

Effect of Drought Stress in *Paspalum Scrobiculatum* L. Biochemical and Compatible Solute Accumulation

G. Marimuthu, P. V. Murali*

Stress Physiology Lab, Department of Botany, Annamalai University, Annamalai Nagar, Tamil Nadu, India

ABSTRACT

Drought stress is a major constraint for crop production to the world. Plants response to drought stress by different adaptation and metabolic alternations. A pot culture experiment was conducted to investigate the effect of Different Day Interval Drought (DID) stress on biochemical and compatible solute accumulation of *Paspalum Scrobiculatum* (L.). The *P. scrobiculatum* variety CO- 1 and CO - 3 were obtained from Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu and India. The plants were exposed to 3, 4, 5 and 6DID stress from 20 to 60 Days after sowing (DAS) plants. Irrigation at one day interval was kept as control. The plant sample were collected on 40, 60 and 80 DAS and estimate the biochemical contents like protein, proline, amino acid, glycine betaine, total sugar and sucrose were analysed. Drought stress significantly increased in biochemical contents like proline, amino acid, glycine betaine, total sugar and sucrose content but, the protein content were decreased. These results are indicate that plants are altering their osmoregulation and to balance the water content by lowering the water potential. From the results of this investigation it is clear that the CO - 3 variety were more tolerance then CO - 1 variety plants.

Keywords: Compatible Solute, Drought, Paspalam scrobiculatum.

I. INTRODUCTION

Plant growth and productivity is adversely affected by various abiotic and biotic stress factors. Plants are frequently exposed to many stress conditions such as low temperature, salt, drought, heat, oxidative stress and heavy metal toxicity. Drought stress is certainly one of the most serious environmental factors limiting the productivity of crop plants. Drought stress is affecting about 50% of rice production in the world (Mostajean and Eichi, 2009).

Drought stress results in various physiological changes plants include, reduction in that may in photosynthetic rate, transpiration, stomatal conductance, pigment degradation and relative water content (RWC) resulting in decreased water use efficiency (WUE) and growth reduction prior to plant senescence (Cattivelli et al., 2008; Tuna et al., 2010). The typical first response of all plants to drought stress

osmotic adjustment. Compatible solutes is accumulation in the cytoplasm is considered as a mechanism to contribute stress tolerance (Hare et al., 1998). To counter with abiotic stress, plants increase the osmotic potential of their cells by synthesizing and accumulating compatible osmolyte such as proline (PRO) and glycine betaine (GB) that participates in the osmotic adjustment (Kavikishore et al., 2005). PRO and GB are thought to function as osmoprotectants for proteins (Bohnert and Jenson, 1996).

Millets are staple foods that supply a major portion of calories and protein to large segments of populations in the semi-arid tropical regions of Africa and Asia (O'Kennedy et al., 2006). The grass genus Paspalum is represented by 300 to 400 species in warm temperate, subtropical, and tropical regions throughout the world (Allen and Hall, 2003). It is commonly called as Kodo millet (*Paspalum scrobiculatum*) is a hardiest drought

resistant small seeded crop grown under poor resource based situations by tribal farmers in quite large area of the country. The *P. scrobiculatum* is a minor grain crop in India and in the Deccan plateau. The fiber content of the whole grain is very high. Kodo millet has around 11% protein and the nutritional value of the protein has been found to be slightly better than that of other small millets (Yadava and Jain 2006). *P. scrobiculatum* is widely distributed in damp habitats across the old world tropics. It grows in a range of sea–level to 3000 m altitude. It is harvested as a wild cereal in West Africa and in India. It is cultivated as grain and fodder crop in south Asia including India, China and Japan and more recently in America. This species thrives even in very poor soils.

The present study aimed to evaluate and to assess the drought tolerance variety of *P. scrobiculatum* on different days interval drought stress.

II. MATERIALS AND METHODS

The experimental part of this work were carried out in Botanical Garden and Stress Physiology Lab, Department of Botany, Annamalai University, Tamil Nadu, India. The P. scrobiculatum variety CO-1 and CO - 3 were obtained from Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India. The plants were raised in plastic pots of 30cm diameter and 40cm height size were used for the study. The pots were filled with homogenous mixture of garden soil containing red soil, sand along with farmyard manure in the ratio of 1:1:1. The pots were arranged in Completely Randomized Block Design (CRBD). The experimental seeds were surface sterilized with 0.2% Mercuric chloride solution for five minutes with frequent shaking and thoroughly washed with tap water. The plants were allowed to grow up to 30 days with regular water irrigation. After 30days, well established plants were selected for treatments. The drought stress given on 3 DID (Days Interval Drought), 4 DID, 5 DID and 6 DID then one day interval irrigation on ground water was kept as

control. The plant samples were collected on 40, 60 and 80 DAS.

A. Estimation of protein content

Extraction and estimation of the protein content was followed by the method of Lowry et al. (1951). Five hundred mg of plant material was ground with 10ml of 20% TCA. The homogenate was centrifuged at 800 rpm for 10 mts. The supernatant was discarded and to the pellet, 5 ml of 0.1 N NaOH was added to solubilize the protein and the solution was centrifuged again at 800 rpm for 5 minutes. The supernatant was made up to 10 ml with 0.1 N NaOH and used for the estimation of protein content. 1 ml of the extract was added with 5 ml of reagent 'C' than incubated at dark for 10mts. After 10 minutes, 1ml of Folin ciocalteae reagent was added and it was incubated at dark for 30mts. The absorbance was read at 660 nm using a spectrophotometer.

B. Estimation of Proline content

The Proline content was estimated by the method of Bates et al., (1973). The plant material (0.5 g) was homogenized in 3% aqueous sulfosalicylic acid and the homogenate was centrifuged. Then the homogenate was filtered through Whatman No.1 filter paper. 2 ml of supernatant was taken in a test tube, and 2 ml of acid ninhydrin and 2 ml of glacial acetic acid was added to it and boiled at 100° C for 1 h. After termination of reaction in ice bath, the reaction mixture was extracted with 4 ml of toluene and absorbance was read at 520 nm. The free proline was calculated, using L-proline as a standard.

C. Estimation of Amino acids content

The amount of amino acid content was estimated according to the method of Moore and Stein (1948). Five hundred milligrams of fresh plant material was homogenized in a mortar and pestle with 80% ethanol. The extract was centrifuged at 800 rpm for 15 min and the supernatant was made up to 10 ml with 80% ethanol. In a 25 ml test tube, ethanol extract was taken and it was 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 min, and then 5 ml of diluting solution was added, cooled and made up to 25 ml with distilled water. The absorbance was read at 570 nm in a spectrophotometer.

D. Estimation of Glycine betaine content

Glycine betaine contents were estimated by the method of Grieve and Grattan (1983). Finely ground dry tissue was diluted with equal volume of 1M H₂SO₄, made into aliquots of 0.5 ml in micro centrifuge tubes, cooled over ice for 1 h and to each of these tube, 0.2 ml of cold potassium iodide reagent was added. The reactants were gently stirred, stored at 4°C overnight and centrifuged at 10,000 rpm for 15 min at 4°C to get the precipitated per iodide crystals. The crystals were dissolved in 1,2-dichloroethane, and the absorbance was measured at 365 nm after 2 h. Glycine betaine was dissolved in 1 M H₂SO₄ and it was served as a standard.

E. Estimation of sucrose content

Sucrose content was estimated by the method of Bernt and Bergmayer (1970). For estimating sucrose, 1 ml of invertase (prepared by dissolving 250 units of yeast invertase in 500 ml of 0.2M sodium acetate buffer = pH 5.0) was added to 1ml of sugar extract and incubated at 37°C for 1h 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 Total soluble sugar content

Total soluble sugar contentwas assayed as described by Nelson (1944). 0.5 gm of fresh plant material was homogenized in a mortar and pestle with 80% ethanol.

The extract was evaporated to dryness in a water bath. To the residue, 1 ml of distilled water and 1 ml of 6 N H₂SO₄ were added. The mixture was incubating in a water bath at 50°C for an hour. The solution was cooled and 1 N NaOH was added then made up to 10 ml of H₂O. 1 ml of fresh copper reagent and 1 ml of extract were added. The mixture was heated mouth covered with a boiling water bath for 20 minutes, then cooled and 1 ml of Arsenomolybdate reagent was added. The final volume was made up to 20 ml with distilled water. The resultant blue colour was read at 520 nm in a spectrophotometer against the appropriate blank. The sugar content was expressed in milligram per gram dry weight.

G. Statistical analysis

Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The values are mean ± S.E. for seven samples in each group. Pvalues≤0.05 was considered as significant.

III. RESULT AND DISCUSSION

During drought stress, the compatible solutes like Proline, Glycine betaine and amino acid were increased. Under drought stress the maintenance of leaf turgor could be achieved by the way of osmotic adjustment in response to the accumulation of proline, sucrose, carbohydrate, glycine betaine and other solutes in cytoplasm improving water uptake from drying soil. When the water stress increases, osmotic pressure was adjusts by the mechanism of this osmotic, incompatible solute accumulation enhanced in cytoplasm.

A decrease in protein content was observed in *P. scrobiculatum* subjected to drought stress. In this study drought stress decrease in protein content was recorded at all level of drought stress and all sampling days when compared to control (Tab. 1). The proteins were suggested to have important roles during stress

as osmotic adjustment and available sources of carbon and nitrogen (Misra and Gupta, 2006). In the present study drought treatments resulted in reduction of total protein content. Similar findings were observed by (Mafakheri et al., 2011) in chickpea and Osman et al. (2007). As suggested by earlier workers, protein degradation might be the result of increased

DAS	Water	Root		Shoot	
	irrigation	Co-1	Co-3	Co-1	Co-3
	Control	4.878 ± 0.191	4.61 1±0.259	5.026 ± 0.274	5.256 ± 0.498
	3 DID	4.488 ± 0.160	4.307 ± 0.287	4.970 ± 0.248	4.778 ± 0.325
40	4 DID	4.079 ± 0.088	3.773 ± 0.407	4.520 ± 0.272	4.486 ± 0.215
	5 DID	3.483 ± 0.259	3.470 ± 0.460	4.338 ± 0.304	4.168 ± 0.151
	6 DID	3.378 ± 0.139	3.175 ± 0.436	3.917 ± 0.219	3.583 ± 0.781
	Control	5.571 ± 0.472	5.020 ± 0.036	5.645 ± 0.453	5.523 ± 0.28
	3 DID	4.956 ± 0.381	4.576 ± 0.145	5.270 ± 0.307	5.219 ± 0.40
60	4 DID	4.587 ± 0.240	4.223 ± 0.183	$4.896 \pm \ 0.40$	4.780 ± 0.240
	5 DID	4.100 ± 0.124	4.007 ± 0.104	4.594 ± 0.310	4.495 ± 0.235
	6 DID	3.846 ± 0.058	3.736 ± 0.187	4.166 ± 0.253	$4.087{\pm}~0.166$
	Control	6.393 ± 0.537	5.37 ± 0.201	5.660 ± 0.595	5.559±0.386
	3 DID	5.715 ± 0.296	5.060 ± 0.221	5.589±0.563	5.397± 0.401
80	4 DID	5.272 ± 0.242	4.668 ± 0.228	5.135 ± 0.337	5.023 ± 0.388
	5 DID	4.841 ± 0.222	4.406 ± 0.265	4.710 ± 0.310	4.627 ± 0.182
	6 DID	4.544 ± 0.207	4.086 ± 0.266	4.467 ± 0.182	4.621± 0.255

 Table 1 .Drought stress induced changes in Root and Shoot Protein content in *P. scrobiculatum*.

Table 2. Drought stress induced changes in Root and Shoot Proline content in *P. scrobiculatum*.

DAS	Water	Root		Shoot	
	irrigation	Co-1	Co-3	Co-1	Co-3
	Control	0.400 ± 0.041	0.407 ± 0.043	0.386 ± 0.026	0.399 ± 0.026
	3 DID	0.423 ± 0.042	0.428 ± 0.043	0.399 ± 0.027	0.419 ± 0.026
40	4 DID	0.443 ± 0.045	0.457 ± 0.046	0.414 ± 0.024	0.445 ± 0.025
	5 DID	0.470 ± 0.053	0.477 ± 0.046	0.441 ± 0.030	0.463 ± 0.027
	6 DID	0.490 ± 0.050	0.493 ± 0.048	0.460 ± 0.028	0.489 ± 0.025
	Control	0.494 ± 0.038	0.508 ± 0.044	0.468 ± 0.023	0.492 ± 0.032
	3 DID	0.503 ± 0.038	0.525 ± 0.044	0.494 ± 0.022	0.508 ± 0.030
60	4 DID	0.507 ± 0.050	0.548 ± 0.044	0.516 ± 0.022	0.537 ± 0.029
	5 DID	0.542 ± 0.035	0.567 ± 0.045	0.537 ± 0.021	0.558 ± 0.032
	6 DID	0.557 ± 0.034	0.598 ± 0.062	0.560 ± 0.021	0.584 ± 0.034
	Control	0.643 ± 0.056	0.661 ± 0.059	0.592 ±0.037	0.597 ± 0.036
	3 DID	0.648 ± 0.039	0.662 ± 0.093	0.610 ± 0.036	0.617 ± 0.036
80	4 DID	0.666 ± 0.040	0.681 ± 0.090	0.631 ± 0.035	0.637 ± 0.038
	5 DID	0.681 ± 0.042	0.696 ± 0.087	0.653 ± 0.040	0.656 ± 0.035
	6 DID	0.693 ± 0.056	0.701 ± 0.059	0.669 ± 0.042	0.673 ± 0.039

	Water	Root		Shoot	
DAS	irrigation	Co-1	Co-3	Co-1	Co-3
	Control	4.897 ± 0.318	5.355 ± 0.627	6.307 ± 0.197	6.658 ±0.615
	3 DID	5.119 ± 0.332	5.753 ± 0.683	6.786 ± 0.146	7.591 ± 0.913
40	4 DID	5.353 ± 0.271	6.054 ± 0.685	7.193 ± 0.125	7.921 ± 0.938
	5 DID	5.568 ± 0.261	6.396 ± 0.725	7.513 ± 0.194	8.338 ±0.973
	6 DID	5.808 ± 0.272	6.816 ± 0.785	8.018 ± 0.131	<i>8.</i> 776 ± 0.956
	Control	5.243 ± 0.309	6.399 ± 0.944	7.341 ± 0.054	7.895 ±0.955
	3 DID	5.440 ± 0.278	6.616 ± 1.005	7.890 ± 0.023	8.613 ± 1.313
	4 DID	5.685 ± 0.287	6.941 ± 1.091	8.339 ± 0.024	9.002 ± 1.336
60	5 DID	5.894 ± 0.292	7.245 ± 1.091	8.766 ± 0.063	9.219 ± 1.307
	6 DID	6.095 ± 0.285	7.518 ± 1.159	9.350 ± 0.026	9.549 ± 1.304
	Control	5.644 ± 0.271	7.185 ± 1.016	10.405±0.590	10.745±0.208
	3 DID	5.933 ± 0.208	7.537 ± 1.055	11.201±0.623	12.287±0.476
	4 DID	6.311 ± 0.148	8.320 ± 1.242	11.893±0.444	13.361±0.192
80	5 DID	6.741 ± 0.151	8.683 ± 1.242	13.581±0.268	14.509±0.518
	6 DID	6.937 ± 0.107	9.107 ± 1.345	14.351±0.452	15.806±0.183

Table 3. Drought stress induced changes in Root and Shoot Amino acid content in *P. scrobiculatum*.

Table 4. Drought stress induced changes in Root and Shoot Glycine betaine content in *P. scrobiculatum*.

DAS	Water	Root		Shoot	
	irrigation	Co-1	Co-3	Co-1	Co-3
	Control	0.349 ± 0.032	0.417 ± 0.024	0.337 ± 0.016	0.349 ± 0.027
	3 DID	$0.398 {\pm}~0.034$	0.444 ± 0.030	0.370 ± 0.018	0.383 ± 0.029
40	4 DID	$0.417{\pm}\ 0.033$	0.464 ± 0.028	0.397 ± 0.020	0.408 ± 0.024
	5 DID	$0.467{\pm}\ 0.032$	0.489 ± 0.027	0.409 ± 0.019	0.430 ± 0.028
	6 DID	0.476 ± 0.031	0.511 ± 0.026	0.430 ± 0.020	0.451 ± 0.027
	Control	$0.417{\pm}0.024$	0.484 ± 0.031	0.445 ± 0.029	0.425 ± 0.046
	3 DID	0.506 ± 0.031	0.533 ± 0.042	0.473 ± 0.025	0.483 ± 0.031
60	4 DID	0.530 ± 0.032	0.560 ± 0.042	0.505 ± 0.022	0.510 ± 0.029
	5 DID	0.545 ± 0.033	0.578 ± 0.042	0.526 ± 0.023	0.527 ± 0.028
	6 DID	0.562 ± 0.033	0.604 ± 0.045	0.550 ± 0.029	0.551 ± 0.020
	Control	0.560 ± 0.035	0.623 ± 0.051	0.547 ± 0.030	0.557 ± 0.037
80	3 DID	0.569 ± 0.017	0.643 ± 0.030	0.549 ± 0.067	0.580 ± 0.039
	4 DID	0.590 ± 0.016	0.667 ± 0.047	0.569 ± 0.028	0.617 ± 0.033
	5 DID	0.607 ± 0.018	0.686 ± 0.046	0.590 ± 0.024	0.640 ± 0.035
	6 DID	0.620 ± 0.018	0.699 ± 0.042	0.617 ± 0.032	0.661 ± 0.035

DAS	Water	Root		Shoot	
	irrigation	Co-1	Co-3	Co-1	Co-3
	Control	6.246 ± 0.224	6.308 ± 0.261	5.855 ±0.293	6.001 ± 0.211
	3 DID	$7.657{\pm}0.262$	7.770 ± 0.315	7.366 ± 0.266	7.471 ± 0.322
40	4 DID	8.618 ± 0.375	8.772 ± 0.276	8.343 ± 0.371	$8.524 \ \pm 0.294$
	5 DID	9.593 ± 0.260	9.811 ± 0.206	9.331 ± 0.258	9.521 ± 0.228
	6 DID	10.596±0.179	10.802±0.138	10.333±0.156	10.553 ±0.137
	Control	7.233 ± 0.393	7.366 ± 0.057	7.090 ± 0.093	7.221 ± 0.167
	3 DID	7.928 ± 0.350	8.544 ± 0.140	8.250 ±0.141	$8.596 \ \pm 0.202$
60	4 DID	8.861 ± 0.392	9.210 ± 0.167	8.927 ± 0.167	9.902 ± 0.389
	5 DID	9.700 ±0. 434	10.490±0.186	9.454 ± 0.450	10.831 ± 0.480
	6 DID	10.896±0.456	11.699±0.159	11.400±0.164	12.515 ± 0.620
	Control	9.560 ± 0.323	9.952 ±0.392	9.634 ± 0.377	10.767 ± 0.314
	3 DID	10.518±0.182	10.849±0.060	10.563±0.056	11.303 ± 0.425
80	4 DID	11.513±0.116	12.508±0.232	12.217±0.232	12.302 ± 0.409
	5 DID	12.820±0.187	13.250±0.198	12.982±0.196	13.423 ± 0.427
	6 DID	14.007±0.335	14.141±0.355	13.901±0.358	14.531 ± 0.635

 Table 5. Drought stress induced changes in Root and Shoot Sucrose content in *P. scrobiculatum*.

 Table 6. Drought stress induced changes in Root and Shoot Total Soluble Sugare content in *P. scrobiculatum*.

DAS	Water	Root		Shoot	
	irrigation	Co-1	Co-3	Co-1	Co-3
	Control	4.368 ± 0.306	4.402 ± 0.264	4.152 ± 0.238	4.198 ± 0.338
	3 DID	4.549 ± 0.299	4.551 ± 0.242	4.326 ± 0.333	4.562 ± 0.472
40	4 DID	4.782 ± 0.326	4.794 ± 0.260	4.517 ± 0.341	4.678 ± 0.262
	5 DID	4.993 ± 0.393	4.999 ± 0.314	4.672 ± 0.253	4.777 ± 0.345
	6 DID	5.082 ± 0.300	5.208 ± 0.337	4.829 ± 0.228	4.989 ± 0.338
60	Control	5.214 ± 0.262	5.127 ± 0.282	4.998 ± 0.222	5.208 ± 0.303
	3 DID	5.236 ± 0.297	5.357 ± 0.300	5.138 ± 0.230	5.387 ± 0.313
	4 DID	5.321 ± 0.470	5.401 ± 0.313	5.299 ± 0.299	5.515 ±0.347
	5 DID	5.390 ± 0.484	5.711 ±0.245	5.623 ± 0.240	5.675 ±0.349
	6 DID	5.447 ± 0.486	5.983 ± 0.231	5.784 ± 0.342	5.882 ± 0.225
	Control	5.851 ± 0.340	6.237 ± 0.195	5.729 ± 0.252	6.242 ± 0.185
80	3 DID	5.948 ± 0.323	6.321 ± 0.197	5.808 ± 0.241	6.311 ± 0.188
	4 DID	6.056 ± 0.306	6.395 ± 0.198	5.864 ± 0.245	6.396 ± 0.197
	5 DID	6.116 ± 0.305	6.491 ± 0.195	5.893 ± 0.245	6.431 ± 0.201
	6 DID	6.212 ± 0.290	6.572 ± 0.409	5.926 ± 0.238	6.553 ± 0.142

activity of protease or other catabolic enzymes, which were activated under drought stress, or due to fragmentation of proteins due to toxic effects of

reactive oxygen species resulting in reduced protein content (Davies 1987).

The drought stressed plants proline content was significantly decreased when compared to control (Tab. 2) plant at all the sampling days. Enhancement of proline with increasing time of water limitation occurred. Proline is an amino acid which is well known as an osmotic protectant, which keeps the osmotic balance between plant cells and the outer environment when plants are subjected to water stress (Jungklang and Saengnil, 2012). The similar report was observed by (Lum et al., 2014) in rice, (Abbas et al 2014) in sugarcane. Sankar et al. (2007) reported that high proline accumulation in plants could provide energy for growth and survival and thereby help the plant to tolerate stress. It is now well known that proline accumulation in plant leaf cells, as a compatible solute, plays an important role in regulating water loss from the cells under water deficit and osmotically stressful conditions (Bayoumi et al., 2008).

Amino acid content has been shown an increase trend under drought condition in Paspalum scrobiculatum. The same report was observed on accumulated amino acid may be occurring in response to the change in osmotic adjustment of their cellular contents (Shao et al., 2007). It is shown that plants have evolved a great number of adaptive mechanisms that allow the biochemical systems to cope with increased water deficit. The complexity of tolerance to water deficit and supports the statements of many authors that the flexibility of cell metabolism and its fast acclimation to changes in environmental conditions is a first essential step in stress avoidance (Zlatev and Lidon, 2012). The accumulation of free amino acid under stress indicates the possibility of their involvement in osmotic adjustment (Yadav et al., 2005).

Glycine betane content showed increase in *P. scrobiculatum* under drought stress condition when compared to control plants (Tab. 4). Similar report was observed by (Arivalagan and Somasundaram, 2016) in *Sorghum bicolor*. Plants were known to

accumulate glycine betane naturally have been reported to grow well under stressful environment. The enzyme of choline monooxyygenase (CMO) first converts choline into betaine aldehyde and then a NAD dependent enzyme, betaine aldehvde dehydrogenase (BADH) produces glycine betaine found in chloroplast stroma and their activity was increased in response to stress (Arakawa et al., 1990). GB may maintain the osmoticum, provided that the basal metabolism of the plant can sustain a high rate of synthesis of these compounds to facilitate osmotic adjustment for tolerance to water stress [Kavikishore et al., 1995].

During drought stress the sucrose content of shows an increase trend. When plants were subjected to stress and the stimulation of sugar accumulation was proportional to osmotic adjustment. Similar observation made in *Sorghum bicolor* (Arivalagan and Somasundaram, 2016a). The sucrose has important role, as well as is the main photo assimilated exported of the synthesis sites as leaves from consumes sites as flowers, buds and stem, besides it is kept during light and moderate water restriction and consumed under severe water deficit (Pimentel, 2004).

The drought stress caused a significant increase in total sugar content of *P. scrobiculatum* plants (Tab. 6). The accumulation of sugars in plants under stress conditions might be involved in the osmotic adjustment was reported (Perez-Lo pez et al., 2010). Sugar accumulated under drought stress are likely to stabilize membranes and prevent membrane fusion, together with other macromolecules such as late embryogenesis abundant protein (LEA Proteins), Trehalose, Disaccharide is accumulated under drought stress and functions during embryo and flower development, as well as in the regulation of carbon metabolism and photosynthesis (Phillips et al., 2009).

These results are indicate that plants are altering their osmoregulation and to balance the water content by lowering the water potential. From the results of this investigation it is clear that the CO - 3 variety was more tolerance than CO - 1 variety plants.

IV. CONCLUSION

In two varieties of *P. scrobiculatum* the protein and starch decreased under drought stress. However, amino acid, total sugar, sucrose, proline and glycine betaine content increased. The two varieties of *P. scrobiculatum* showed an inhibited altered biochemical activities under drought conditions. Among the two varieties studied, *P. scrobiculatum* showed better drought tolerance capacity Co-3 variety under pot culture.

V. REFERENCES

- Abbas, S.R., S.D. Ahmad, S.M. Sabir and A.H. Shah. (2014). Detection of drought tolerant sugarcane genotypes (Saccharum officinarum) using lipid peroxidation, antioxidant activity, glycine-betaine and proline contents. Journal of Soil Science and Plant Nutrition, 14(1), 233-243.
- [2]. Allen, C.M. and D.W. Hall. (2003). Paspalum. Flora of North America north of Mexico, 25, 566-599.
- [3]. Arakawa, K., M. Katayama and T. Takabe, (1990). Levels of betaine and betaine aldehyde dehydrogenase activity in the green leaves, and etiolated leaves and roots of barley. Plant and cell physiology, 31(6), 797-803.
- [4]. Arivalagan. M and R. Somasundaram. (2016). Induction of drought stress tolerance by propiconazole and salicylic acid in Sorghum bicolor is mediated by enhanced osmoregulation, compatible solutes and biochemical accumulation. Journal of Applied and Advanced Research, 1, 41–52.
- [5]. Arivalagan. M. and R. Somasundaram. (2016). Propiconazole and salicylic acid alleviate effect of drought stress in Sorghum (Sorghum bicolor (L.) Moench) through biochemical and some

physiological characters. Journal of Applied and Advanced Research, 2016, 1(3), 1-7.

- [6]. Arivalagan. M. and R. Somasundaram. (2017). Exogenous application of triazoles modifies growth and biochemical characteristics of Lycopersicon esculentum Mill. under water limited conditions. Journal of scientific Agriculture, 1, 171–181.
- [7]. Bates. L.S, R.P. Waldron and I.D. Teare. (1973). Rapid determination of free proline for water stress studies. Plant Soil, 39, 205-208.
- [8]. Bayoumi. T.Y., M.H. Eid and E.M. Metwali. (2008). Application of physiological and biochemical indices as a screening technique for drought tolerance in wheat genotypes. African Journal of Biotechnology, 7 (14)
- [9]. Bernt, E. and H.U. Bergmeyer. (1970). l-Glutamat. Bestimmung mit glutamatdehydrogenase und NAD. Methoden der enzymatischen Analyse, 2, 1659-1663.
- [10]. Bohnert. H.J and R.J. Jenson. (1996). Strategies for engineering water-stress tolerance in plants, Trends Biotechnology, 14, 89–97.
- [11]. BOTANIK, Z. (1937). Journal of the Arnold Arboretum, 18, 54.
- [12]. Cattivelli. L., F. Rizza, F.W. Badeck, E. Mazzucotelli, A.N. Mastrangelo, E. Francia, C. Mare, A. Tondelli and A.M. Stanca. (2008). Drought tolerance improvement in crop plants. Anintegrated view from breeding to genomics. Field Crops Research, 105, 1-14.
- [13]. Davies. K.J.A. (1987). Protein damage and degradation by oxygen radicals. I. General aspects. Journal of Biochemistry, 262, 9895– 9901.
- [14]. Grieve. C.M. and S.R. Grattan. (1983). Rapid assay for determination of water soluble quaternary ammonium compounds. Plant and Soil, 70(2), 303-307.
- [15]. Hare. P.D., W.A. Cress and J. Van Staden. (1998). Dissecting roles of osmolyte accumulation during stress. Plant Cell Environment, 21, 535–553.
- [16]. Iturriaga, G., R. Suárez and B. Nova-Franco. (2009). Trehalose metabolism: from osmoprotection to signaling. International journal of molecular sciences, 10(9), 3793-3810.

- [17]. Jain, S., G. Bhatia, R. Barik, P. Kumar, A. Jain and V.K. Dixit. (2010). Anti-diabetic activity of *Paspalum Scrobiculatum* Linn. In alloxan induced diabetic rats. Journal of Ethno pharmacology, 127, 325-338.
- [18]. Jungklang. J. and K. Saengnil. (2012). Effect of paclobutrazol on patumma cv. Chiang Mai Pink under water stress. Songklanakarin Journal of Science and Technology, 34, 361-366.
- [19]. Kavikishore. P.B., S. Sangam, R.N. Amrutha, P. Srilaxmi, K.R. Naidu, K.R.S.S. Rao, S.Rao, K.J. Reddy, P. Theriappan and N. Sreenivasulu. (2005). Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance, Current Science, 88, 424–438.
- [20]. Kavikishore. P.B., Z. Hong, G.U. Miao, C.A.H. Hu and D.P.S. Verma. (1995). Over expression of 1-pyroline-5-carboxylase synthetase increases proline production and confers osmotolerance in transgenic plants, Plant Physiology, 108, 1387– 1394.
- [21]. Kiran, P., M. Denni and M. Daniel. (2014). Antidiabetic Principles, Phospholipids and Fixed Oil of Kodo Millet (*Paspalum Scrobiculatum* Linn.). Indian Journal of Applied Research, 4, 13-15.
- [22]. Lowry. O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall. (1951). Protein measurement with the Folin phenol reagent. Journal of biological chemistry, 193(1), 265-275.
- [23]. Lum. M.S., M.M. Hanafi, Y.M. Rafii and A.S.N. Akmar. (2014). Effect of drought stress on growth, proline and antioxidant enzyme activities of upland rice. Journal of Animal and Plant Science, 5, 1487-1493.
- [24]. Mafakheri. A., A. Siosemardeh, B. Bahramnejad, P.C. Struik and Y. Sohrabi. (2011). Effect of drought stress and subsequent recovery on protein, carbohydrate contents, catalase and peroxidase activities in three chickpea (Cicer arietinum) cultivars. AJCS, 5, 1255-1260.
- [25]. Misra. N. and A.K. Gupta. (2006). Effect of salinity and different nitrogen sources on the activity of antioxidant enzymes and indole alkaloid content in Catharanthus roseus seedlings. Journal of plant physiology, 163(1), 11-18.

- [26]. Moore, S. and W.H. Stein. (1948). Photometric nin-hydrin method for use in the ehromatography of amino acids. Journal of biological chemistry, 176, 367-388.
- [27]. Mostajean, A and V.R. Eichi. (2009). Effects of drought stress on growth and yield of rice (Oryza sativa L.) Cultivars and accumulation of proline and soluble sugars in sheath and blades of their different ages leaves American-Evras. Journal of Agriulture and Environmental Science, 5, 264-272.
- [28]. Murthy, A.V.S and N.S.A. Subramanyam. (1989). Textbook of Economic Botany. (Wiley Eastern Limited), New Delhi.
- [29]. Nelson, N. (1944). A photometric adaptation of the Somogyi method for the determination of glucose. Journal of biological chemistry, 153(2), 375-380.
- [30]. O'Kennedy. M.M., A. Grootboom and R.R. Shewry. (2006). Harnessing sorghum and millet biotechnology for food and health. Journal of Cereal Science, 44, 224–235.
- [31]. Osman. M.E.H., S.S. Elfeky, K. Abo El-Soud and A.M. Hasan. (2007). Response of Catharanthus roseus Shoots to Salinity and Drought in Relation to Vincristine Alkaloid Content. Asian Journal of Plant Sciences, 6(8), 1223-1228.
- [32]. Perez-Lopez, U., A. Robredo, M. Lacuesta, A. Munoz-Rueda and A. Mena-Petite. (2010). Atmospheric CO 2 concentration influences the contributions of osmolyte accumulation and cell wall elasticity to salt tolerance in barley cultivars. Journal of plant physiology, 167(1), 15-22.
- [33]. Phillips. R.L., D.R. Zak, W.E. Holmes and D.C. White. (2002). Microbial community composition and function beneath temperate trees exposed to elevated atmospheric carbon dioxide and ozone. Oecologia, 131(2), 236-244.
- [34]. Pimentel. C. (2004) The relation of the plant with water. EDUR, Seropedica
- [35]. Sankar, B., C.A. Jaleel, P. Manivannan, A. Kishorekumar, R. Somasundaram and R. Panneerselvam. (2007). Effect of Paclobutrazol on water stress amelioration through antioxidants and free radical scavenging enzymes in Arachis hypogaea L. Colloids and Surfface. B: Biointerfaces, 60, 229–235

- [36]. Shao. H.B., L.Y. Chuc, G. Wu, J.H. Zhang, Z.H. Lua and Y.C. Hug. (2007). Changes of some antioxidative physiological indices under soil water deficits among 10 wheat (Triticum aestivum L.) genotypes at tillering stage. Colloids Surfface B: Biointerfaces, 54, 143–149.
- [37]. Tan, Y., Z. Liang, H. Shao and F. Du. (2006). Effect of water deficits on the activity of antioxidative enzymes and osmoregulation among three different genotypes of Radix Astragali at seeding stage. Colloids and surfaces B: Biointerfaces, 49(1), 60-65.
- [38]. Tuna. A.L., C. Kaya and M. Ashraf. (2010). Potassium sulfate improves water deficit tolerance in melon plants grown under glasshouse conditions. Journal of Plant Nutrition, 33, 1276-1286.
- [39]. Yadav. S.K., N. Jyothi Lakshmi, M. Maheswari, M. Vanaja and B. Venkateswarlu. (2005). Influence of water deficit at vegetative, an thesis and grain filling stages on water relation and grain yield in sorghum. Indian Journal of Plant Physiology, 10, 20-24.
- [40]. Yadava, H. S., & Jain, A. K. (2006). Advances in kodo millet research. Directorate of Information and Publications of Agriculture, Indian Council of Agricultural Research.
- [41]. Zhang. L.H., J.H. Lai, Z.S. Liang and M. Ashraf. (2014). Interactive Effects of Sudden and Gradual Drought Stress and Foliar-applied Glycine betaine on Growth, Water Relations, Osmolyte Accumulation and Antioxidant Defence Systemin Two Maize Cultivars Differing in Drought Tolerance. Journal of Agriculture and Crop Science, 200, 425–433.
- [42]. Zlatev. Z and F.C. Lidon. (2012). An overview on drought induced changes in plant growth, water relations and photosynthesis. Emirate Journal of Food and Agriculture, 24, 57-72.