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Influence of drought at anthesis on physiological traits of barnyard millet (*Echonochloa frumentacaea* L.) genotypes

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Abstract

Barnyard millet (*Echonochloa frumentacaea*) is one of the minor millet and used for multi-purpose which is cultivated for food and fodder. It is a good source of carbohydrate, proteins and minerals which is highly digestible and is an excellent source of dietary fiber with good amount of soluble and insoluble fractions. It is moderately drought resistant crop but changing climate is a major factor for causing the occurrence of water scarcity, which leads to drastic changes in the growth and final yield of the crop. Also the physiological and molecular mechanism of drought tolerance was not yet identified in barnyard millet. The present study was conducted to evaluation of 18 genotypes of barnyard millet to screen the drought tolerance under the pot culture experiment. Drought stress was imposed by withholding of water for ten days during anthesis stage and various physiological traits measures such as, chlorophyll fluorescence, relative water content (RWC), chlorophyll stability index (CSI), soluble protein, photosynthetic rate and nitrate reductase (NR) activity were measured. Among the eighteen barnyard millet genotypes, ESLG– 94, ESD-83, ERP – 100, ESD – 102 and ESLG – 58 were selected as physiologically efficient plants to tolerate drought at anthesis stage. These selected genotypes be used in breeding programme to develop drought tolerant varieties.

Keywords: Barnyard millet, drought, anthesis, physiological traits

Introduction

Barnyard millet is an important minor millet crop well adapted to low and moderate rainfall areas (500-700 mm) due to its early maturity character. The grains of barnyard millet are low in phytic acid and rich in iron and calcium (Sampath *et al.*, 1986) [16]. In general small millets had a lots of health benefits. Among the small millets, barnyard millet place a most important role and have high micronutrient content, particularly calcium and iron, high dietary fiber, higher amount of essential amino acids and low glycemic index thus play an important role in the food and nutritional security of the poor (Bhag mal *et al.*, 2010). According to Resources Council Science and Technology Agency, Japan, (1982) the nutritional aspect, its grains are highly nutritious for example rich in protein, lipid, vitamins B1 and B2, and nicotinic acid compared with other cereals, such as rice and wheat grains. Barnyard millet have lots of health benefits but the awareness and health benefits of barnyard millets are very less in India compare to the developed countries thereby indicating a greater scope for exploitation of this millet under Indian condition. In our country, malnutrition is a major issue and commonly spread in Indian children’s. Barnyard millets have high production potential under optimum conditions and millets have diverse adaptation mechanisms to grow and survive under relatively marginal environments. And also present day’s climate change is one of the alarming issues which have capability to alter the whole reproductive processes of the plant system and drastically affects seventy percent yield of the crop. To increase the productivity and stabilize the production in the changing climate, development of abiotic stress tolerant genotypes is essential. Therefore, it is crucial to understand the physiological and yield response of barnyard millet genotypes to drought especially during anthesis.

Materials and methods

A pot culture experiment was conducted at the department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore. Eighteen barnyard millets genotypes *viz.*,ESLG–104, ESLG–94, ESD–83, ELB–58, ESLG – 35, ESLG – 58, SEJ–194, ELB – 91, ELB–125, ELB–32, ELB–114, ELB–110, ESD–92, ESD–63, ESD–102, ERP–100, ESD-85 and CO–2and the genotypes were collected from Center of Excellence in Millets, Athiyandal, Thiruvannamalai. Two treatments viz., control and drought potted plants were uniformly irrigated upto panicle initiation stage and the irrigation was withheld 10 days at anthesis stage in drought treatment.

Experiment was replicated thrice and adopted factorial randomized block design (FCRD) the soil moisture was measured by 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. The physiological parameters were taken during the drought stress period in both control and treatment.

The photosynthetic rate was measured using portable photosynthesis system (PPS) (Model LI-6400 of LICOR inc., Lincoln, Nebraska, USA) and expressed as µmol CO_2 $m^{-2}s^{-1}$. Nitrate reductase (NR) activity in young leaves was estimated as per the method described by Nicholas *et al.* (1976) [13] and expressed as µg NO2 $g^{-1}h^{-1}$. Chlorophyll fluorescence was recorded by using Junior Pulse Amplitude Modulation Fluorometer (PAM wincontrol-3.16, Germany). Chlorophyll stability index (CSI) was measured by using the protocol of Koleyoras, (1958) [7] is followed to estimate CSI and expressed in per cent. The Soluble protein content was estimated from the leaf samples by Lowry *et al.* (1951) [10] method. The RWC was calculated by the formula given by Barrs and Weatherly (1962) expressed as per cent.

$$\text{RWC} = \frac{\text{Fresh weight - dry weight}}{\text{Turgid weight - dry weight}} \times 100$$

The analysis of variance (ANOVA) was executed by using SPSS 16.0 version.

Results and Discussion

Decreasing Photosynthetic rate is a common problem of plants to water deficit condition. This problem could be attributed to either stomatal closure or metabolic impairment was reported by Franca *et al.,* (2000) [3]. Similar results were also reported by Kawamitsu *et al.,* (2000). In the present study, under drought condition, genotype ESLG – 94, ESD – 83 recorded higher photosynthetic rate of (28.35 and 27.49µmol CO_2 $m^{-2}s^{-1}$) and in the case of percent reduction over control was higher in SEJ – 94 (34.21%) compare to other genotypes (Table 1). Boyer, (1970) was revealed that the diffusive resistance of stomata to CO_2 entry is the main factor limiting photosynthesis under drought stress condition. The present investigations also support the earlier findings.

NR enzyme activity is coordinated with the rate of photosynthesis and the availability of carbon skeletons by both transcriptional and post translational controls as reported by Huber *et al.,* (1996) [5]. In the present study, the genotypes ESD-83 (146.84µg NO2 $g^{-1}h^{-1}$) and ESLG -94 (139.37µg NO2 $g^{-1}h^{-1}$) recorded maximum NR activity (Table 1) which indicated that imposed stress did not have a major detrimental effect on NR activity of the tolerant genotypes and thus, helps to maintain photosynthetic efficiency. As reported by Sivaramakrishnan *et al.* (1988) [18] NR activity was sensitive to water deficit and found to decline rapidly with a slight change in leaf water potential.

Fluorescence yield will be high when PS II reaction centre is least damaged by photo inhibition which is caused by abiotic stress. According to Gitelson *et al.* (1998) [4] Fv/Fm ratio indicated the photosynthetic efficiency of photo system II. The high Fv/Fm ratio is positively proportional to quantum yield and showing high degree of photosynthesis. Chlorophyll fluorescence values showed significant difference between treatments and under the drought imposed plants value get decreased when compared to control. Among the genotypes ESLG – 94 (0.74) and ESD – 83 (0.72) recorded maximum values of Fv/Fm ratio and the genotypes ELB – 58 and ELB – 125 recorded the least values (0.586, 0.593) but per cent decrease over control was high in genotype SEJ – 194 (20.730) under drought at anthesis stage (Table 2).

Chlorophyll stability index is a single factor was used to measure the temperature and drought tolerance of a crop. As reported by Todorov *et al.* (2003) [19] drought and high temperature enhanced chlorophyllase activity and decreased photosynthetic pigments concentrations. In the present study, CSI was showed decreasing trend line under drought stress at anthesis stage. The genotype ESL-94 recorded maximum CSI per cent (85.58%) followed by ESD – 83 (80.88%) and the genotype SEJ – 94 showed minimum CSI per cent (62.18%) compared to other genotypes under stress condition. High per cent of CSI were helped the plants to withstand stress through better availability of chlorophyll. Therefore, Madhan Mohan *et al.,* (2000) [12] suggested that higher CSI per cent under stress leads to increased photosynthetic rate, more dry matter production and maximum productivity of the crop. The positive correlation between chlorophyll stability index and photosynthetic rate was observed under drought condition in the present study also.

Soluble protein, being a measure of Rubisco activity and which is considered as an good indicator for photosynthetic efficiency. Rubisco enzyme forms nearly 40 percent of soluble protein in leaves of crop plants. Under drought stress condition the RuBp carboxylase activity was affected, which leads to reduce the soluble protein content. In current study indicated that, water stress at anthesis stage causes the maximum of soluble protein content reduction (Table 3) in SEJ – 94 (36.191%) and percent reduction noted in ESLG - 94 (14.170%). Kramer, (1983) [8] indicated that, drought causes impaired protein synthesis, protein degradation also enhanced under prolonged drought situation.

Sinclair and Ludlow (1986) [17] proposed that RWC was better measure for plant's water status than thermodynamic state variable (water potential, turgor potential and solute potential). In the present study, drought stress at anthesis stage had a significant inhibitory effect on RWC in all the genotypes compared to control SEJ - 194 genotype recorded 24.32 per cent reduction in RWC (64.35 %) and ESLG - 94 recorded minimum percent reduction (14.43%) compared to other genotypes during drought. Similar to the above findings Vinothini *et al.* (2016) [20] reported that reduction in RWC values in loss of turgidity which leads to stomatal closure and in turn to reduce photosynthetic rates in foxtail millet. As observed by Liu *et al.* (2002) [9] decrease in RWC in plants under drought stress may depend on plant vigour reduction and have been observed in many plants.

Table 1: Effect of drought stress on Photosynthetic rate (µmol CO_2 $m^{-2}s^{-1}$) and NR activity (µg NO2 $g^{-1}h^{-1}$) of barnyard millet genotypes

Genotypes	**Photosynthetic rate (µmol CO_2 $m^{-2}s^{-1}$)**			**NR activity (µg NO2 $g^{-1}h^{-1}$)**		
	Control	**Drought stress**	**% decrease over control**	**Control**	**Drought stress**	**% decrease over control**
ESLG - 104	29.09	22.35	23.16	133.14	107.30	19.41
ESLG - 94	35.09	28.35	19.20	165.21	144.37	12.62
ESD - 83	34.23	27.49	19.68	163.68	141.84	13.34
ELB - 58	28.12	20.34	27.66	150.10	119.26	20.55
SEJ - 194	25.53	16.80	34.21	134.66	88.82	34.04
ESLG -58	27.06	20.32	24.90	109.80	78.96	28.09
ELB - 32	24.92	18.13	27.24	103.14	72.30	29.90
ELB - 114	24.89	18.16	27.04	100.03	69.19	30.83
ELB - 110	28.96	21.23	26.71	139.90	109.06	22.05
ESLG - 35	29.19	21.25	27.19	102.60	66.76	34.93
ESD - 63	35.90	29.16	18.77	128.31	95.47	25.60
ESD - 102	30.46	23.72	22.12	148.92	118.08	20.71
ESD - 92	25.57	18.83	26.35	103.01	72.17	29.94
ERP - 100	27.30	20.56	24.67	143.82	115.98	19.36
ESD - 85	25.48	19.74	22.52	109.08	78.24	28.27
ELB - 91	26.98	20.24	24.98	99.67	64.83	34.96
ELB - 125	28.63	21.64	24.43	97.08	66.24	31.77
CO 2	25.96	18.23	29.79	80.57	51.73	35.80
Mean	28.52	21.48	25.03	122.93	92.25	26.23
	G	T	G X T	G	T	G X T
SEd	0.48	0.16	0.68	1.97	0.66	2.78
CD (0.05)	0.96	0.32	1.36	3.92	1.31	5.54

Table 2: Effect of drought stress on Chlorophyll fluorescence and CSI (%) of barnyard millet genotypes

Genotypes	**Chlorophyll fluorescence**			**CSI (%)**		
	Control	**Drought stress**	**% decrease over control**	**Control**	**Drought stress**	**% decrease over control**
ESLG - 104	0.739	0.6	18.809	78.96	64.31	18.55
ESLG - 94	0.849	0.74	12.839	95.23	85.58	10.13
ESD - 83	0.83	0.721	13.133	90.93	80.88	11.05
ELB - 58	0.725	0.586	19.172	79.91	65.26	18.33
SEJ - 194	0.767	0.608	20.730	87.83	62.18	29.20
ESLG -58	0.786	0.647	17.684	78.92	65.27	17.29
ELB - 32	0.732	0.593	18.989	69.03	57.38	16.88
ELB - 114	0.741	0.602	18.758	68.29	50.64	25.84
ELB - 110	0.734	0.625	14.850	69.93	55.28	20.95
ESLG - 35	0.788	0.649	17.640	78.09	63.44	18.76
ESD - 63	0.736	0.627	14.810	86.81	72.16	16.87
ESD - 102	0.781	0.642	17.798	87.73	73.08	16.70
ESD - 92	0.742	0.633	14.690	85.22	70.57	17.19
ERP - 100	0.791	0.652	17.573	78.01	63.36	18.78
ESD - 85	0.759	0.62	18.314	81.34	66.69	18.01
ELB - 91	0.741	0.602	18.758	79.32	61.67	22.25
ELB - 125	0.732	0.593	18.989	77.35	66.70	13.77
CO 2	0.745	0.606	18.658	73.29	57.64	21.35
Mean	0.762	0.630	17.344	80.34	65.67	18.44
	G	T	G X T	G	T	G X T
SEd	13.64	4.55	19.29	1.40	0.47	1.98
CD (0.05)	27.19	9.06	38.45	2.79	0.93	3.95

Table 3: Effect of drought stress on soluble protein ($mg^{-1}g$) and RWC (%) of barnyard millet genotypes

Genotypes	**Soluble protein**			**RWC (%)**		
	Control	**Drought stress**	**% decrease over control**	**Control**	**Drought stress**	**% decrease over control**
ESLG - 104	20.91	16.24	22.334	94.73	81.06	14.43
ESLG - 94	27.9	24.23	13.154	92.73	78.06	15.82
ESD - 83	26.69	22.83	14.462	70.93	55.26	22.10
ELB - 58	19.01	14.34	24.566	85.02	64.35	24.32
SEJ - 194	20.43	13.76	32.648	78.96	63.29	19.85
ESLG -58	23.32	18.65	20.026	67.06	51.39	23.37
ELB - 32	21.8	17.13	21.422	69.12	53.45	22.68
ELB - 114	20.99	16.32	22.249	70.15	54.48	22.34
ELB - 110	20.54	15.87	22.736	82.09	66.42	19.09
ESLG - 35	23.77	19.1	19.647	85.01	69.34	18.44
ESD - 63	24.93	20.26	18.732	87.04	71.37	18.01
ESD - 102	22.74	18.07	20.536	80.01	64.34	19.59

ESD - 92	20.97	16.3	22.270	79.02	63.35	19.84
ERP - 100	25.83	21.16	18.080	74.93	59.26	20.92
ESD - 85	23.6	18.93	19.788	72.98	55.31	24.22
ELB - 91	20.02	15.35	23.327	77.65	60.98	21.47
ELB - 125	23.92	19.25	19.523	71.90	56.23	21.80
CO 2	21.56	16.89	21.660	78.88	62.93	20.43
Mean	22.718	18.038	20.953	80.53	64.86	19.46
	G	T	G X T	G	T	G X T
SEd	10.50	3.50	14.85	22.79	7.60	32.22
CD (0.05)	20.93	6.98	29.60	45.42	15.14	64.24

Conclusion

Considering the above results, present study was concluded that tolerant barnyard millet genotypes being adapted to changing environments and have inheritability to withstand water scarcity situation at reproductive stage which was very sensitive period for barnyard millets. The genotypes ESLG – 94 and ESD – 83 followed by ESLG – 58, ESD – 102 and ERP – 100 were identified as physiologically efficient plants to maintain yield under drought situation and these genotypes are highly tolerant to drought. These may be studied further to unravel the metabolomics and molecular actual mechanisms responsible for drought tolerance.

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