

Drought Stress Induced Alterations in Compatible Solute accumulation and yield of six *Paspalum scrobiculatum* Land Races of Tamilnadu

Pallipalayam Varadharajan Murali¹, Gurusamy Marimuthu²

^{1,2}Stress Physiology Lab, Department of Botany
Annamalai University, Annamalai Nagar – 608 002, Tamil Nadu, India

Abstract-Drought is a serious threat to world food security. Plants respond to drought stress by different adaptations and metabolic alterations. A pot culture experiment was conducted to investigate effect of different levels of drought stress on photosynthetic pigments, compatible solute accumulations and yield parameters of six Kodo millet landraces. Plants were exposed to different levels of drought stresses (3, 4, 5 and 6 DID) from 20th to 60th DAS and samples were harvested on 40th and 60th DAS to estimate photosynthetic pigments and compatible solutes. Drought stress significantly decreased photosynthetic (chl a, chl b and carotenoids). However, organic solutes such as free amino acid, sucrose, total soluble sugars, proline, and glycinebetaine contents significantly increased under drought stress treatment. On the other hand, drought stress markedly decreased protein and starch content and yield parameters. Therefore, these plants possess different metabolic adaptation mechanisms which favored them to withstand the drought stress conditions.

Keywords-Compatible Solute, Drought, Kodo millet, *Paspalum scrobiculatum*, Proline.

I. INTRODUCTION

Drought, a major abiotic stress, adversely affects food productivity by limiting plant growth and development (Shao et al., 2009; Moussa, 2011). Drought influences plant growth, yield, membrane integrity, pigment content, osmotic adjustment, water relations, and photosynthetic activity (Praba et al., 2009). The range of plant susceptibility to drought stress depends on stress degree, associated stress factors, plant species, and their developmental stages (Demirevska et al., 2009). Drought stress induces many physiological, biochemical and molecular responses in crop plants, which would help them to adapt to such limiting environmental conditions (Arora et al., 2002). It inhibits the photosynthesis, causes changes of chlorophyll contents and damages the photosynthetic apparatus (Escuredo et al., 1998). Drought stress reduces respiration, translocation, ion uptake, carbohydrates, nutrient metabolism and growth promoters (Praba et al., 2009). It interrupts the balance between the

generation of reactive oxygen species (ROS) and the antioxidant defense system causing the accumulation of ROS which induces oxidative stress to protein, membrane lipids and disruption of DNA strands (El Tayeb, 2006).

A major effect of drought is reduction in photosynthesis which takes place by a decrease in leaf expansion, impaired photosynthetic machinery and premature leaf senescence. Drought stress produced changes in photosynthetic pigments and components. Decrease in chlorophyll content of leaves under water stress is well known fact. Low concentrations of photosynthetic pigments can directly limit photosynthetic potential and hence primary production. From a physiological perspective, leaf chlorophyll content is a parameter of significant interest in its own right. Studies by majority of chlorophyll loss in plants in response to water deficit occurs in the mesophyll cells with a lesser amount being lost from the bundle sheath cells (Anjum et al., 2011). The photosynthesis inhibition may be associated with limited CO₂ availability due to decreased stomatal conductance and disturbances in carbon assimilation metabolism (Peeva and Cornic, 2009).

Several plant species accumulate organic solutes such as sugars and proline in response to water stress (Szabados and Savoure, 2010; Mohamed and Abdel-Hamid, 2013). Proline accumulation is the first response of plants exposed to water-deficit stress in order to reduce injury to cells. Proline can act as a signaling molecule to modulate mitochondrial functions, influence cell proliferation or cell death and trigger specific gene expression, which can be essential for plant recovery from stress (Szabados and Savoure, 2009). Proline also contributes to stabilizing sub-cellular structures, scavenging free radicals, and buffering cellular redox potential under stress conditions. Accumulation of free proline is likely a part of general adaptation of plants to drought stress, as it promotes water retention in plants thereby alleviating negative effects of water deficit (Ashraf and Foolad, 2007). Thus, the concentration of free proline in plants has been suggested as a metabolic measure of drought tolerance (Farooq and Bano, 2006). Water stress also increases the levels of soluble sugars (compatible solutes), while it

reduces the amounts of polysaccharides and starch (El Tayeb, 2006). These compatible solutes protect plants against stresses by cellular adjustment through the protection of membranes integrity and enzymes stability (Farooq et al., 2009).

The degree of drought for a given location depends on the crop, rainfall and its distribution, soil type, and various management practices. Drought occurs frequently in Tamilnadu – some part of the state experiences drought every year. The state of Tamilnadu is located in the southernmost tip of peninsular India. Its lies between 8°5" and 13°35" latitude north and 76°15" and 80°20" longitude east, covering an area of 0.13 million Km² and including a long coastline (about 1,000 Km)(Kulandaivelu and Jayachandran, 1994). The conservation, use and availability of millet genetic diversity are increasingly important in the view of the evolving needs and main food challenges of small scale farmer in the arid and semi-arid lands. Millet biodiversity represent extraordinary genetic resources available at the gross root level, to address and cope with unpredictable climatic condition, desertification processes and developing crop diversification in the most challenging agro-ecological areas through the optimal valuation and is of local agro-biodiversity and the associate indigenous knowledge (Josep A. Gari, 2002).

Paspalum scrobiculatum is a loosely tufted, shallow rooting short – lived perennial or annual with ascending, somewhat succulent branched stem. Plants are slender to stout, up to 90 cm tall and often root from the lower nodes. Tufts are up to 60 cm in diameter, culms with 4-nodes. The Kodo millet is a food grain crop of minor importance. The husked grain, which is white in color, is cooked and used as rice. The kodo millet is cultivated in many countries both for the grain and for fodder. Though widely distributed, the area under this crop is not high and is often limited to particular regions (De wet et al., 1983).

Millet produce multiple securities, such as securities of food, nutrition, fodder, fiber, health, livelihood and ecology. Due to all the qualities mentioned above, Millets remain our agricultural answer to the climate crisis that the world is facing. Climate Change is expected to confront us with challenges. Only millets have the capacity to meet this challenge (Rao, 1986; Millets Future of Food and Farming). Kodo millet forms an important component of dry land, tribal and hilly agriculture, mostly cultivated in rain-fed areas. So, it is important to understand its drought adaption mechanism for better yield. Therefore, this experiment was conducted by considering the importance of Kodo millet in rain-fed areas and hence the aim of the present study was to investigate the effect of drought stress on photosynthetic pigment contents

and alteration in compatible solute accumulations of six Kodo millet (*Paspalum scrobiculatum* L.) land races of Tamil Nadu.

II. MATERIALS AND METHODS

The seeds of *Paspalum scrobiculatum* L. were collected from local farmers of Kovilpatty, Trichy, Kollimalai, Vadalur and Virudhunagar areas of Tamil Nadu. The research work was conducted in the Botanical Garden and Stress – Physiology Laboratory, Department of Botany, Annamalai University, Tamil Nadu, India (11°23'59"N, 79°41'37"E). Six land races of *Paspalum scrobiculatum* L., (S1, S2, S3, S4, S5 and S6), were selected for the study. Plastic pots of 30 cm diameter and 40 cm height size were used for the study. The pots were filled with 8 kg of soil mixture containing red soil, sand and farm yard manure in the ratio 2:1:1 and 180 pots were arranged in completely randomized block design. One set of 36 pots were kept as control (6 pots for each land race) and the other 4 sets (3, 4, 5, and 6 DID) of 144 pots were used for drought stress treatments for each variety. The seeds were sown and the seedlings were thinned to 05 per pot on 10th DAS (days after sowing). The plants were allowed to grow up to 20 DAS and were watered regularly. From 20th day onwards, all the potted plants were grown under poly – house to avoid any type of atmospheric precipitation. The control plants were irrigated every day. Drought stresses 3 DID (irrigation once in 3 days), 4 DID (irrigation once in 4 days), 5 DID (irrigation once in 5 days) and 6 DID (irrigation once in 6 days) were imposed from 20th to 60th DAS. The samples were collected on 40th and 60th DAS for analysis. Plants were uprooted randomly, washed carefully and were separated into root and shoot for the estimation of photosynthetic pigments and compatible solutes.

A. Estimation of photosynthetic pigments

Chlorophyll contents were measured from the A. cepa leaves according to Arnon (1949) method. Fresh leaves (1g) were extracted with 80 % acetone (v/v) and chlorophyll contents were estimated spectrophotometrically at 645 and 663 nm using Hitachi U-2000 spectrophotometer and were expressed in terms of mg chlorophyll present g⁻¹ fresh mass. Carotenoid content was estimated using the method of Horvath et al. (1972) and expressed in milligrams per gram fresh weight.

B. Estimation of Protein content

Protein content was estimated according to Bradford (1976). 0.1 ml of protein extract was pipetted in test tubes and to it 5 ml of protein reagent was added and the contents were mixed. The absorbance at 595 nm was measured after 2

minutes against a reagent blank-reagent blank was prepared with distilled water 0.1 ml of 0.1 N NaOH and 5 ml of Bradford reagent. The weight of protein was plotted against the corresponding absorbance resulting in a standard curve and it was used to determine the protein in unknown samples and the results were expressed in milligrams per gram dry weight.

C. Estimation of Amino acid content

Total free amino acids were extracted and estimated by following the method of Moore and Stein (1948). Five hundred milligrams of fresh plant material was homogenized in a mortar and pestle with 80% boiled ethanol. The extract was centrifuged at 800 g for 15 minutes and the supernatant was made up to 10 ml with 80% ethanol. In 25 ml test tube, ethanol extract was taken and neutralized with 0.1 N NaOH using the methyl red indicator to which ninhydrin reagent was added. The contents were boiled in a boiling water bath for 20 minutes, and then 5ml of diluting solution was added, cooled and made up to 25 ml with distilled water. The absorbance was read at 570 nm.

D. Estimation of Starch content

Starch content was estimated by following the method of Clegg (1956). 10 ml of cold anthrone reagent was added to the 1 ml of sample perchloric acid extract and it was diluted with 5 ml of deionized water. The test tube was heated for 10 min at 100°C in a boiling water bath. The test tube was cooled rapidly and the absorbance was read at 630 nm in a spectrophotometer.

E. Estimation of Sucrose content

Sucrose content was estimated by the method of Bernt and Bergmayer, 1970. 1 ml of invertase (prepared by dissolving 250 units of yeast invertase in 500 ml of 0.2 M sodium acetate buffer, pH 5.0) was added to 1 ml sugar extract and incubated at 37°C for 1 hour and, thereafter, the reaction was stopped by keeping the tubes in boiling water bath for 10 min. Under these conditions, sucrose was completely hydrolyzed. Glucose was determined by the glucose oxidase and peroxidase reaction (sigma) (Gascon and Lampen, 1968) before and after invertase hydrolysis and the difference between these values was taken as the actual amount of sucrose in the sample.

F. Estimation of Soluble sugar content

Soluble sugars were quantified following the phenolsulfuric acid method (Robyt and White, 1987). 100 mg

dry weight of shoots was extracted in 80% (v/v) methanol heated to 70°C in a water bath. The extract was then centrifuged at 5,000 × g for 10 min. The supernatant was used for the estimation of soluble sugar concentrations. The reaction mixture consisted of 5% phenol and 98% sulfuric acid. Once the extract had cooled, its absorbance was determined at 490 nm using D-glucose as standard.

G. Estimation of Proline content

Free proline was assayed spectrophotometrically by the ninhydrin method (Bates et al, 1973). The plant material was homogenized in 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 14,000 rpm. The supernatant was used for the estimation of the proline concentration. The reaction mixture consisted of acid ninhydrin and glacial acetic acid, which was boiled at 100°C for 1 h. After termination of reaction in ice bath, the reaction mixture was extracted with toluene, and absorbance was read at 520 nm using L-proline as standard.

H. Estimation of Glycine betaine content

Glycine-betaine was estimated by the method of Grieve and Grattan (1983). Briefly, finely ground dried plant tissue (0.5 g) was stirred with 20 cm³ distilled water for 24 h and filtered. The filtrate was diluted with equal volume of 1 M H₂SO₄, made into aliquots of 0.5 cm³ in microcentrifuge tubes, cooled over ice for 1 h and to each of these were added 0.2 cm³ cold KI-I₂ reagent. The reactants were gently stirred, stored at 4 °C overnight and centrifuged at 12000 g for 15 min at 4 °C to get the precipitated periodide crystals. The crystals were dissolved in 1, 2-dichloroethane, and absorbance was measured at 365 nm after 2 h. Glycine-betaine dissolved in 1 M H₂SO₄ served as standard.

I. Yield parameters

For yield parameters such as, spike length and seed weight per spike, plants were harvested 110th DAS and spikes were separated from the rest of the plant. The spike length was measured by using meter scale and the data were presented as mean of five replicates in centimeters per spike. Seeds were separated from complete dried spikes and seeds collected from each spike were weighed using electronic balance (Citizen Ltd) and the data were expressed as mean of five replicates in grams per spike.

J. Statistical analysis

The data represent means calculated from six replicates. Variance analysis of mean values was performed

with Duncan Multiple Comparison test (one-way ANOVA) using SPSS software for Microsoft Windows (Ver. 16.0, SPSS Inc., USA) and significance level was determined at the 5% ($P < 0.05$) level.

III. RESULTS

A. Effect on Photosynthetic pigments

The data revealed that drought stress caused a marked reduction ($P < 0.05$) in chlorophyll 'a' and chlorophyll 'b' contents as compared to control in all six Kodo millet landraces (Table 1 and 2). Among drought stress treatments, lowest chlorophyll content was recorded in 6 DID on all growth stages. From Table (3), it is clear that drought stress decreased carotenoid contents in the leaves of all Kodo millet land races on all growth stages. 6 DID caused higher reduction as compared to other drought stress treatments. It is further confirmed from the data that carotenoid content was less affected as compared to chlorophyll contents.

B. Effect on Protein content

Data presented in Table (4 and 5) showed that root and shoot protein content significantly decreased ($P < 0.05$) under drought stress as compared to control in all Kodo millet land races. However, 6 DID caused the highest reduction in protein content as compared to other drought stress treatments. Among the six millets, lowest protein content was recorded in S6 and highest protein content in S2 under 6 DID treatment on 60 DAS.

C. Amino acid content

Drought stress caused a marked increase ($P < 0.05$) in amino acid content as compared to control in both root as well as shoot organs of six millet landraces (Fig. 1 A and B). Comparing the drought stress treatments, highest amino acid content was recorded under 6 DID. When comparing the organs, shoots showed higher protein content than roots.

D. Starch content

From Fig. 1 (C and D), it is clear that drought stress significantly ($P < 0.05$) decreased root and shoot starch content as compared to control in all six Kodo millet landraces. Among the drought stress treatments, 6 DID markedly decreased starch content.

E. Sucrose content

Sucrose content increased significantly ($P < 0.05$) under drought stress when compared with control (Fig. 1 E

and F). In all millet landraces, highest sucrose content was recorded under 6 DID as compared to other drought stress treatments. The highest root sucrose content was recorded in S6 (12.87), whereas lowest was recorded in S1 (10.44) on 60 DAS under 6 DID.

F. Total sugar content

Fig. 2 (a and b) depicted that drought stress drastically increased ($P < 0.05$) root and shoot total sugar content as compared to control in all Kodo millet landraces on all growth stages. However, total sugar content was found highest under 6 DID followed by 5, 4, and 3 DID treatments. In roots, the highest total sugar was recorded in S6 (0.839), whereas lowest total sugar content was observed in S2 (0.572) under 6 DID on 60 DAS.

G. Proline content

Drought stress significantly increased ($P < 0.05$) proline content in roots and shoots of Kodo millet landraces on all growth stages Fig. 2 (c and d). Among the drought stress treatments, 6 DID resulted in higher proline content both in root and shoot organs. The highest root proline content was observed in S2 (5.845) and lowest was recorded in S6 (3.901) under 6 DID on 60 DAS.

H. Glycine betaine content

The data presented in Fig. 2 (e and f) revealed that drought stress significantly increased ($P < 0.05$) root and shoot glycine betaine (GB) content as compared to control in all Kodo millet landraces. Among the drought stress treatments, highest GB content was recorded under 6 DID. When comparing the Kodo millet landraces, S1 showed highest and S3 showed lowest GB content under 6 DID on 60 DAS.

I. Yield

Table (6) depicted that drought stress significantly decreased the spike length and seed weight per plant. The 6 DID treatment showed highest reduction in spike length and seed weight as compared to other drought stress treatments in all Kodo millet land races studied. The maximum reduction in yield was recorded in S2 as compared to other millet landraces studied.

IV. DISCUSSION

The present investigation revealed that increasing levels of drought stress caused significant decrease in photosynthetic pigments in Kodo millet landraces. These

results are in harmony with Abass and Mohamed (2011) who reported that chlorophylls and carotenoid content decreased significantly with increased levels of drought stress in common bean. A reduction in chlorophyll and carotenoid content was reported in wheat seedlings (Heshmat et al., 2012) under drought stress. Photo-oxidation and pigment degradation which is caused by drought stress induced oxidative stress might be responsible for reduction in chlorophyll content. Under drought stress condition, the decline in photosynthetic pigment contents may be due to both stomatal and non-stomatal mechanisms. Stomata closure, very first response to drought stress, reduces the rate of photosynthesis in plants. Reduction in chlorophyll content may be due chloroplast membrane injury and distortion of the lamellae vesiculation (Heba and Samia, 2014). It can be further assumed that the pigment degradation under drought stress may be caused due the loss of balance between reactive oxygen species (ROS) and antioxidant defense (Reddy et al., 2004), which can cause accumulation of excessive ROS causing oxidative stress which affects proteins, membrane lipids and other cellular components.

Drought stress significantly decreased protein content both in roots and shoots of six millet landraces. These results are consistent with those observed in chickpea (Mafakheri et al., 2010). The reduction in quantity of soluble proteins observed in present experiment can be related to the reduced rate of protein biosynthesis and increased breakdown of protein under limited environment (Chen et al., 1999). Under water limited conditions, plants activate the pathway of protein breakdown, because the plants use the proteins for the synthesis of nitrogen compounds as amino acids that might support the plant osmotic adjustment (Sankar et al., 2007).

Accumulation of compatible solutes is a common phenomenon and most common stress tolerance strategies in plants exposed to drought stress. Osmotic adjustment is a very important process to maintain water relations under stressful conditions (Serraj and Sinclair, 2002). It involves the accumulation of osmotically active molecules/ions like soluble sugars, proline, glycinebetaine, organic acids etc. The results revealed that compatible solute accumulation such as amino acid, sucrose, total sugar, proline, and glycine betaine content significantly with the increasing levels of drought stress in all Kodo millet landraces. These results are in line with those observed under drought stress in sorghum (Yadav et al., 2005) and Marsh grasses (Maricle et al., 2008). Accumulation of amino acids may be due to the hydrolysis of proteins and/or may be occurring in response to the change in osmotic adjustment of the cellular contents (Shao et al., 2007). In plants, sugars play an important role osmoregulation under drought stress. From the present study, it is clear that drought

stress caused accumulation of total sugar and sucrose content and at the same time caused degradation of starch. Similar results were found in *Oryza sativa* cultivars (Mostajeran and Rahimi, 2009) and tomato (Mohamed et al., 2011). The reducing sugar content increased in parallel with the invertase activity under drought stress in Alfalfa seedlings (Zeid and Shedeed, 2006). The increased total sugar and sucrose content might be due to the degradation of starch under drought stress.

Proline accumulation is the first response of plants exposed to water-deficit stress in order to reduce cell injury. These results are consistent with those of Anjum et al. (2011a) who observed that progressive drought stress induced a considerable accumulation of proline in drought stressed maize plants. Proline accumulation in plants might act as a scavenger of ROS and acting as an osmo-protectant to reduce water potential which in turn helps to retain water content inside the cell. The reduced proline oxidase may be the reason for increasing proline accumulation. Under stressful conditions, proline accumulation supplies energy for the growth and survival and thereby helps the plant to tolerate stress (Jaleel et al., 2007). Glycine betaine is considered to be one of the most abundant quaternary ammonium compounds produced in higher plants under stressful environment (Yang et al., 2003). There are similar evidences that glycine betaine content increased under drought stress in barley (Nakamura, 2001). The accumulation of solutes under drought stress helps in plant cells lowers the water potential which helps the cell to retain water inside it and further attracts water into cell. With the help of osmotic adjustment, cell functions about normally and helps plant to perform better under stressful conditions (Subbarao et al., 2000).

Drought stress significantly decreased spike length and total seed weight per spike of Kodo millet landraces. Shao et al. (2008) reported that, seed yield and yield components are severely affected by drought stress. Water stress reduced the seed weight and yield per plant in sunflower (Blumward et al., 2004). In crop plants, water deficit leads to harsh reduction in yield traits probably by disrupting leaf gas exchange properties which not only limits source and sink tissue size but the phloem loading, assimilate translocation and impaired dry matter partitioning (Farooq et al., 2009).

V. CONCLUSION

From the above it may be concluded that drought stress caused a higher reduction in photosynthetic pigments and yield components in all six Kodo millet landraces. However, accumulation of compatible solutes such as amino acids, sucrose, total sugar, proline, and glycinebetaine were found enhanced under drought stress which indicates that how these plants adapt themselves and cope with drought stress

conditions. Osmotic adjustment is an important phenomenon to maintain water relations by lowering the water potential to retain maximum water inside the tissues and cells itself in stressful environment. This can be achieved by the accumulation of organic solutes that plays an important role in osmoregulation. Similar phenomenon has been observed in the Kodo millet landraces used in this study. Therefore, it can be assumed that these plants possess different adaptations which favored them to withstand the drought stress conditions.

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Table-1: Drought stress induced changes in chlorophyll 'a' content of six Kodo millet landraces (values are the mean \pm SE of 6 replicates and expressed in mg g⁻¹ fresh weight).

Growth Stages	Treatments	S1	S2	S3	S4	S5	S6
40 DAS	C	3.28 \pm 0.31	2.51 \pm 0.43	3.58 \pm 0.44	3.83 \pm 0.39	2.85 \pm 0.28	3.62 \pm 0.24
	3DID	2.87 \pm 0.24	2.01 \pm 0.38	3.07 \pm 0.41	3.15 \pm 0.26	2.40 \pm 0.24	3.40 \pm 0.27
	4DID	2.56 \pm 0.26	1.68 \pm 0.18	2.02 \pm 0.28	2.50 \pm 0.33	2.11 \pm 0.19	2.96 \pm 0.21
	5DID	2.10 \pm 0.21	0.93 \pm 0.36	1.63 \pm 0.21	1.87 \pm 0.23	1.60 \pm 0.30	2.12 \pm 0.18
	6DID	1.78 \pm 0.17	0.70 \pm 0.12	1.16 \pm 0.25	0.80 \pm 0.16	1.26 \pm 0.14	1.60 \pm 0.15
60 DAS	C	4.49 \pm 0.41	3.73 \pm 0.33	4.84 \pm 0.36	4.22 \pm 0.40	4.03 \pm 0.32	4.89 \pm 0.36
	3DID	3.31 \pm 0.38	3.28 \pm 0.36	4.20 \pm 0.31	3.31 \pm 0.37	3.61 \pm 0.34	4.63 \pm 0.31
	4DID	2.83 \pm 0.27	2.69 \pm 0.28	3.62 \pm 0.26	2.80 \pm 0.21	3.29 \pm 0.25	4.32 \pm 0.23
	5DID	2.26 \pm 0.18	2.02 \pm 0.14	3.12 \pm 0.22	2.35 \pm 0.30	2.82 \pm 0.18	4.01 \pm 0.19
	6DID	1.63 \pm 0.17	1.11 \pm 0.15	2.50 \pm 0.16	2.19 \pm 0.22	2.41 \pm 0.20	3.83 \pm 0.12

DAS-Days After Sowing, DID-Days Interval Drought.

Table-2: Drought stress induced changes in chlorophyll 'b' content of six Kodo millet landraces (values are the mean \pm SE of 6 replicates and expressed in mg g⁻¹ fresh weight).

Growth Stages	Treatments	S1	S2	S3	S4	S5	S6
40 DAS	C	0.432 \pm 0.09	0.310 \pm 0.07	0.441 \pm 0.05	0.519 \pm 0.08	0.521 \pm 0.05	0.658 \pm 0.07
	3DID	0.328 \pm 0.05	0.214 \pm 0.05	0.360 \pm 0.06	0.440 \pm 0.05	0.447 \pm 0.04	0.570 \pm 0.05
	4DID	0.336 \pm 0.06	0.190 \pm 0.03	0.338 \pm 0.04	0.423 \pm 0.06	0.429 \pm 0.05	0.550 \pm 0.06
	5DID	0.311 \pm 0.03	0.169 \pm 0.02	0.320 \pm 0.03	0.401 \pm 0.04	0.414 \pm 0.03	0.532 \pm 0.04
	6DID	0.292 \pm 0.04	0.148 \pm 0.02	0.299 \pm 0.02	0.387 \pm 0.03	0.390 \pm 0.02	0.514 \pm 0.05
60 DAS	C	0.784 \pm 0.08	0.430 \pm 0.05	0.533 \pm 0.06	0.628 \pm 0.09	0.662 \pm 0.07	0.790 \pm 0.06
	3DID	0.645 \pm 0.08	0.336 \pm 0.04	0.457 \pm 0.04	0.550 \pm 0.05	0.580 \pm 0.06	0.710 \pm 0.08
	4DID	0.564 \pm 0.07	0.310 \pm 0.03	0.438 \pm 0.05	0.534 \pm 0.05	0.561 \pm 0.04	0.691 \pm 0.05
	5DID	0.390 \pm 0.04	0.281 \pm 0.04	0.413 \pm 0.04	0.519 \pm 0.03	0.538 \pm 0.03	0.672 \pm 0.06
	6DID	0.353 \pm 0.06	0.215 \pm 0.03	0.390 \pm 0.02	0.505 \pm 0.04	0.519 \pm 0.04	0.651 \pm 0.03

Table-3: Drought stress induced changes in carotenoid content of six Kodo millet landraces (values are the mean \pm SE of 6 replicates and expressed in mg g⁻¹ fresh weight).

Growth Stages	Treatments	S1	S2	S3	S4	S5	S6
40 DAS	C	0.253 \pm 0.04	0.267 \pm 0.05	0.282 \pm 0.05	0.248 \pm 0.06	0.295 \pm 0.04	0.316 \pm 0.06
	3DID	0.182 \pm 0.02	0.193 \pm 0.04	0.203 \pm 0.04	0.192 \pm 0.05	0.203 \pm 0.05	0.241 \pm 0.05
	4DID	0.171 \pm 0.03	0.178 \pm 0.02	0.180 \pm 0.05	0.179 \pm 0.03	0.185 \pm 0.04	0.228 \pm 0.04
	5DID	0.156 \pm 0.02	0.165 \pm 0.03	0.172 \pm 0.03	0.166 \pm 0.04	0.173 \pm 0.03	0.205 \pm 0.03
	6DID	0.144 \pm 0.02	0.149 \pm 0.01	0.158 \pm 0.02	0.153 \pm 0.02	0.163 \pm 0.04	0.192 \pm 0.04
60 DAS	C	0.307 \pm 0.05	0.315 \pm 0.07	0.345 \pm 0.07	0.304 \pm 0.05	0.349 \pm 0.07	0.374 \pm 0.07
	3DID	0.248 \pm 0.03	0.234 \pm 0.04	0.272 \pm 0.06	0.231 \pm 0.04	0.288 \pm 0.05	0.305 \pm 0.05
	4DID	0.221 \pm 0.02	0.211 \pm 0.03	0.250 \pm 0.03	0.219 \pm 0.04	0.266 \pm 0.04	0.283 \pm 0.06
	5DID	0.204 \pm 0.03	0.198 \pm 0.04	0.242 \pm 0.05	0.197 \pm 0.03	0.254 \pm 0.03	0.270 \pm 0.03
	6DID	0.188 \pm 0.02	0.186 \pm 0.02	0.226 \pm 0.03	0.185 \pm 0.02	0.238 \pm 0.03	0.258 \pm 0.02

Table-4: Drought stress induced changes in root protein content of six Kodo millet landraces (Values are the mean \pm SE of 6 replicates and expressed in mg g⁻¹ dry weight).

Growth Stages	Treatments	S1	S2	S3	S4	S5	S6
40 DAS	C	3.76 \pm 0.16	4.96 \pm 0.13	4.08 \pm 0.17	3.92 \pm 0.12	4.10 \pm 0.19	3.10 \pm 0.15
	3DID	3.51 \pm 0.12	4.62 \pm 0.15	3.45 \pm 0.12	3.85 \pm 0.14	3.80 \pm 0.17	2.83 \pm 0.13
	4DID	2.85 \pm 0.10	3.85 \pm 0.18	2.56 \pm 0.13	3.42 \pm 0.15	3.56 \pm 0.14	2.45 \pm 0.12
	5DID	2.48 \pm 0.08	3.30 \pm 0.13	2.15 \pm 0.14	2.78 \pm 0.10	3.02 \pm 0.11	1.92 \pm 0.13
	6DID	2.34 \pm 0.11	3.05 \pm 0.12	1.75 \pm 0.09	2.30 \pm 0.11	2.85 \pm 0.09	1.58 \pm 0.10
60 DAS	C	4.56 \pm 0.20	5.27 \pm 0.19	4.55 \pm 0.19	4.82 \pm 0.15	4.72 \pm 0.21	3.76 \pm 0.18
	3DID	4.10 \pm 0.18	4.82 \pm 0.15	3.84 \pm 0.17	4.33 \pm 0.12	4.15 \pm 0.18	2.91 \pm 0.15
	4DID	3.79 \pm 0.17	4.20 \pm 0.11	2.91 \pm 0.13	3.53 \pm 0.11	3.76 \pm 0.16	2.73 \pm 0.14
	5DID	3.24 \pm 0.13	3.80 \pm 0.14	2.65 \pm 0.16	3.01 \pm 0.14	3.30 \pm 0.14	2.69 \pm 0.09
	6DID	3.11 \pm 0.15	3.47 \pm 0.12	2.59 \pm 0.15	2.75 \pm 0.12	2.96 \pm 0.10	2.57 \pm 0.10

Table-5: Drought stress induced changes in shoot protein content of six Kodo millet landraces (Values are the mean \pm SE of 6 replicates and expressed in mg g⁻¹ dry weight).

Growth Stages	Treatments	S1	S2	S3	S4	S5	S6
40 DAS	C	5.11 \pm 0.21	6.29 \pm 0.19	5.09 \pm 0.20	4.93 \pm 0.18	4.69 \pm 0.17	4.31 \pm 0.19
	3DID	4.88 \pm 0.12	5.32 \pm 0.21	4.85 \pm 0.15	4.35 \pm 0.14	4.06 \pm 0.18	4.19 \pm 0.13
	4DID	4.42 \pm 0.15	4.68 \pm 0.18	4.49 \pm 0.14	3.82 \pm 0.16	3.87 \pm 0.15	3.75 \pm 0.16
	5DID	3.92 \pm 0.11	3.91 \pm 0.12	4.33 \pm 0.16	3.51 \pm 0.15	3.39 \pm 0.13	3.16 \pm 0.15
	6DID	3.18 \pm 0.12	3.51 \pm 0.14	3.09 \pm 0.13	3.07 \pm 0.13	2.77 \pm 0.09	2.66 \pm 0.13
60 DAS	C	5.25 \pm 0.18	6.53 \pm 0.20	5.76 \pm 0.19	5.21 \pm 0.21	5.22 \pm 0.19	4.43 \pm 0.20
	3DID	4.89 \pm 0.17	5.50 \pm 0.15	5.49 \pm 0.18	4.82 \pm 0.17	4.88 \pm 0.12	4.27 \pm 0.14
	4DID	4.73 \pm 0.16	5.02 \pm 0.17	4.82 \pm 0.13	4.28 \pm 0.18	4.61 \pm 0.14	3.83 \pm 0.17
	5DID	4.66 \pm 0.20	4.85 \pm 0.15	4.64 \pm 0.15	3.85 \pm 0.16	4.30 \pm 0.16	3.25 \pm 0.11
	6DID	3.41 \pm 0.15	3.67 \pm 0.11	3.36 \pm 0.10	3.31 \pm 0.15	2.94 \pm 0.13	2.89 \pm 0.10

Table-6: Drought stress induced changes in Yield parameters of six Kodo millet landraces (values are the mean \pm SE of 6 replicates).

	Treatments	S1	S2	S3	S4	S5	S6
Spike Length (cm/spike)	C	16.2 \pm 0.51	14.5 \pm 0.62	16.3 \pm 0.56	17.1 \pm 0.60	16.4 \pm 0.25	17.5 \pm 0.48
	3DID	14.8 \pm 0.35	12.7 \pm 0.44	14.6 \pm 0.50	15.3 \pm 0.52	14.2 \pm 0.35	15.6 \pm 0.52
	4DID	12.1 \pm 0.37	11.1 \pm 0.41	13.9 \pm 0.32	14.4 \pm 0.41	13.3 \pm 0.43	13.9 \pm 0.42
	5DID	10.9 \pm 0.27	9.5 \pm 0.39	13.0 \pm 0.43	13.5 \pm 0.46	12.0 \pm 0.32	12.8 \pm 0.35
	6DID	10.1 \pm 0.21	9.0 \pm 0.25	12.2 \pm 0.36	11.9 \pm 0.33	11.4 \pm 0.29	11.5 \pm 0.24
Seed Weight (g/spike)	C	17.6 \pm 0.59	13.6 \pm 0.53	15.2 \pm 0.47	16.7 \pm 0.45	16.1 \pm 0.55	18.3 \pm 0.64
	3DID	14.7 \pm 0.45	11.5 \pm 0.26	12.4 \pm 0.38	13.5 \pm 0.052	12.9 \pm 0.48	15.1 \pm 0.56
	4DID	13.6 \pm 0.48	10.3 \pm 0.31	10.9 \pm 0.34	11.7 \pm 0.31	11.5 \pm 0.34	13.8 \pm 0.45
	5DID	12.2 \pm 0.32	9.1 \pm 0.29	9.7 \pm 0.27	10.3 \pm 0.25	10.2 \pm 0.41	12.5 \pm 0.37
	6DID	10.4 \pm 0.25	7.8 \pm 0.33	8.5 \pm 0.24	8.8 \pm 0.30	8.6 \pm 0.28	10.8 \pm 0.31

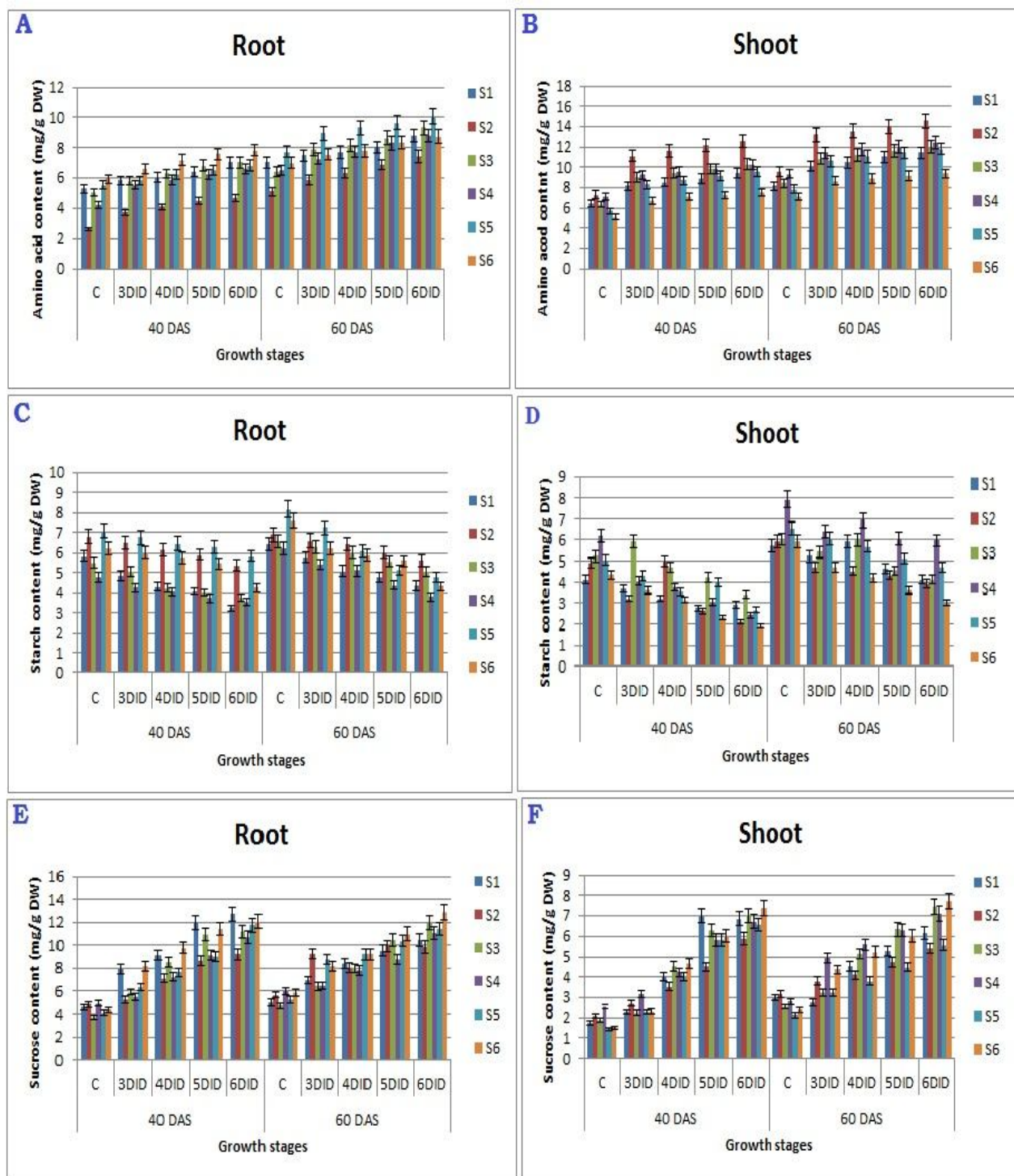


Fig. 1. Effect of drought stress on compatible solute accumulation such as Amino acid (A, B), Starch (C, D), and Sucrose (E, F) of six Tamilnadu land races of *P. scrobiculatum*. DAS – days after sowing; DID – days interval drought; FW – fresh weight; DW – dry weight.

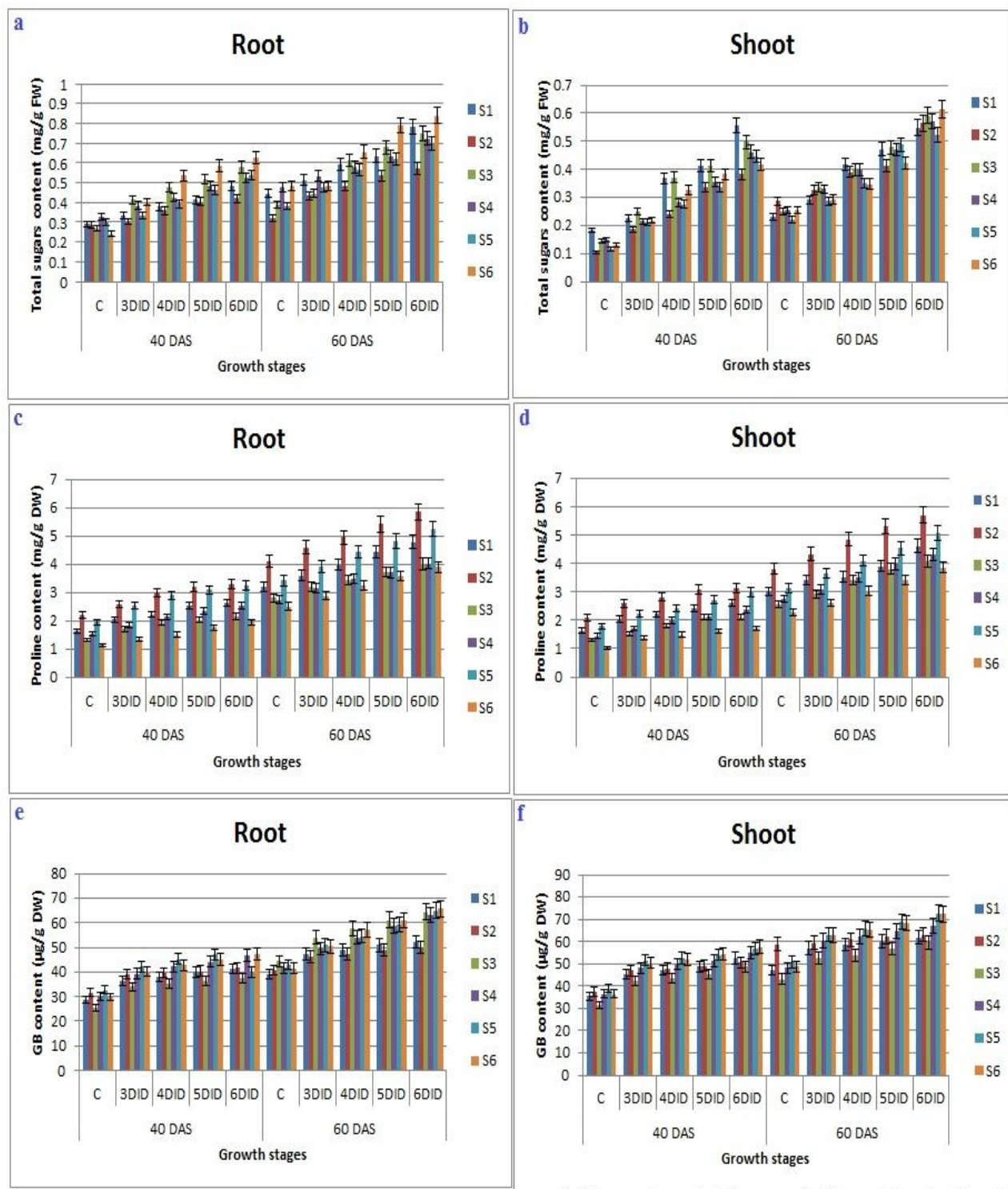


Fig. 2. Effect of drought stress on compatible solute accumulation such as Sugar (A, B), Total sugar (C, D), and Proline (E, F) of six Tamilnadu land races of *P. scrobiculatum*. DAS – days after sowing; DID – days interval drought; GB – glycine betaine; FW – fresh weight; DW – dry weight.