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## Screening for drought and heat tolerance in foxtail millet by physiological and biochemical indices

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### Abstract

A pot culture study was conducted to screen seventeen foxtail millet genotypes for drought coupled with heat stress tolerance at anthesis stage under open top chamber. The results revealed that the genotypes TNAU-159 was found to be the tolerant followed by GS – 77 and GS/15/1 based on the physiological and biochemical traits such as photosynthetic rate, chlorophyll fluorescence, relative water content (RWC), chlorophyll stability index (CSI), Nitrate reductase (NR) activity. The combined stress at anthesis stage brought about more severe yield reduction compared to single stress either by drought or heat at anthesis stage. The combined stress at anthesis stage caused reduction in photosynthetic rate, chlorophyll fluorescence (Fv/Fm values), RWC, CSI and NR activity which reduces grain yield. Among the genotypes, TNAU-159 showed its supremacy in recording high drought and heat tolerance with more photosynthetic rate ( $16.79 \mu\text{mol CO}_2\text{m}^{-2}\text{s}^{-1}$ ), chlorophyll fluorescence Fv/Fm (0.751), RWC (38.07%), CSI (53.95%) and NR activity ( $55.54 \mu\text{g NO}_2 \text{g}^{-1}\text{hr}^{-1}$ ) and 29.41 percent of yield reduction while susceptible genotype GS – 199 recorded lower values of photosynthetic rate ( $6.59 \mu\text{mol CO}_2\text{m}^{-2}\text{s}^{-1}$ ), chlorophyll fluorescence Fv/Fm values (0.629), RWC (19.15), CSI (10.49) and NR activity of  $17.64 \mu\text{g NO}_2 \text{g}^{-1}\text{hr}^{-1}$  with 93.16 percent of yield reduction during anthesis stage. These physiological and biochemical traits can be used to screen foxtail millet genotypes for drought and heat stress tolerance.

**Keywords:** Chlorophyll, chlorophyll fluorescence, drought and heat stress, foxtail millet

### Introduction

Small millets are considered as nutri cereals and are a source of food, feed and fodder. The total area of 23 million ha under millets in India, small millets account for only about 3.5 million ha as reported by Padulosi *et al.* (2015) [22]. Small millets are a group of six crops comprising of finger millet, kodo millet, little millet, foxtail millet, barnyard millet and proso millet. Foxtail millet (*Setaria italica* L.) is second most widely cultivated species and the most important in East Asia. Bhag mal *et al.* (2010) [16] reported that among the small millets foxtail millet place a most important role and have high micronutrient content, particularly protein, fat, carbohydrate and ash thus play an important role in the food and nutritional security of the poor. As observed by Malleshi *et al.* (1986) [17], the protein content of foxtail millet is greater than rice and is comparable to wheat and the fat content of foxtail millet is eight and three times more than rice and wheat respectively.

The average global temperature is reported to be increasing at a rate of  $0.18^\circ\text{C}$  every decade (Hansen *et al.*, 2012) [11]. Future climates will also be affected by greater variability in temperature and increased frequency of warm days (Pittock, 2003) [23]. To adapt new crop varieties to the future climate, we need to understand how crops respond to elevated temperatures and how tolerance to heat can be improved (Halford, 2009) [10]. Water scarcity, being also a very important environmental stress, severely impairs plant growth and development, limits plant production and the performance of crop plants, more than any other environmental factor (Shao *et al.* 2009) [26].

Drought and high temperature has adverse effect on the growth and development of plants (Noohi, *et al.*, 2009) [21]. and the yield may be reduced by  $101 \text{ kg ha}^{-1} \text{ day}^{-1}$  when the temperature reaches up to  $35^\circ\text{C}$  during pollination and grain filling stage in rice. Furthermore, higher temperature with water scarcity ( $45\text{-}48^\circ\text{C}$  at flowering and grain formation stages) is the most alarming factor that determines the crop growth and ultimate yields. The impacts of environmental stress, particularly those of drought and heat have been studied, independently. Hence, overcoming the effects of high temperature and water stress on foxtail millet production is essential for food security in the future.

In plant physiology photosynthesis is an essential process to maintain plant growth and

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development, and it is well known that photosynthetic systems and enzymes in higher plants are most sensitive to drought and temperature stress. Rate of photosynthesis, stability of chlorophyll, water potential, photosynthetic enzymes is one of the essential components of plants that reduction affects the growth, development and the yield of the crops. Unfortunately, slight is known about changes of these traits such as photosynthetic rate, CSI, RWC, NR activity and chlorophyll fluorescence parameters under drought and heat stress in foxtail millet. Hence, the present study was aimed to investigate the influence of drought and heat stress during anthesis on physiological parameters, biochemical traits and yield of foxtail millet.

### Materials and methods

A pot culture experiment was conducted at the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore. Seeds of sixteen foxtail millet genotypes with one variety (Assam local 1, GS-77, GS/15/1, TNAU-159, TNAU-161, TNAU-164, GS-520, GS-470, GS-20, GS-199, GS-474/1, GS-495, GS-84/4, GS-73, GS-415, GS-108/1, CO7) obtained from Centre of Excellence in Millets, Athiyanthal, Thiruvannamalai and used for the study. Stress treatments were imposed in the open top chamber, while a similar area of control was maintained adjacent to the ambient OTC facility. The design of pot culture experiment was Factorial Completely Randomized Block Design (FCRD) was consisting control, drought and heat stress treatments with three replications. The treatments T<sub>1</sub> (Control) well watered throughout the life cycle, T<sub>2</sub> Drought and heat stress at anthesis stage in this treatment drought stress was imposed by withholding of water for 10 days and heat stress imposed by the temperature range in the OTC was maintained from 30.4 to 40.0°C during anthesis stage. The following parameters were measured after the stress imposition (10 days) in both control and stress pots.

The photosynthetic rate was measured using portable photosynthesis system (PPS) (Model LI-6400 of LICOR Inc, Lincoln, Nebraska, USA) and expressed as  $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ . Chlorophyll fluorescence measurements were recorded by using Junior Pulse Amplitude Modulation Fluorometer (PAM wincontrol-3.16, Germany). NR activity in young leaves was estimated as per the method described by Nicholas *et al.* (1976) [20], and the enzymes were expressed as  $\mu\text{g NO}_2 \text{ g}^{-1}\text{h}^{-1}$ . Chlorophyll fluorescence was measured using chlorophyll fluorescence meter (opti-sciences OS-5p). The key fluorescence parameters F<sub>o</sub> (Initial fluorescence), F<sub>m</sub> (Maximum fluorescence), F<sub>v</sub> (Variable fluorescence) and the ratio of F<sub>v</sub>/ F<sub>m</sub> were measured. The Relative Water Content (RWC) was estimated according to Barrs and Weatherley (1962) [3], and calculated by using following formula and expressed as per cent.

$$\text{Relative Water content} = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Turgid weight} - \text{dry weight}} \times 100$$

Chlorophyll Stability Index (CSI) protocol of Koleyoras (1958) [12].

$$\text{Chlorophyll Stability Index (CSI)} = \frac{\text{Total chlorophyll content (treated)}}{\text{Total chlorophyll content (control)}} \times 100$$

For the yield analysis four plants each treatment randomly selected and assessed all the parameters. The data arrived and

collected on various parameters from the pot culture experiment and statistically designed in Factorial Completely Randomized Block Design (FCRD) were analyzed by using SPSS 16.0 version.

### Results and discussion

The mean values of photosynthetic rate under combined stress showed declining trend lines compared to control. Among the genotypes, TNAU 159/1 recorded minimum reduction of 44.46 percent followed by GS 77 (46.26 %) and TNAU 161 (46.67 %) and the genotype, GS 199 showed the maximum reduction of 72.55 percent in photosynthetic rate (Table 1). Results indicated that drought and heat stresses had a significant inhibitory effect on photosynthetic rate in all the genotypes compared to control. Heat stress primarily deactivates Rubisco by inhibiting the enzyme Rubisco activase (Crafts-Brandner and Salvucci, 2000) [6]. Zhou *et al.* (2007) [29] reported that both diffusive limitation through stomatal closure and non stomatal limitation (such as oxidative damage to chloroplast) under drought or heat stress are responsible for decline in photosynthesis. Tezara *et al.* (2002) [27], also expressed their view on leaf water potential ( $\Psi_L$ ) and stomatal closure under drought or heat stress leads lowering of less CO<sub>2</sub> availability and rubisco activity which cause low photosynthetic rate. Chaves *et al.* (2003) [5], also confirms that as limitation of CO<sub>2</sub>, leads to inactivation of electron transfer reactions, an excess of reducing power is frequently generated in drought plants. The present study also corroborated with the earlier findings.

### NR activity

Generally drought induced a rapid reduction in NR activity observed in maize (Foyer *et al.*, 1998) [9]. It is an important enzyme for nitrate assimilation ultimately protein synthesis in plant cell. NR activity is highly sensitive under drought and heat stress and its play a vital role in metabolic and physiological plant status (Azcon and Ruiz Lozano *et al.*, 1996) [1]. In the present investigation, sharp decline in NR was observed in all the genotypes under drought and heat stress condition when compared to control. The reduction in enzyme activity might be either due to reduction in enzyme level or due to the inactivation of the enzyme caused by combined stress. The genotypes GS - 77 (31.29 %) and TNAU - 159/1 (47.56 %) recorded lesser per cent reduction in (Table 1) which indicating the drought and heat stress while GS - 474/1 and GS 199 recorded higher per cent reduction of 78.09 and 79.66 percent respectively.

Chlorophyll fluorescence provides direct information on functionality and the effectiveness of photosynthesis (Lichtenthaler *et al.*, 2005) [13]. The mean value of chlorophyll fluorescence decreased under drought and heat stress when compared to control (Table 2). Among the genotypes, TNAU -159/1 recorded higher fluorescence value of 0.751 and GS-199 recorded the lower ratio of (0.629) F<sub>v</sub>/ F<sub>m</sub>. Fluorescence yield will be high when PS II reaction centre is less damaged by photo inhibition. F<sub>v</sub>/F<sub>m</sub> values indicated the photosynthetic efficiency of photosystem II. In the present study indicated that, the fluorescence value of all the genotypes of foxtail millet get decreased under stress condition. Chlorophyll fluorescence parameters were strongly correlated with whole-plant mortality in response to environmental stresses and were reliable indicators of stress tolerance. (Baker and Rosenqvist, 2004; Valladares, *et al.*, 2005) [2, 28]. The present study also supports the earlier researchers view.

Plant water stress was measured in terms of leaf water potential or leaf relative water content (Deivanai *et al.*, 2010) [7]. Farooq *et al.* (2010) [7], observed that the most important and primary effects of drought stress includes reduction in leaf water status. Liu *et al.* (2002) [14], that also suggested that decrease in RWC in plants under drought and heat stress might be depend on plant vigour reduction and which was observed in many plants. Reduction in RWC results loss of turgidity which leads to stomatal closure and in turn to reduce photosynthetic rates. In the present study also, the genotypes, TNAU - 159/1 and GS-77 maintained higher relative water content (48.07 and 43.15 %) compared to control conditions (Table 2). Thus, higher rate of water flow from the soil to plant helps in better stomatal conductance and more leaf area which help to sustain better transpiration thereby improving the ear head numbers, its size (in terms of length) and final grain yield. These might be the reason to maintain high RWC in TNAU - 159/1 and GS-77 under stress.

Chlorophyll stability index (CSI) is an important parameter that reflects the ability of the affected plant to sustain photosynthesis under stress and also which is a measure of integrity of membrane (Sayed, 1999) [25]. In present study, the genotypes TNAU-159/1 (and GS-77 (showed higher CSI values (Table 3) of 53.95 and 50.92 percent respectively indicates imposed stress did not have a major detrimental effect on chlorophyll content of the tolerant genotypes and

thus, helps to maintain photosynthetic machinery. Reduced CSI in susceptible genotypes during drought stress was also observed in maize which was reported by Meenakumari *et al.*, (2004) [18]. Therefore, the high CSI helps the plants to withstand drought and heat stress through better availability of chlorophyll, which leads to increase the photosynthetic rate as reported by Mohan *et al.* (2000) [19]. These earlier studies confirmed the present investigation in maintaining CSI.

Significant reduction in yield and yield components were observed in all the genotypes of foxtail millets which was subjected to combined stresses. Heat and drought stress at anthesis stage caused a reduction in final grain yield of foxtail millet (Table 3). Among the genotypes TNAU-159 (16.9 g<sup>-1</sup>plant), GS-77 (15.56 g<sup>-1</sup>plant) and GS-15/1 (10.09 g<sup>-1</sup>plant) showed maximum grain yield under combined stress condition and in the case of GS 199 (0.98 g<sup>-1</sup>plant) and GS-474/1 (0.99 g<sup>-1</sup>plant) recorded minimum grain yield when compare to other genotypes. Mahalakshmi and Bidinger (1985) [15], Suggested that drought stress at seed filling stage reduced seed yield up to 50 percent. The measurement of yield and yield components in the current study indicated that, the decline in grain yield was mainly due to reduction of seed number per ear head and weight of grains. Seed reduction could be as result of stress effect on pollination and floret abortion and the weight of grain reduction under stress condition (Bradford, 1994) [4].

**Table 1:** Effect of drought and heat stress on Photosynthetic rate ( $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ ) and NR activity ( $\mu\text{g NO}_2 \text{ g}^{-1}\text{h}^{-1}$ )

Genotypes	Photosynthetic rate			NR activity ( $\mu\text{g NO}_2 \text{ g}^{-1}\text{h}^{-1}$ )		
	Control	Drought + Heat stress	% decrease	Control	Drought + Heat stress	% decrease
Assam local 1	28.90	15.48	46.44	96.96	49.64	48.80
GS - 77	29.01	15.59	46.26	90.00	61.84	31.29
GS/15/1	30.91	16.18	47.65	109.12	44.18	59.51
TNAU -159	30.23	16.79	44.46	105.91	55.54	47.56
TNAU -161	30.90	16.48	46.67	90.74	40.83	55.00
TNAU - 164	22.96	9.54	58.45	96.25	48.62	49.49
GS - 520	20.79	7.37	64.55	67.80	29.10	57.08
GS - 470	23.02	9.60	58.30	81.76	24.38	70.18
GS - 20	25.92	12.50	51.77	83.61	28.28	66.18
GS - 199	24.01	6.59	72.55	86.71	17.64	79.66
GS - 474/1	24.91	7.49	69.93	83.61	18.32	78.09
GS - 495	21.92	8.50	61.22	73.24	21.74	70.32
GS - 84/4	26.97	13.55	49.76	85.85	24.69	71.24
GS - 73	23.21	9.79	57.82	69.59	20.96	69.88
GS - 415	20.31	6.89	66.08	68.28	22.14	67.57
GS - 108/1	21.29	9.87	53.64	79.43	30.25	61.92
CO7	22.13	10.90	50.75	65.98	42.09	36.21
Mean	25.14	11.35	55.66	84.40	34.13	60.00
	G	T	G X T	G	T	G X T
SEd	0.38	0.13	0.54	1.25	0.43	1.77
CD (0.05)	0.76	0.26	1.07	2.50	0.86	3.54

**Table 2:** Effect of drought and heat stress on Chlorophyll fluorescence  $F_v/F_m$  and RWC (%)

Genotypes	Chlorophyll fluorescence			RWC (%)		
	Control	Drought + Heat stress	% decrease	Control	Drought + Heat stress	% decrease
Assam local 1	0.743	0.718	3.365	56.82	39.96	29.67
GS - 77	0.794	0.745	6.171	60.01	43.15	28.10
GS/15/1	0.781	0.742	4.994	55.04	40.18	27.00
TNAU -159	0.826	0.751	9.080	63.91	48.07	24.78
TNAU -161	0.779	0.723	7.189	56.26	36.42	35.26
TNAU - 164	0.766	0.707	7.702	53.21	32.35	39.20
GS - 520	0.748	0.718	4.011	46.26	26.55	42.61
GS - 470	0.765	0.678	11.373	41.02	20.16	50.85
GS - 20	0.731	0.687	6.019	51.23	30.37	40.72
GS - 199	0.774	0.629	18.734	49.01	19.15	60.93
GS - 474/1	0.773	0.631	18.370	47.13	22.27	52.75

GS - 495	0.765	0.721	5.752	43.10	23.24	46.08
GS - 84/4	0.749	0.705	5.874	48.02	27.16	43.44
GS - 73	0.775	0.679	12.387	58.05	37.19	35.93
GS - 415	0.743	0.682	8.210	45.64	24.78	45.71
GS - 108/1	0.757	0.726	4.095	52.21	31.35	39.95
CO7	0.737	0.629	14.654	49.54	20.64	58.34
Mean	0.765	0.698	8.705	51.56	30.76	41.25
	G	T	G X T	G	T	G X T
SEd	0.02	0.01	0.03	0.84	0.29	1.19
CD (0.05)	0.04	0.01	0.05	1.68	0.57	2.37

**Table 3:** Effect of drought and heat stress on chlorophyll stability index (%) and Grain yield (g/plants)

Genotypes	Chlorophyll stability index (%)			Grain yield (g/plants)		
	Control	Drought + Heat stress	% decrease	Control	Drought + Heat stress	% decrease
Assam local 1	60.40	45.95	23.92	13.13	9.01	31.38
GS - 77	64.37	50.92	20.89	23.9	15.56	34.90
GS/15/1	62.10	47.65	23.27	21.34	10.09	52.72
TNAU -159	65.09	53.95	17.11	23.94	16.9	29.41
TNAU -161	58.52	44.07	24.69	22.38	13.7	38.78
TNAU - 164	50.10	36.65	26.85	23.24	4.67	79.91
GS - 520	48.63	34.18	29.71	18.39	3.76	79.55
GS - 470	49.96	36.51	26.92	19.24	3.30	82.85
GS - 20	52.60	39.15	25.57	17.09	4.91	71.27
GS - 199	53.94	10.49	80.55	14.32	0.98	93.16
GS - 474/1	57.90	15.45	73.32	17.49	0.99	94.34
GS - 495	60.08	46.63	22.39	18.32	3.17	82.70
GS - 84/4	42.40	28.95	31.72	19.32	7.61	60.61
GS - 73	51.22	36.77	28.21	23.13	6.10	73.63
GS - 415	45.80	31.35	31.55	15.3	3.31	78.37
GS - 108/1	49.20	35.75	27.34	16.43	4.09	75.11
CO7	48.34	25.64	46.96	12.78	6.34	50.39
Mean	54.16	36.474	33.00	18.81	6.730	65.24
	G	T	G X T	G	T	G X T
SEd	0.90	0.31	1.28	0.29	0.10	0.40
CD (0.05)	1.80	0.62	2.55	0.57	0.20	0.81

## Conclusion

The present investigation concluded that reduction of chlorophyll fluorescence, photosynthetic rate, RWC, CSI and NR activity due to combined stress and this leads to decrease in final grain yield of foxtail millet genotypes. The effect was more pronounced at combined stresses during anthesis stage. The lower per cent reduction in yield of TNAU - 159/1, GS 77 and GS 15/1 might be linked to maintenance of high chlorophyll fluorescence, photosynthetic rate, RWC, CSI and NR activity under stress and classified as a drought and heat stress tolerant genotypes. These physiological and biochemical traits could be good indicators to screen foxtail millet genotypes to combined stresses. Foxtail millet genotypes TNAU-159/1, GS 77 and GS 15/1 can be used as tolerant for breeding programme to develop drought and heat stress tolerant varieties.

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