Journal of Stress Physiology & Biochemistry, Vol. 19, No. 2, 2023, pp. 16-24 ISSN 1997-0838

ORIGINAL ARTICLE

OPEN ACCESS

Accumulation of Osmoprotectants Acclimating Proso millet (*Panicum miliaceum* L.) to Drought Stress Tolerance

Reyaz Ahmad Mir*, R. Somasundaram

[1] *Department of Botany, Annamalai University, Tamilnadu, India*

*E-Mail: **reyazmaqbool07@gmail.com** ; ORCID ID 0000-0002-7096-8741

Received November 4, 2022

Drought is one of the environmental stressors that limits plant growth, production, and is a great threat to the rising population and security throughout the globe. In the present work, we aimed to check the impact of drought stress given at different days interval drought (DID) on proso millet and its tolerance capability. The pot culture experiment was conducted and drought stress was given at 3 DID, 5 DID, and 7 DID respectively, and sampling was done on 15, 30, and 60 DAS, and unstressed plants were irrigated regularly. Drought stress results in decreased protein contents in both the roots and shoots of *P. miliaceum* on all sampling days. However, with increasing drought intensity and duration there was a tremendous increase in amino acids, and proline contents as well. Therefore, increasing osmolytes like proline with an increased drought period proves the role of proline in stress tolerance of proso millet and helps it to survive by maintaining osmotic potential, water influx, and detoxifying ROS. Thus, proso millet can be cultivated in drought-prone areas, and used for the breeding program.

Key words: Drought stress, Proline, Osmoprotectants, Proso millet, ROS.

Plants in nature may be exposed to a wide range of biotic or abiotic stress factors. Environmental stresses, such as drought, extreme temperature, cold or high salinity, severely impair plant growth and productivity worldwide. The most significant environmental stressor, drought, drastically hinders plant growth and development, limits plant yield, and negatively affects the performance of crop plants more than any other environmental factors (Shao *et al.,* 2009). It is the most restrictive environmental element for crop quality and production, including economic output and the provision of food for people globally (Roche *et al.,* 2009). However, in India, different parts have varied drought trends and frequencies (Thomas *et al.,* 2015). By the end of the 21st century, drought episodes are projected to occur in India's peninsular, central northeast, and west-central regions (Ojha *et al.,* 2012). Its impacts in India are worsened by the monsoon's deviation (Thomas and Prasannakumar, 2016), groundwater depletion (Panda and Wahr, 2016), and growing population (Lobell *et al.,* 2008).

Water deficit can be defined as "Any water content of a tissue or cell that is below the highest water content exhibited at the most hydrated state". Water stress is a common phenomenon and it severely reduces the yield of crops (Jangpromma *et al.,* 2010a). Drought may occur at any time of the cultivation season because of variable climatic changes associated with increasing temperature than average and this may cause a profound decrease in yield (Parry *et al.,* 1999). Drought stress impacts include reduction of growth attributes, diminished productivity and its quality, pigment composition, and enhanced antioxidant activities (Silva *et al.,* 2012; Mir *et al.,* 2019). Such alterations are typically brought on by tissue dehydration (Dobra *et al.,* 2010).

Improving the efficiency of water use in agriculture is an accomplice to increasing the fraction of the available water resources that transpired, because of the unavoidable association between yield and water use (Lawlor *et al*., 2002). To improve production, it is important to comprehend how plants react to drought situations with the ultimate goal of improving crop performance in the vast regions of the globe where rainfall is limiting or unreliable. Although, the drought stress tolerance mechanism is not clearly understood, may be explained by stress adaptation effectors that mediate osmolyte biosynthesis, toxic radical scavenging, ion homeostasis, water transport, and long-distance response coordination (Reddy *et al.,* 2004). One mechanism utilized by crop plants to overcome the drought effects might be via the assimilation of osmoprotectants, including soluble sugars, and amino-acid based constituents including proline (Vendruscolo *et al.,* 2007; Ghosh *et al.,* 2021). These molecules are also known as cytoprotectants due to their capability to prevent cell contents against abiotic stresses (Groppa and Benavides, 2008; Khan *et al.,* 2010) by enhancing stress tolerance through maintaining ion-homeostasis, and scavenging ROS by upregulating antioxidant activities.

Millet is an important genetic resource that conducts C4 photosynthesis and requires less water. They are of potential value particularly in semi-arid regions due to their short life span. Millets either have the ability to face drought and extreme heat or they can avoid these conditions by maturing very quickly, a novel adaptive approach of millet crops (Baltensperger, 2002; Benhin, 2008). Therefore, millets could be the answer to fighting climate change, poverty, and malnutrition globally. Proso millet (*Panicum miliaceum* L.) is one of the important grass species of the largest genus *Panicum*, which include more than 400 species (Roshevits, 1980), widely cultivated in India, Russia, Turkey, Romania, and China (Nazifi *et al*., 2009; Chai, 1999). The fact being it uses soil water very effectively, is acclimated to sandy, dry soils, and thrives in dry climates (Baltensperger, 2002). Therefore, generally considered an anti-famine and relief crop. Proso millet is grown mostly in Southern India, south of the Krishna River, although it may be cultivated in scattered locales in central and hilly tracts of the North.

Proso millet grain is rich in nutrient, which is used as a staple food in Africa (Medonough, 2000) treated allergies in Japan (Nishizawa *et al.*, 1990), and is processed into convenience foods that is suitable for infants and young children in India (Srivastava *et al.,* 2001). The protein content in the grains of proso millet usually ranges from 11.3-12.7%. Broomcorn millet is used for medicine as well. For instance, proso millet protein has a role in regulating cholesterol metabolism (Nishizawa and Fudamo, 1995), and preventing liver injury (Nishizawa *et al*., 2002). Therefore, in near future, when the food and water crisis stares us in the face; millets can become the food of security. In view of all these features that they so amazingly combine, millets can only be called “Miracle Grains”. So, the present work was carried out with the aim to study the biochemical’s role in drought tolerance in proso millet.

MATERIALS AND METHODS

SEED COLLECTION AND EXPERIMENTAL DESIGN

Economically important common millet seeds (*Panicum miliaceum* L.), family *Poaceae* were collected from Kolimalai of Salem district, Tamilnadu, and were identified botanically by Tamilnadu Agricultural University, Coimbatore, Tamil Nadu, India.

Experimental Design

The research work was conducted in the Botanical Garden and biochemical analysis was conducted at Stress Physiology Laboratory, Department of Botany, Annamalai University. Healthy seeds were surface sterilized with 0.2 % Mercuric chloride solution for five minutes with frequent shaking and thoroughly washed with tap water. The experiment was laid out in a Completely Randomized Block Design (CRBD). To determine the most favorable periods that can be applied for drought stress withdrawal; 1, 2, 3, 4, 6, 7, 8, 9, or 10 days and which can influence growth. The preliminary experiment 1, 2, 3, …., and 10 days interval drought stress was used for the experiment. Among these treatments, based on the reduction in dry weight significant to 50%, 3 DID, 5 DID, and 7 DID (days interval drought) were chosen and used for all the experiments. Plastic pots of size 45 cm diameter and 60 cm height were used for the present study.

The pots were filled with 10 kg of soil mixture containing red soil, sand, and farm yard manure in the ratio of 1:1:1 ratio, and 44 pots were arranged in a completely randomized block design (CRBD). One set of 11 pots was kept as control and the other 3 sets of 33 pots (11 pots for each treatment) were used for drought treatments. Twelve selected seeds were cultivated in each pot. Formerly all pots were irrigated daily with 500 ml of water to keep the water content of each pot at 70% of the total holding capacity of the used soil, and then on the 8th day of plant growth, thinning was done so as to leave 5 uniform plants in each pot days after sowing (DAS). The plants were allowed to grow up to 15 DAS. The non-stressed plants (control) were irrigated daily. Mild stress (Irrigation 3 days later), moderate stress (Irrigation 5 days later), and severe stress (Irrigation 7 days later) were imposed from 15th DAS to 60th DAS. The drought was imposed and continued until the last of the experiment i.e., 15th to 60th DAS. Plants were selected randomly, uprooted on 30th, 45th and 60th DAS, washed carefully, and separated into the root and shoot for estimating biochemical constituents.

BIOCHEMICAL CONSTITUENTS

Estimation of free amino acids, soluble proteins, and proline contents

Soluble Protein content was estimated by Lowry *et al.* (1951) and observed at 595 nm using a spectrophotometer(U-2001–Hitachi). The results were expressed in mg/g FW. Free amino acids were estimated by following the previous protocol of Moore and Stein, (1948). The absorbance was read at 570 nm in a spectrophotometer. Proline contents were estimated by following the protocol of Bates *et al*. (1973). The absorbance was measured at 520 nm in a spectrophotometer respectively, using a ninhydrin reagent. Further L-leucine and L-proline were used as the standards and the results were expressed in milligrams per gram dry weight.

STATISTICAL ANALYSIS

The data pertained to all the characters studied were subjected to statistical analysis by One-Way ANOVA Statistical software version 22.0. Values in the figures indicate the mean ‘±’ Standard error of three replicates (n = 3). Differences between means were evaluated for significance using DMRT at 5% probability level.

RESULTS

Effect of Drought Stress on Biochemical Constituents

Protein

It is apparent from (fig. 2) that the protein content decreased progressively in all parts of *P. miliaceum* L. with increased periods of water withdrawal from 3 to 7 days above those of control plants. This decrease was observed higher on 60 DAS, particularly in 5 DID and 7 DID treatments than in 3 DID treatments (mild stress) respectively. When related to control, it was noted 83.63, 47.04, and 53.00 percent over control in shoots, and 90.27, 79.11, and 72.04 percent over control in roots on 60 DAS in 3 DID, 5 DID, and 7 DID respectively.

Amino acids

It is evident from (fig. 3) that drought stress caused a significant increase in free amino acids in shoots of *P. miliaceum* L. with an increased drought period. Thus, an increasing trend was observed between free amino acids and the severity of the drought period in all parts of *P. miliaceum* L. However, it was noted higher in 5 DID and 7 DID on 60 DAS, than in unstressed plants, and was 120.31, 137.67, and 151.10 percent over control in shoots and 112.59, 130.12, and 153.33 percent over control in roots on 60 DAS in 3 DID, 5 DID, and 7 DID respectively.

Proline (Pro)

Drought stress increased proline content in all parts of *P. miliaceum* L. with increased duration of drought treatments. It is evident from (fig. 4) that there is a positive correlation between the proline contents and the severity of drought in *P. miliaceum* L. plants. However, this increased amount was found higher in 7 DID on 60 DAS than in unstressed plants. In comparison to non-stressed plants, it was 140.38, 165.41, and 169.23 percent over control in shoots, and 145.16, 225.80, and 258.06 percent over control in root on 60 DAS in 3 DID, 5 DID and 7 DID respectively.

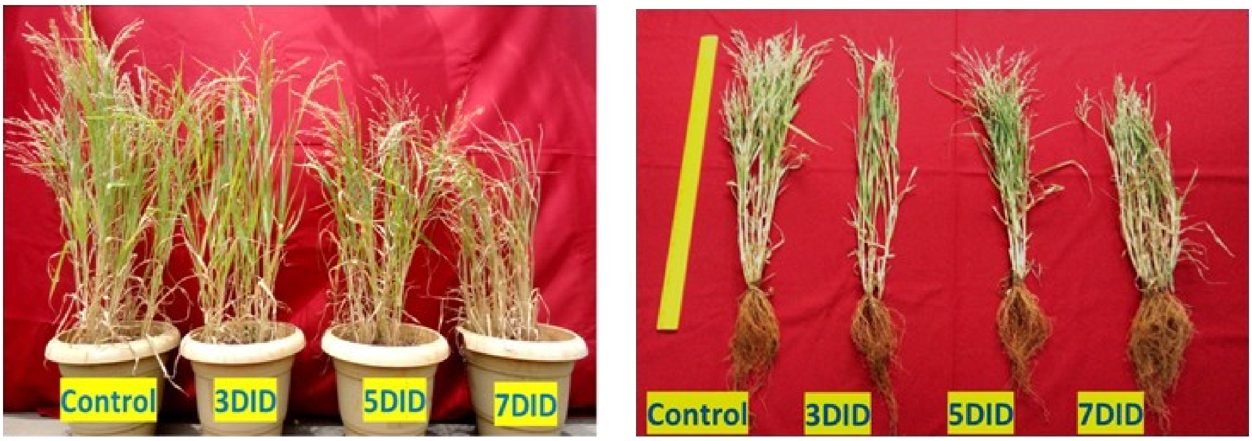

Figure 1 Drought stress and *Panicum miliaceum* L. on 60 DAS

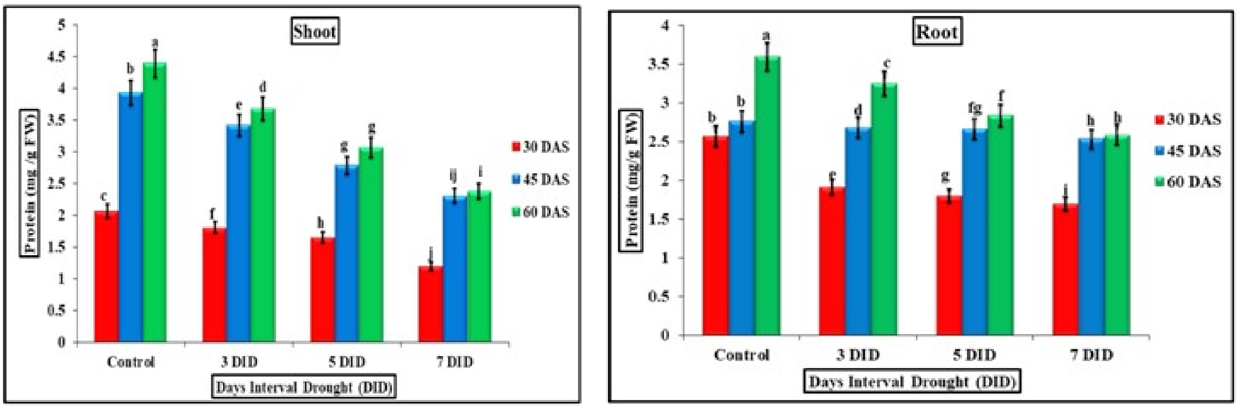

Figure 2 Impact of drought stress on protein content in shoot and root of *P. miliaceum* L. Values are expressed as mean ± standard error of three replicates (n=3). Error Bars with different lowercase alphabetic letters (a, b, c, d…..j) represent a significant difference at P ≤ 0.05% by DMRT.

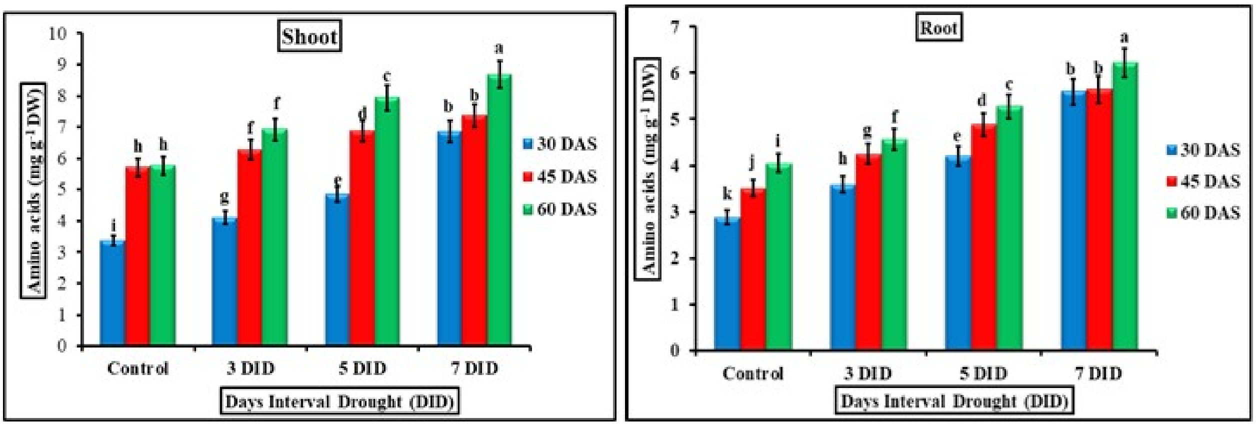

Figure 3 Impact of drought stress on amino acid contents in shoot and root of *P. miliaceum* L. Values are expressed as mean ± standard error of three replicates (n=3). Error Bars with different lowercase alphabetic letters (a, b, c, d….. k) represent a significant difference at P ≤ 0.05% by DMRT.

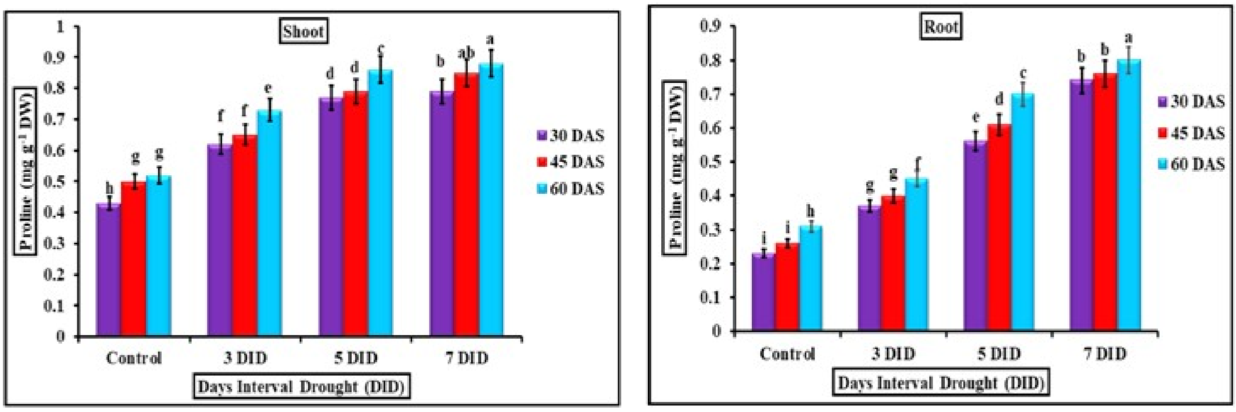

Figure 4 Impact of drought stress on proline contents in shoot and root of *P. miliaceum* L. Values are expressed as mean ± standard error of three replicates (n=3). Error Bars with different lowercase alphabetic letters (a, b, c, d….. i) represent a significant difference at P ≤ 0.05% by DMRT.

DISCUSSION

Drought is one of the challenging environmental factors that shows a negative impact on plant growth performance as seen in the above (fig. 1). It directly reduced the growth of plants by diminishing photosynthetic pigments and causes biomass reduction in *P. miliaceum* plants (Mir *et al.,* 2019). Therefore, exploring millets to drought is a promising strategy to get rid of food security and overcome malnutrition.

A negative correlation was progressively found between the severity of the drought period and protein content in *P. miliaceum* L. In comparison to unstressed plants, the decline was noted higher at maturity (fig. 2). Because plants use proteins to accomplish nitrogen deficiency by creating nitrogen molecules such as amino acids, which may assist little millet plants to adjust osmotic pressure and water intake capacity under salinity stress (Mir and Somasundaram, 2020) and the protein breakdown process becomes more active under insufficient circumstances seen in *A. esculentus* L. (Sankar *et al.,* 2007). The reduction in protein content in the chilling-stressed tomato plants was correlated with water deficit conditions (Bauer *et al.,* 1997). Similar results are reported in Cowpea (Manaf, 2016), and rice (Frukh *et al.,* 2020).

Drought stress caused an increase in amino acid contents than unstressed plants at all stages of *P. miliaceum* (fig.03) Free amino acid accumulation is more important to account for most of the changes in osmotic potential. The accumulation of amino acids may be for two reasons one being the hydrolysis of protein and another reason being the osmotic adjustment changes of their cellular contents (Greenway and Munns, 1980). Therefore, free amino acid accumulation under stress at all the growth stages shows the potential for their participation in osmotic adjustment in sorghum (Yadav *et al.,* 2005). Similar results of enhanced amino acids were obtained in *Arachis hypogaea* (Asha and Rao, 2002), pepper (Nath *et al.,* 2005), and barley (Noreen *et al.,* 2021) respectively.

Drought stress increased proline content significantly both in the roots and shoots of *P. miliaceum* (fig. 4). However, it was observed that there is a directly proportional relationship between the proline contents of shoots and roots and the severity of drought than the control one. Concerning drought impact on proline content, it was documented by many workers that it accumulated intensively in all stressed organs of plants especially in leaves as a consequence of the increased breakdown of proteins with a simultaneous decline in its synthesis in addition to conversion of some of amino-acids as ornithine, arginine and glutamic acid to proline (Yoshiba *et al.,* 1997). Moreover, Yoshiba, *et al.* (1997) and Phutela, *et al.* (2000) suggested that proline accumulated in tissues of stressed plants is by an increased rate of its synthesis by pyrroline-5-carboxylate synthetase and the decreased rate of its degradation by proline oxidase enzyme. Increased proline accumulation was noted in bell pepper (Nath *et al*., 2005), lentil (Bekka *et al.,* 2018), wheat (Vendruscolo *et al.,* 2007), common bean (Sofy *et al.,* 2020), and canola cultivars (Javeed *et al.,* 2021) respectively. Increased proline plays a vital and diversified role in plants exposed to various stresses such as drought stress (Iqbal *et al.,* 2019). Therefore, higher proline content buildup in proso millet under drought helps it to acquire tolerance and complete the life span by early maturation.

CONCLUSION

In the present work, it was concluded that when *P. miliaceum* plants were subjected to drought treatments resulted in an upsurged osmoprotectants, especially proline. This proves osmolytes as useful tools for plants to determine their health conditions when subjected to drought stress, thereby maintaining osmotic adjustment. Therefore, *P. miliaceum* showed resilience to prolonged drought conditions and can be exploited for the genetic program.

ACKNOWLEDGMENT

The author is grateful to the authorities of Annamalai University for providing facilities to complete this work.

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

REFERENCES

Asha, S., and Rao, K.N. (2002). Effect of simulated water logging on the levels of amino acids in groundnut at the time of sowing. *Indian J Plant Physiol.,* 7, 288–291.

Baltensperger, D. (2002). Progress with proso, pearl and other millets. In Janick, J. and Whipkey, A. (eds). Trends in New Crops and New Uses. ASHS Press, Alexandria, VA, USA, 100-103.

Bates, S., Waldren, R.P., and Teare, I.D. (1973). Rapid determination of the free proline in water stress studies. *Plant and Soil*., 39, 205–208.

Bauer, D., Biehler, K., Fock, H., Carrayol, E., Hirel, B., Migge, A., and Becker, T.W. (1997). A role for cytosolic glutamine synthetase in the remobilization of leaf nitrogen during water stress in tomato. *Physiol. Plant.,* 99, 241-248.

Bekka, S., Abrous-belbachir, O., and Djebbar, R. (2018). Effects of exogenous proline on the physiological characteristics of *Triticum aestivum* L. and *Lens culinaris* Medik. under drought stress. *Acta Agric. Slov.,* 111(2), 477–491.

Benhin, J.K.A. (2008). South African crop farming and climate change: An economic assessment of impacts. *Glob. Environ. Change*.,18, 666-678.

Chai, Y. (ed.) (1999). Broomcorn Millet. *China Agriculture Press*, Beijing, China, 13.

Dobra, J., Motyka, V., Dobrev, P., Malbeck, J. Prasil, IT., Haisel, D., Gaudinova, A., Havlova, M., Gubis, J., and Vankova, R. (2010). Comparison of hormonal responses to heat, drought and combined stress in tobacco plants with elevated proline contents. *J Plant Physiol.,* 167, 1360–1370.

Frukh, A., Siddiqi, T.O., Khan, M.I.R., and Ahmad, A. (2020). Modulation in growth, biochemical attributes and proteome profile of rice cultivars under salt stress. *Plant Physiol. Biochem*. 146, 55-70.

Ghosh, U.K., Islam, M.N., Siddiqui, M.N., and Khan, M.A.R. (2021). Understanding the roles of osmolytes for acclimatizing plants to changing environment: a review of potential mechanism. *Plant Signal. Behav.,* 16(8), 1913306.

Greenway, H., and Munns, R. (1980). Mechanism of salt tolerance in non-halophytes. *Ann. Rev. Plant Physiol.,* 31(1), 149-190.

Groppa, M., and Benavides, M. (2008). Polyamines and abiotic stress: recent advances. Amino Acids. 34 (1), 35.

Iqbal, N., Fatma, M., Khan, N.A., and Umar, S. (2019). Regulatory role of proline in heat stress tolerance: modulation by salicylic acid. In: Plant Signaling Molecules. *Woodhead Publishing*., 437–448.

Jangpromma, N., Kitthaisong, S., Lomthaisong, K., Daduang, S., Jaisil, P., and Thammasirirak, S. (2010a). A Proteomics Analysis of Drought Stress-Responsive Proteins as Biomarker for Drought-Tolerant Sugarcane Cultivars. *Am. J Biochem. Biotechnol.,* 6, 89-102.

Javeed, H. M. R., Ali, M., Skalicky, M., Nawaz, F., Qamar, R., Faheem, M., ... and EL Sabagh, A. (2021). Lipoic Acid Combined with Melatonin Mitigates Oxidative Stress and Promotes Root Formation and Growth in Salt-Stressed Canola Seedlings (*Brassica napus* L.). *Molecules*., 26(11), 3147.

Khan, S.H., Ahmad, N., Ahmad, F., and Kumar, R. (2010). Naturally occurring organic osmolytes: from cell physiology to disease prevention. IUBMB Life., 62(12),891–895.

Lobell, D.B., Burke, M.B., Tebaldi, C., Mastrandrea, M.D., Falcon, W.P., and Naylor, R.L. (2008). “Prioritizing climate change adaptation needs for food security in 2030.’’ *Science*, 319 (5863), 607–610.

Lawlor, D.W., Tezara, W., Mitchell, V., and Driscoll, S. P. (2002). Effects of water deficit and its interaction with CO_2 supply on the biochemistry and physiology of photosynthesis in sunflower. *J Exp. Bot.*, 53 (375), 1781-1791.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951). Protein measurement with folin-phenol reagent. *J Biol. Chem.,* 193, 265–275.

Manaf, H. H. (2016). Beneficial effects of exogenous selenium, glycine betaine and seaweed extract on salt stressed cowpea plant. *Ann. of Agric. Sci.,* 61(1), 41-48.

Medonough, C. (2000). The millets. In Kulp, K. (ed.). Handbook of Cereal

Mir, R.A., and Somasundaram, R. (2020). Effect of NaCl stress on pigment composition, membrane integrity and proline metabolism of little millet (*Panicum sumatrense* L.) CO-4 variety. *Int. Journal Bot. Stud.,* 5 (3), 586-593.

Mir, R. A., Somasundaram, R., and Panneerselvam, R. (2019). Changes in antioxidant enzymes activities mitigates deleterious effects of ROS in *Panicum miliaceum* (L.) under drought stress. *J Stress Physiol. Biochem.,* 15(3), 81-91.

Moore, S., and Stein, W.H. (1948). Photometric ninhydtherin method for use in chromatography of amino acids. *J Biol. Chem.,* 176, 367–388.

Nath, A.K., Kumari, S., and Sharma, D.R. (2005). *In vitro* selection and characterization of water stress tolerant cultures of bell pepper. *Indian J Plant Physiol.,* 10, 14–19.

Nazifi, S., Ghane, M., Fazeli, M., Ghafaria, N., Azizi, S., and Mansourian, M. (2009). Proso millet *(Panicum miliaceum* L.) poisoning in Iranian fat-tailed sheep. *Comp. Clin. Pathol.,* 18, 249-253.

Nishizawa, N., and Fudamoto, Y. (1995). The elevation of plasma concentration of high-denisity lipoprotein cholesterol in mice fed with protein from proso millet. *Biosci. Biotechnol. Biochem.* 59, 333-335.

Nishizawa, N., Oikawa, M., and Hareyama, S. (1990). Effect of dietary protein from proso millet on the plasma cholesterol metabolism in rats. *Agro. Bio. Chem.* 54, 229-230.

Nishizawa, N., Sato, D., Ito, Y., Nagasawa, T., Hatakeyama, Y., Choi., M.R., Choi, Y.Y., and Wei, Y.M. (2002). Effect of dietry protein of proso millet on liver injury induced by D-glactosamine in rats. *Biosci. Biotechnol. Biochem.,* 66, 92-96.

Noreen, S., Sultan, M., Akhter, M.S., Shah, K.H., Ummara, U., Manzoor, H., ... and Ahmad, P. (2021). Foliar fertigation of ascorbic acid and zinc improves growth, antioxidant enzyme activity and harvest index in barley (*Hordeum vulgare* L.) grown under salt stress. *Plant Physiol. Biochem.,* 158, 244-254.

Ojha, R., Nagesh Kumar, D., Sharma, A., and Mehrotra, R. (2012). “Assessing severe drought and wet events over India in a future climate using a nested bias-correction approach.” *J Hydrol. Eng.,* 18(7),760–772.

Panda, D.K., and Wahr, J. (2016) “Spatiotemporal evolution of water storage changes in India from the updated GRACE-derived gravity records. *Water Resources Research*, 52 (1),135–149.

Parry, M., Rosenzweig, C., Iglesias, A., Fischer, G., and Livermor, M. (1999). Climate change and world food security: a new assessment. *Glob. Environ. Change.,* 9, 51-67.

Phutela, A., Jain, V., Dhawan, K., and Nainawatee, H.S. (2000). Proline metoblism under water stress in the leaves and roots of *Brassica junce* cutltivars differing in drought tolerance. *J Plant Biochem. Biotechnol.,* 9, 35-39.

Reddy, A.R., Chiatanya, K.V., and Vivekanandan, M. (2004). Drought induced responses of photosynthesis and antioxident metabolism in higher plants. *J. Physiol.,* 161, 1189-1202.

Roche, J., Hewezi, T., Bouniols A., and Gentzbittel, L. (2009). Real-time PCR monitoring of signal transduction related genes involved in water stress tolerance mechanism of sunflower. *Plant Physiol. Biochem.* 47, 139-145.

Roshevits, R.Y. (1980). Grasses: An introduction to the study of fodder and cereal grasses. *Indian National Scientific Doc. Center*, New Delhi.

Sankar, B., Jaleel, C.A., Manivannan, P., Kishorekumar, A., Somasundaram, R., and Panneerselvam, R. (2007). Drought induced biochemical modifications and proline metabolism in *Abelmoschus esculentus* (L.) Moench. *Acta Bot. Croatica,* 66, 43–56.

Shao, H.B., Chu, L.Y., Jaleel, C.A., Manivannan, P., Panneerselvam, R., and Shao, M.A. (2009). Understanding water deficit stress-induced changes in the basic metabolism of higher plants-biotechnologically and sustainably improving agriculture and the ecoenvironment in arid regions of the globe. *Crit. Rev. Biotechnol.,* 29, 131-151.

Silva, D.D., Kane, M.E., and Beeson, R.C. (2012). Changes in root and shoot growth and biomass partition resulting from different irrigation intervals for *Ligustrum japonicum* Thunb. *Hortic. Sci.,* 47, 1634–1640.

Sofy, M.R., Elhawat, N., and Alshaal, T. (2020). Glycine betaine counters salinity stress by maintaining high K^+/Na^+ ratio and antioxidant defense via limiting Na^+ uptake in common bean (*Phaseolus vulgaris* L.). *Ecotoxicol. Environ. Saf.,* 200, 110732.

Srivastava, S., Thathola, A., and Batra, A. (2001). Development and nutrition evaluation of proso millet-based convenience mix for infants and children. *J. Food Sci. Technol.,* 38(5), 480-483.

Thomas, J., and Prasannakumar, V. (2016). "Temporal analysis of rainfall (1871-2012) and drought characteristics over a tropical monsoon-dominated State (Kerala) of India." *J Hydrol.,* 534, 266–280.

Thomas, T., Nayak, P.C., and Ghosh, N.C. (2015). "Spatiotemporal analysis of drought characteristics in the bundelkhand region of central India using the standardized precipitation index." *J Hydrol. Eng.,* 20(11), 1–12.

Vendruscolo, E.C.G., Schuster, I., Pileggi, M., Scapim, C.A., Molinari, H.B.C., Marur, C.J., and Vieira, L.G.E. (2007). Stress-induced synthesis of proline confers tolerance to water deficit in transgenic wheat. *J plant physiol.,* 164(10), 1367-1376.

Yadav, S.K., Jyothi Lakshmi, N., Maheswari, M., Vanaja, M., and Venkateswarlu, B. (2005). Influence of water deficit at Vegetative, Anthesis and grain filling stages on water relation and grain yield in sorghum. *Indian J. Plant Physiol.,* 10, 20–24.

Yoshiba, Y., Kiyosea, T., Nakashima, K., Yamaguchi-shinozaki, K., and Shino-zaki, K. (1997). Regulation of levels of proline as an osmolyte in plants under water stress. *Plant Cell Physiol.,* 38(10), 1095-1102.