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Physiological Efficiency of Small Millets under Drought Condition

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An experiment was conducted to study the physiological and biochemical traits contributing for better yield under reproductive stage drought in selected small millets *viz.,* finger millet (CO 15), little millet (CO 4) and barnyard millet (CO 2). Drought was imposed during flowering stage of the crops by withholding irrigation till the soil moisture reaches below 20 per cent. The physiological parameters considered as indicators of drought tolerance *viz*., LA, LAI, LAD, NAR, soluble protein content and NR (Nitrate Reductase) activity measured during drought at reproductive stage depicted the relative ability of small millets to endure the water stress. Among the small millets, barnyard millet recorded the highest values for growth attributes such as LA (561.10 cm^2 $plant^{-1}$), LAI (2.24), NAR (1.52 mg g^{-1} day^{-1}) and LAD (53.63 days) under stress at reproductive stage. Drought stress imposed at reproductive stage had an adverse effect on soluble protein content with considerable reduction in all small millets. Among them, barnyard millet recorded the highest soluble protein content (9.35 mg g^{-1}) but the lowest reduction in soluble protein content due to water stress. Similar trend was observed for NR activity also.

Key words: Small millets, Physiological parameters, Growth attributes, NR activity

Small millets are a group of annual grasses found mainly in the arid and semi-arid areas and are nutrient-rich food sources traditionally grown and consumed by subsistence farmers in Asia and Africa. They are also unique due to their short growing season (65 -120 days). Hence, they can be very well fitted into multiple cropping systems both under irrigated as well as dry farming conditions (Stanly and Shanmugam, 2013). Of the total area of 23 million ha under millets in India, small millets account for only about 3.5 million ha (Padulosi *et al*., 2015). Small millets have high production potential under optimum conditions and millets have diverse adaptation mechanisms to grow and survive under relatively marginal environments. Currently, drought is one of the most important limiting factors for crop production in many regions of the world (Aslam *et al.,* 2006). To advance crop productivity in such drought prone areas, it is necessary to understand the mechanism of plant responses with the ultimate goal of improving crop performance. Understanding of the physiological, biochemical and molecular mechanisms of drought stress perception, manifestation and tolerance is still a major challenge in biology. Attempts to measure the degree of tolerance with single parameter have limited value because of the multiplicity of the factors and their interactions contributing to drought tolerance. Hence, the present study was designed with finger millet, barnyard millet and little millet to assess the various traits contributing to drought tolerance during reproductive stage of the crop.

Material and Methods

The pot culture experiment was conducted in the Rainout shelter located at Field No. 75 of Eastern Block, Department of Farm Management, Tamil Nadu Agricultural University, Coimbatore during *Rabi*, 2015. The location is in North Western Agro-Climatic Zone of Tamil Nadu at 11°N latitude and 77°E longitude and at an altitude of 426.7 m above MSL. During the cropping period in 2015-16 the average maximum and the minimum temperature were 30°C and 21°C, respectively. The total rainfall 3.1 mm was received during the cropping season (*Rabi*, 2015). The relative humidity ranged from 55.9 to 88.9 per cent. The mean evaporation rate ranged from 2.9 to 4 mm day^{-1}. The average bright sunshine hours were 5.8 hours day^{-1} and mean wind velocity was 4.7 km hr^{-1} during the cropping period. The experiment was conducted with seven replications in three small millets *viz*., finger millet (CO 15), little millet (CO 4), barnyard millet (CO 2).

Red sandy soil was used for pot culture experiment. Pot mixture was prepared by using red soil, sand and vermicompost in the ratio of 3:1:1 and the pots (D26×H30 cm size) were filled with 14 kg of soil. The seeds were directly sown in the pot since minor millets used for the study are direct seeded one. After the establishment of seedlings, thinning was done to maintain three seedlings per pot uniformly across the replications. Fertilizer dosage for pot culture was calculated using per hectare recommendations of small millets and other cultivation operations including plant protection measures were carried out as per recommended package of practices of Tamil Nadu Agricultural University, Coimbatore. All plants, in both the control and treatment groups, were watered regularly up to the flowering stage of small millets and then the irrigation was with held to create drought at

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reproductive stage. Since, the selected small millets have different maturity duration, the flowering stage was not synchronized and hence, the drought was imposed after flowering of individual millets. Soil moisture content was measured using moisture meter (Delta-T Soil moisture kit - Model: SM150, Delta-T Devices, Cambridge) periodically and rewatering was done, when the soil moisture reached below 20 per cent and 1/3rd of leaves started drying. The growth parameters like leaf area, leaf area duration, leaf area index, net assimilation rate and biochemical characters, soluble protein content and nitrate reductase activity were measured at the crop stages under both control and stressed condition. Since the crops taken for study were having different maturity duration, they were harvested in different dates.

Results and Discussion

Leaf area

From the experimental data, we found that there was a significant decrease in leaf area due to drought which was imposed during reproductive stage and the impact was also observed after rewatering in all the small millets taken for this study (Table. 1).

Table 1. Effect of reproductive stage drought on leaf area (cm^2 plant^{-1}) of small millets

Crop	Vegetative stage	Reproductive stage			Maturity stage		
		Control	Stress	Mean	Control	Stress	Mean
Finger millet	310.81	382.73	341.25	361.99	299.55	284.34	291.95
Little millet	235.28	294.32	259.24	276.78	226.21	201.12	213.67
Barnyard millet	535.87	590.29	561.10	575.70	528.16	512.43	520.30
Mean	360.65	422.45	387.20	404.82	351.31	332.63	341.97
	G	G	T	G x T	G	T	G x T
SEd	2.68	2.97	2.43	4.20	2.56	2.09	3.63
CD (0.05)	5.48	6.07	4.95	NS	5.24	4.27	NS

The barnyard millet recorded the highest leaf area of 561.10 and 512.43 cm^2 plant^{-1} at reproductive and maturity stages respectively, while little millet recorded the lowest leaf area (259.24, 201.12 cm^2 plant^{-1}). Also, the reduction of leaf area under stress over its control was less in barnyard millet (4.95%) compared to little millet (11.92%) and finger millet (10.84%) due to drought at reproductive stage. The source, leaf area is an important component that is closely related to the physiological processes controlling dry matter production and yield. It is an important photosynthetic parameter in crop plants and shows positive relationship with net photosynthetic activity. Leaf area, as an index of its actively growing material, was significantly reduced under drought conditions (Deshmukh *et al*., 2004). Under water deficits, reduction in leaf area was attributed by the restricted leaf growth and accelerated leaf senescence (Ludlow and Muchow, 1990).

Table 2. Effect of reproductive stage drought on leaf area index of small millets

Crop	Vegetative stage	Reproductive stage			Maturity stage		
		Control	Stress	Mean	Control	Stress	Mean
Finger millet	1.04	1.28	1.04	1.16	0.99	0.90	0.95
Little millet	0.94	1.18	1.01	1.09	0.90	0.80	0.85
Barnyard millet	2.14	2.36	2.24	2.30	2.11	2.06	2.08
Mean	1.37	1.61	1.43	1.52	0.95	1.25	1.29
	G	G	T	G x T	G	T	G x T
SEd	0.010	0.012	0.009	0.016	0.010	0.008	0.014
CD (0.05)	0.021	0.024	0.019	NS	0.020	0.016	NS

Water stress reduced the leaf area in all the small millets. The reduction in leaf area due to moisture stress might be attributed to the negative influence on the cell enlargement and cell division causing reduced leaf growth according to Ludlow and Muchow (1990). The reduction of leaf area under stress over its control was less in barnyard millet (4.95 %) compared to little millet (11.92 %) and finger millet (10.84 %) due to drought at reproductive stage. Observation on leaf area at maturity stage after re watering showed that the barnyard millet registered better recovery after drought by means of significantly lesser reduction in leaf area followed by finger millet and the highest reduction was observed in little millet at maturity stage. This was in corroboration with the suggestions of Chazen and Neumann, (1994) that drought tolerant varieties, for example, show relatively less reduction in leaf area relative to sensitive lines, associated in large part with maintenance of cell wall plasticity and continued leaf growth. Several morphological and physiological adaptations are associated with increased WUE in foxtail millet including small leaf area, thickening of the cell walls, and ability to form a dense root system (Li, 1997). There was a decline in leaf production and notable restriction in canopy size as a result of drought especially beyond 56 DAS in finger millet (Muhammad and Azam, 2007). The reduction of leaf area in drought affected plants, as observed in this study, could probably be one of the mechanisms for reducing the total leaf area under the limited soil moisture content, a drought tolerance mechanism that Jones (1992) considered as a water conservation strategy. With a small leaf area a crop is able to limit water loss because the size of the evaporating surface is small.

Leaf area index

Analysis of leaf area index (LAI) revealed that there was a significant difference among the small millets under drought at reproductive stage and after recovery of drought. In our study there was a significant decrease in LAI due to drought which was imposed during reproductive stage and the impact was also observed after re watering in all the small millets taken for this study. The barnyard millet recorded the highest LAI of 2.24 and 2.06 at reproductive and maturity stages, respectively while little millet recorded the lowest (1.01, 0.80). The percent reduction of LAI under stress over its control was lesser in barnyard millet (5.08 %) compared to little millet (14.40 %) and finger millet (18.75 %) during drought at reproductive stage. Leaf area index (LAI) is the principle factor influencing net photosynthesis which in turn is related to dry matter production and yield (Hansen, 1972). Similar results were also observed by Muhammad and Azam (2007) in LAI of finger millet genotypes due to moistures stress. In general, the reduction in LAI was primarily due to leaf abscission. Water stress imposed particularly during flowering stage, accelerated the senescence process which was evident in this study. The LAI declined during reproductive development and maturity stage as the leaf number and leaf area was reduced by water stress. Water stress reduced the mean LAI at reproductive stage (8.69 %) and maturity stage (25.19 %) over control, irrespective of small millets studied. The reduction of LAI under stress over control was lesser in barnyard millet (5.08 %) compared to little millet (14.40 %) and finger millet (18.75 %) due to drought at reproductive stage (Table 2). Observation on LAI at maturity stage after rewatering showed that the barnyard millet registered better recovery after drought by means of significantly lesser reduction in LAI compared to finger millet and little millet at maturity stage. Barnyard millet having the highest LAI compared to other small millets under drought stress, could harvest higher solar radiation than others. Stress caused by single or multiple factors suppresses leaf initiation and expansion and leaf area index (Kumar *et al*., 2006). Hence, water stress induced changes in leaf area and LAI ultimately reflected on biomass production (Kerby *et al*., 1990).

Table 3. Effect of reproductive stage drought on NAR (mg g^{-1} day^{-1}) and LAD (days) of small millets

Crop	NAR			LAD		
	Control	Stress	Mean	Control	Stress	Mean
Finger millet	1.70	1.24	1.47	45.40	41.80	43.60
Little millet	2.36	1.19	1.78	26.00	23.00	24.50
Barnyard millet	2.49	1.52	2.01	55.88	53.63	54.76
Mean	2.18	1.32	1.75	42.43	39.48	40.95
	G	T	G x T	G	T	G x T
SEd	0.01	0.01	0.02	0.30	0.25	0.43
CD (0.05)	0.03	0.02	0.04	0.61	0.50	NS

Net assimilation rate and leaf area duration

Analysis of net assimilation rate (NAR) and leaf area duration (LAD) revealed that there was a significant difference among the small millets and showed decreasing trend due to drought stress compared to control from reproductive stage to maturity stage (Table 3). Among the small millets, barnyard millet recorded the highest values for NAR (1.52 mg g^{-1} day^{-1}) and LAD (53.63 days) when drought was imposed at reproductive stage while the lowest NAR and LAD were recorded by little millet (1.52 and 23, respectively). The percent reduction of NAR due to drought in finger millet over the control was 27.06 %, in little millet 49.58 % and in barnyard millet 38.96 % at reproductive to maturity stage. The per cent reduction in LAD was highest (11.54 %) in little millet over its control followed by finger millet (7.93 %) under drought condition and the barnyard millet recorded lesser reduction (4.02 %) in LAD. Net assimilation rate (NAR) indicates the assimilatory capacity of the leaves for biomass production in unit time. Singh and Singh (1995) found that, agronomic and physiological response of sorghum, maize and pearl millet showed that NAR reduced when drought stress prolonged.

The percent reduction of NAR due to drought in finger millet over the control was 27.06 %, in little millet 49.58 % and in barnyard millet 38.96 % at reproductive to maturity stage. Similar reduction in NAR due to water stress was observed by Williams and Shapter (1955) in barley and rye. Moisture stress resulted in reduction of LAD in all the small millets. LAD increased with the advancement of crop growth up to flowering and then decreased due to the drought accelerated senescence. In this study, barnyard millet registered higher LAD of 53.63 days at reproductive to maturity stage with a lesser reduction (4.02 %) over its control compared to finger millet (41.8 days and 7.93 %) and little millet (23 days and 11.54 %) (Table 3). Such results was also observed by Gonzalez and Paez (1996) reported that the LAD reduced significantly when drought stress occurred during reproductive stage. The reduction of LAD under moisture stress was also reported in ragi by Krishnasastry *et al*. (1982). The higher reduction of LAD in little millet might be due to the rapid decrease in leaf number and leaf area in the later stages.

The lesser reduction in LAD in barnyard millet showed its tolerance to water stress among the small millets evaluated. This might be due to sustained extension of leaf area and slow rate of senescence due to drought compared to other millets. The result of present study was confirmed with the findings of Duncan *et al*. (1981) who recorded higher LAD, LAI and LAR in non-senescent genotypes than in senescent ones. Similar results were obtained by Singh and Singh (1982) in green gram.

Table 4. Effect of reproductive stage drought on soluble protein content (mg g^{-1}) of small millets

Crop	Vegetative stage	Reproductive stage			Maturity stage		
		Control	Stress	Mean	Control	Stress	Mean
Finger millet	10.57	12.64	9.22	10.93	10.03	7.58	8.81
Little millet	9.83	11.58	8.59	10.09	9.54	7.00	8.27
Barnyard millet	10.96	12.77	9.35	11.06	10.27	7.81	9.04
Mean	10.45	12.33	9.05	10.69	9.95	7.46	8.71
	G	G	T	G x T	G	T	G x T
SEd	0.07	0.08	0.06	0.11	0.06	0.05	0.09
CD (0.05)	0.15	0.16	0.13	NS	0.12	0.10	NS

Soluble protein content

The soluble protein content in leaves was found declining under drought condition and there was a significant difference for soluble protein content among the small millets was observed at reproductive and maturity stages. In the present study, the soluble protein content showed increasing trend towards reproductive stage and thereafter declined under drought condition. The quantum of decrease was higher during drought condition at reproductive stage compared to recovery at maturity stage. Maximum soluble protein content was observed in barnyard millet at both the stages compared to other millets in this study and the minimum was measured in little millet. Drought stress imposed at reproductive stage had an adverse effect on soluble protein content and the reduction was 26.60 and 25.03 per cent at reproductive stage and maturity stage, respectively irrespective of small millets. Similar findings were also reported in foxtail millet (Dai *et al*., 2012). In this context the stress assumes importance, as it regulates the carboxylation and ultimately photosynthesis. Kramer (1983) reported that synthesis of protein is impaired in plants under water stress and in extreme stress conditions, protein degradation took place. The decrease in soluble protein under drought stress may be either due to increased proteolysis or decreased synthesis or both (Garg *et al.,* 1981). From the study, it was inferred that among the small millets, barnyard millet registered the highest soluble protein content of 9.35 and 7.81 while little millet recorded the lowest value (8.59, 7.00) under drought at reproductive and maturity stages, respectively. The reduction in soluble protein content due to drought over the respective control was 27.05, 25.82, 26.78 per cent for finger millet, little millet and barnyard millet, respectively at reproductive stage and 24.43, 26.62, 23.95 per cent at maturity stage (Table 5). Garg *et al*. (1998) reported that water deficit stress induced decline in plant water potential and leaf relative turgidity and this led to decline in total chlorophyll and soluble protein to various extent depending upon the genotype and stage of growth, at which drought was experienced. A positive correlation was found between seed yield and soluble protein content. This was in support of the present findings that barnyard millet registered higher soluble protein content and higher yield per plant.

Table 5. Effect of reproductive stage drought on NRase activity (µg of NO_2 $g^{-1}h^{-1}$) of small millets

Crop	Vegetative stage	Reproductive stage			Maturity stage		
		Control	Stress	Mean	Control	Stress	Mean
Finger millet	51.19	64.92	42.57	53.75	39.84	33.24	36.54
Little millet	47.98	62.35	44.71	53.53	37.23	30.21	33.72
Barnyard millet	60.27	69.69	49.42	59.56	41.15	29.85	35.50
Mean	53.15	65.65	45.57	55.61	39.41	31.10	35.25
	G	G	T	G x T	G	T	G x T
SEd	0.37	0.39	0.32	0.56	0.25	0.20	0.35
CD (0.05)	0.76	0.81	0.66	1.14	0.51	0.41	0.72

Nitrate reductase activity

According to experimental results nitrogen reductase (NR) activity was found decreasing due to the effect of drought at reproductive stage and prolonged even after recovery of drought in small millets (Table 5). Though there was a significant reduction in NR activity, barnyard millet recorded higher NR activity (49.42, 29.85) compared to finger millet and little millet during reproductive and maturity stages. Yadav *et al*. (1997) reported that the rapid decline in NR activity was due to decrease in nitrate content, which was caused by reduced nutrient uptake under stress condition in chickpea. At maturity, the reduction percentage was declined in all the small millets. In situations of water deprivation, maximal foliar extractable NR activity has been found to decrease in some cases (Larsson *et al*., 1989). Sivaramakrishnan *et al*. (1988) reported that, NR activity was sensitive to water deficit and found to decline rapidly with a slight change in leaf water potential or relative water content.

Considering the above results of this experiment, it is concluded that, small millets, being adapted to harsh environments, have inherent ability to withstand drought situation at any stage of their phenology, there is a significant variation in tolerance level for water stress at reproductive stage among the small millets, which was evident in this study. Barnyard millet performed better in terms of physiological parameters like leaf area, LAI, NAR, LAD, Soluble protein content and NR activity, which ultimately contributed for better yield compared to finger millet and little millet. Hence, the traits which are conferring better yield in barnyard millet may be studied further to unravel the actual mechanisms responsible for drought tolerance and to exploit these traits for genetic improvement in other millets and cereals.

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