increased the total soluble proteins and total free amino acids. The pre-Soaked proline varieties showed more proteins and amino acids under drought stress. These results are similar to previous studies. The increment in soluble protein by the application of proline is because of de novo synthesis of proteins and amino acid for cell defence[25]. Literature says that the proteins content improves in the plants facing stress and this increased amount of proteins tries to protect the plant from unadorned stress[21, 25-27].

REFERENCES

01. Aldesuquy, H.S., S.A. Abo-Hamed, M.A. Abbas and A.H. Elhakem, 2012. Role of glycine betaine and salicylic acid in improving growth vigour and physiological aspects of droughted wheat cultivars. J. Stress Physiol. Biochem., 8: 149-171.
02. Aldesuquy, H.S., F.I. Ibraheem and H.E. Gahnem, 2014. Comparative morpho-biochemical responses of wheat cultivars sensitive and tolerant to water stress. J. Stress Physiol. Biochem., 10: 168-189.
03. Ali, G.M. and S. Komatsu, 2006. Proteomic analysis of rice leaf sheath during drought stress. J. Proteome Res., 5: 396-403.

04. Anjum, S.A., X.Y. Xie, L.C. Wang, M.F. Saleem, C. Man and W. Lei, 2011. Morphological, physiological and biochemical responses of plants to drought stress. Afr. J. Agric. Res., 6: 2026-2032.
05. Apel, K. and H. Hirt, 2004. Reactive oxygen species: Metabolism, oxidative stress and signal transduction. Annu. Rev. Plant Biol., 55: 373-399.
06. Arnon, D.I., M.B. Allen and F.R. Whatley, 1956. Photosynthesis by isolated chloroplasts IV. General concept and comparison of three photochemical reactions. Biochimica Biophysica Acta, 20: 449-461.
07. Ashraf, M. and M.R. Foolad, 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ. Exp. Bot., 59: 206-216.
08. Aslam, M., M.A. Maqbool and R. Cengiz, 2015. Drought stress in maize (*Zea mays* L.): Effects, Resistance Mechanisms, Global Achievements and Biological Strategies for Improvement. Springer, New York, USA.,.
09. Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.
10. Jaleel, C.A., P. Manivannan, G.M.A. Lakshmanan, M. Gomathinayagam and R. Panneerselvam, 2008. Alterations in morphological parameters and photosynthetic pigment responses of Catharanthus roseus under soil water deficits. Colloids Surf. B: Biointerfaces, 61: 298-303.
11. Chen, Q., H. Zhong, X.W. Fan and Y.Z. Li, 2015. An insight into the sensitivity of maize to photoperiod changes under controlled conditions. Plant Cell Environ., 38: 1479-1489.
12. Chomba, E., C.M. Westcott, J.E. Westcott, E.M. Mpabalwani and N.F. Krebs *et al*., 2015. Zinc absorption from biofortified maize meets the requirements of young rural Zambian children. J. Nutr., 145: 514-519.
13. Davison, P.A., C.N. Hunter and P. Horton, 2002. Overexpression of ß-carotene hydroxylase enhances stress tolerance in Arabidopsis. Nature, 418: 203-206.
14. Harris, B.N., V.O. Sadras and M. Tester, 2010. A water-centred framework to assess the effects of salinity on the growth and yield of wheat and barley. Plant Soil, 336: 377-389.
15. Harris, D., R.S. Tripathi and A. Joshi, 2002. On-farm seed priming to improve crop establishment and yield in dry direct-seeded rice. Proceedings of the International Workshop on Direct Seeding in Asian Rice Systems: Strategic Research Issues and Opportunities, Jan., 25-28, Bangkok, Thailand, pp: 231-240.
16. Irfanullah, H.A., A.A.I. Hussain, M.W. Khan and M.D. Ahmadzai, 2017. Yield and yield attributes of maize (*Zea mays* L.) as affected by detasseling and potassium fertilization. Pure Applied Biol. (PAB.), 6: 958-964.
17. Kaiser, W.M., G. Kaiser, S. Schoner and S. Neimanis, 1981. Photosynthesis under osmotic stress. Planta, 153: 430-435.
18. Kausar, A., M.Y. Ashraf and M. Niaz, 2014. Some physiological and genetic determinants of salt tolerance in sorghum (*Sorghum bicolour* (L.) Moench): Biomass production and nitrogen metabolism. Pak. J. Bot., 46: 515-519.
19. Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.
20. Rahi, A.A., S.M. Mehdi, M.K. Rasheed, B. Butt and R. Ullah *et al*., 2013. Evaluating the salinity tolerance of maize (*Zea mays* L.) genotype under brackish water application in Punjab-Pakistan. Life Sci. J., 10: 913-919.
21. Reid, L.M., M.J. Morrison, X. Zhu, K.K. Jindal and B.L. Ma, 2015. High stalk sugar corn: A potential biofuel crop for Canada. Agron. J., 107: 475-485.
22. Nayyar, H. and D.P. Walia, 2003. Water stress induced proline accumulation in contrasting wheat genotypes as affected by calcium and abscisic acid. Biol. Plant., 46: 275-279.
23. Noreen, S., H.U.R. Athar and M. Ashraf, 2013. Interactive effects of watering regimes and exogenously applied osmoprotectants on earliness indices and leaf area index in cotton (*Gossypium hirsutum* L.) crop. Pak. J. Bot., 45: 1873-1881.
24. Rashid, M., M.A. Sajid, S. Noreen, A. Akrem, S. Mahmood and K.H. Shah, 2018. 17. Study of adverse effects of drought stress on two different hybrids of maize (*Zea mays* L.). Pure Applied Biol. (PAB.), 7: 1316-1325.
25. Teixeira, M. and I.S. Carvalho, 2009. Effects of salt stress on purslane (*Portulaca oleracea*) nutrition. Ann. Applied Bio., 154: 77-86.
26. Stich, B., B.I. Haussmann, R. Pasam, S. Bhosale, C.T. Hash, A.E. Melchinger and H.K. Parzies, 2010. Patterns of molecular and phenotypic diversity in pearl millet [*Pennisetum glaucum* (L.) R. Br.] from West and Central Africa and their relation to geographical and environmental parameters. BMC Plant Biol., Vol. 10. 10.1186/1471-2229-10-216
27. Auge, R.M., 2004. Arbuscular mycorrhizae and soil/plant water relations. Can. J. Soil Sci., 84: 373-381.