Dissecting the tolerance to combined drought and high temperature stress in foxtail millet (*Setaria italica*) using gas exchange response and plant water status

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Abstract

Foxtail millet is an early maturing crop and has high water use efficiency, suitable for arid and semi-arid regions. Climate models predicted that the average rise in global temperature will be 1.5 ^{0}C in the next two decades. There will be an altered rainfall pattern along with a high occurrence of heat waves in foxtail millet growing regions. Therefore, understanding the response of foxtail millet to combined drought and high-temperature stress is the need of the hour. Twenty-four foxtail millet genotypes were sourced from the Indian Institute of Millet Research, Hyderabad and Centre of Excellence, Athiyandhal. The experiment was carried out in rain-out shelter in the Department of Crop Physiology, Tamil Nadu Agricultural University by adapting an augmented design. All the checks alone had two replications. The plants were subjected to combined drought and high-temperature stress from peak vegetative to mid grain filling stage. Physiological and yield traits were evaluated under combined drought and high-temperature stress. The results revealed that Athiyandhal genotype ISe- 15 had better tolerance to combined drought and high-temperature stress than the other genotypes taken for the study. This genotype performed well for physiological traits such as chlorophyll index (45.76), relative water content (RWC) (69.51%) stomatal conductance (0.35 mol m^{-2}s^{-1}), and transpiration rate (3.19 mmol m^{-2}s^{-1}). Better physiological performance resulted in higher 1000 seed weight and grain yield in the tolerant genotype ISe- 15.

Keywords

drought; foxtail millet; high-temperature; plant water status; stomatal conductance; yield

Introduction

Foxtail millet is the staple food crop in arid and semi-arid regions of India. It is cultivated in Karnataka, Tamil Nadu, Andhra Pradesh, Rajasthan, Madhya Pradesh and Chhattisgarh. It is the second most cultivated millet in the world next to pearl millet. The average production of foxtail millet is 800 - 900 kg ha^{-1} (1). India has a wealth of genetic and genomic resources of foxtail millet. Climate models forecast a significant escalation in drought intensity and high-temperature stress over the next four decades, with an anticipated average increase in world temperature of 1.5 ^{0}C (2). Reduced

precipitation and altered rainfall patterns have led to increased temperatures (3). In field scenarios, drought often coincides with high-temperature stress, aggravating yield loss in crop plants. Minimizing the agricultural loss due to changing climatic conditions especially water deficit and high temperature has become a major concern for food security (4).

Foxtail millet has a short life cycle and its high-water use efficiency (WUE) makes it an excellent drought-tolerant crop (5, 6), suitable for arid and semi-arid regions (1). However, it is sensitive to drought at critical crop growth stages like inflorescence and spikelet developmental stage (7). Therefore, understanding the response of foxtail millet genotypes to drought stress at critical stages is necessary to develop tolerant lines. The RWC is a widely accepted indicator for plant water status (8). Therefore, estimating the RWC under drought stress is a valuable screening method to determine the drought tolerance ability of plants. Similarly, photosynthetic rate and transpiration rate could be used as indicators for screening foxtail millet against drought stress (9). The chlorophyll content and photosynthetic rate were found to be altered under drought stress in foxtail millet (10). The chlorophyll index was also used as an early phenotyping tool to identify drought-tolerant sorghum germplasm (11).

Combined drought and high-temperature stress were more detrimental than the individual stress as evidenced by a higher reduction of gas exchange traits such as photosynthetic rate, stomatal conductance and transpiration rate (12). Photosystem II (PS II) is highly susceptible to high-temperature stress and can be used as an indicator for high-temperature stress tolerance (13). Photosynthetic rate and maximum quantum yield of PS II [variable fluorescence (F_v)/maximum fluorescence (F_m) ratio] had a strong positive association under high-temperature stress tolerance (14). Higher leaf temperature and transpiration rate were observed in finger millet genotypes exposed to high-temperature stress. High-temperature stress reduces crop yield by affecting the basic physiological process *viz.,* photosynthesis and respiration and also by reducing the grain filling rate and duration (15). Grain yield was found to be reduced up to 20-60% in foxtail millet exposed to drought and high-temperature stress (16, 17). The synthesis and accumulation of seed reserves are highly governed by the water status of the cell. Drought stress during seed development or seed filling stage invariable affects seed size (18). Drought and high temperatures significantly reduced the grain yield by reducing the seed size and number (19).

There are reports that individual drought and high-temperature stress affect the physiological, biochemical and molecular responses such as photosynthesis, membrane integrity, stomatal conductance, gene expression and ultimately reducing the yield of the crop. Though drought and high-temperature stress often occur in combination, studies on their effect on crop growth and yield are very minimal (20). The combined drought and high-temperature stress is more deleterious than the individual stress affecting the physiological, biochemical and molecular functions which in turn affects the crop growth and

yield (21, 22). Thus, the effect of combined drought and high-temperature stress on foxtail millet has to be addressed to understand the mechanism behind the tolerance/ susceptibility. This will help in breeding programs to develop lines that are tolerant to combined drought and high-temperature stress. Hence, the study was aimed to cluster the available foxtail millet germplasm to drought and high temperature stress tolerance/ susceptibility with the following objectives i) to assess the RWC and leaf temperature in foxtail millet germplasm under combined drought and high temperature stress ii) evaluate the response of foxtail millet genotypes to gas exchange traits and PS II photochemistry under combined drought and high temperature stress iii) to classify the genotypes as tolerant/ susceptible to combined stress based on the physiological and yield traits iv) to correlate the gas exchange traits and PS II photochemistry with yield of foxtail millet germplasm under stress.

Materials and Methods

Plant materials and stress treatments

A field experiment was conducted in the rain out shelter (ROS) at the Department of Crop Physiology, Tamil Nadu Agricultural University (TNAU), Coimbatore. Twenty-four foxtail millet genotypes including four high-yielding checks (Table 1), sourced from the Indian Institute of Millet Research (IIMR), Hyderabad and Centre of Excellence (CoE) for millets, Athiyandhal, TNAU were used for studying the effects of combined drought and high-temperature stress on the growth and physiology of foxtail millet. The experiment was laid out in an augmented design where two replications were maintained only for checks. The seeds (2 - 3 seeds) were sown in line sowing with a spacing of 22.5 cm x 10 cm at a depth of 3 cm. The recommended dose of fertilizer, 44:22 kg of N: P_2O_5 ha^{-1} was applied at the time of sowing as given in the TNAU crop production guide. Plants

Table 1. List of **a**) genotypes and **b**) checks taken for the research

a

S. No	Genotypes	S. No	Genotypes
G01	SEA 12	G11	IC0403440
G02	IC0403470	G12	ISe- 23
G03	ISe- 254	G13	IC0479455
G04	Tenai 2201	G14	ISe- 57/A
G05	Tenai 2202	G15	ISe- 128/1
G06	IC0403487	G16	EC0529793
G07	ISe- 2/3	G17	ISe- 183/1
G08	IC0479711	G18	ISe- 213/1
G09	ISe- 365	G19	IC0479804
G10	ISe- 26	G20	ISe- 15

b

S. No	Checks	S. No	Checks
C 1	SIA 326	C 3	Suryanandi
C 2	Co (Te 7)	C 4	ATL 1

were maintained under normal conditions until peak vegetative stage [30 days after sowing (DAS)]. Thereafter, subjected to two treatments *viz.,* i) control (irrigated), ii) interactive drought and natural high-temperature stress for 30 days from peak vegetative stage (30 DAS) to mid grain filling stage (60 DAS). To impose drought stress irrigation was withdrawn for 30 days for stressed plants alone (Fig. 1). For high-temperature stress the sowing was taken up in such a way that these 30 days coincided with high air temperature (31- 36 ^{0}C). The air temperature and relative humidity (RH) during the stress period were monitored using MINC-ERs (Micrometeorological Instruments to measure Near Canopy in Rice, Japan) and soil moisture using a soil moisture meter and expressed in % (Fig. 2).

Fig. 1. Field view of foxtail millet genotypes exposed to combined drought and high-temperature stress in Rain Out Shelter (ROS).
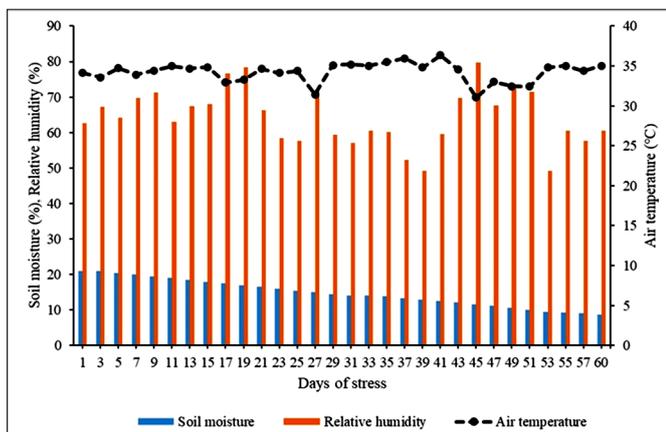
Fig. 2. Weather conditions during the stress period (from peak vegetative to mid-grain filling stage) in rain-out shelter.

Physiological traits

The RWC was measured in the penultimate leaf on 56th DAS (23). The penultimate leaf was excised and leaf bits of uniform size were collected immediately to record the fresh weight. Then the leaf was soaked in distilled water overnight at room temperature to record the turgid weight. The leaf dry weight was measured after drying the samples at 80°C in a hot air oven until there was no change in dry weight. Relative water content was arrived at according to the formula below and expressed as a percentage (%).

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

The chlorophyll index was measured in the flag leaf using self-calibrating chlorophyll meter [Soil Plant Analysis

Development (SPAD); Model 502, 134 Spectrum Technologies, Plainfield, IL, USA] on 56th DAS. The gas exchange traits such as photosynthetic rate (µmol m^{-2}s^{-1}), stomatal conductance (mol m^{-2}s^{-1}) and transpiration rate (mmol m^{-2}s^{-1}) were measured in the flag leaf using portable photosynthesis system (LI-COR 6400XT, Lincoln, NE, USA) between 9.30 - 13.30 hrs on 57th DAS. For each genotype, 3 readings were taken and the mean was computed. The maximum quantum yield of PS II (F_v/F_m ratio) was measured using a chlorophyll fluorometer (OS30p+, Optisciences, Hudson, NH, USA) in 30-minute dark-adapted leaves between 9.30 - 13.30 hrs on 57th and 58th DAS. The leaf temperature was measured in the flag leaf using hand-held infrared radiometer (Apogee, MI-220) on 58th DAS between 10.30 - 12.00 hrs and expressed as °C.

Yield and yield components

Matured plants were harvested and three plants from each genotype were collected and dried at 80 °C for seven days to record the yield components. The panicle weight was recorded and expressed as g panicle^{-1}. 1000 seed weight was estimated following a standardized protocol and expressed as gram (g) (24). The grain yield was recorded and expressed as g plant $^{-1}$. For total dry matter production (TDMP), the dry weight of the whole plant was weighed and expressed as g plant $^{-1}$.

Statistical analysis

The experiment was laid out in an augmented design, used to evaluate germplasm collection with minimum seeds. The design was adopted as it was not feasible to replicate the genotypes. Only four high-yielding checks were replicated twice. The remaining twenty genotypes were unreplicated. The genotypes and checks were evaluated for interactive effects of drought and high-temperature stress. The data was analyzed using the "augmentedRCBD" R package. Tukey's honestly significant difference test (often known as Tukey's HSD) was used to compare means at P < 0.05. The "FactoMineR" and "factoextra" packages were utilized to conduct Principal Component Analysis (PCA) to identify the major physiological characteristics determining stress tolerance in genotypes. All graphical representations were carried out using the "ggplot2" and "gridExtra" packages.

Results

Influence of combined drought and high-temperature stress on physiological traits of S. italica germplasm

Significant differences were observed for genotypes and checks under both irrigated and combined drought and high-temperature stress for RWC. In addition, genotypes Vs checks varied significantly. Among the genotypes, ISe- 15 and ISe-213/1 recorded higher RWCs of 69.51% and 68.92 % respectively and a lower RWC (45.76%) was observed in IC0403440. The check, ATL 1 recorded a higher RWC (86.35%) under interactive drought and high temperature stress. Leaf temperature differed significantly for checks Vs genotypes, and genotypes when subjected to drought and high-temperature stress. Lower leaf temperature was

recorded by ISe- 213/1 and IC0479804 (33.57 °C and 33.77 °C respectively). It was observed that 12 foxtail millet lines (8 genotypes and four checks) recorded RWC values of > 62% (61.98-86.35%) under combined stress. These genotypes also recorded comparatively lower leaf temperatures of 33.57-35.67 °C than the other 12 genotypes (Table 2).

Significant differences in photosynthetic rate were observed for checks Vs genotypes under both irrigated and combined stress treatments. The genotypes IC0479455 and EC0529793 recorded the highest photosynthetic rate of 34.65 μmol m^{-2}s^{-1} and 33.86 μmol m^{-2}s^{-1} respectively. In contrast, a lower photosynthetic rate under combined

Table 2. RWC and leaf temperature of foxtail millet genotypes exposed to combined drought and high-temperature stress.

Genotypes/ checks	RWC (%)		Leaf temperature (℃)	
	Control	Stress	Control	Stress
SEA 12	59.97 ± 1.39[ghi]	57.45 ± 2.04[efgh]	29.71 ± 0.74[d]	36.03 ± 0.59[cdefg]
IC0403470	61.79 ± 1.39[fghi]	55.74 ± 2.04[dfgh]	29.71 ± 0.74[d]	40.28 ± 0.59[b]
ISe- 254	55.69 ± 1.39[i]	53.03 ± 2.04[cdgh]	32.24 ± 0.74[abcd]	38.63 ± 0.59[abg]
Tenai 2201	61.79 ± 1.39[fghi]	58.17 ± 2.04[efgh]	31.11 ± 0.74[abcd]	40.73 ± 0.59[b]
Tenai 2202	63.54 ± 1.39[efh]	50.61 ± 2.04[cdh]	30.21 ± 0.74[cd]	39.43 ± 0.59[ab]
IC0403487	56.67 ± 1.39[gi]	53.38 ± 2.04[cdgh]	30.38 ± 0.74[bcd]	38.23 ± 0.59[abdfg]
ISe- 2/3	59.14 ± 1.39[ghi]	47.21 ± 2.04[cd]	32.21 ± 0.74[abcd]	40.53 ± 0.59[b]
IC0479711	63.72 ± 1.39[defh]	58.48 ± 2.04[befgh]	30.05 ± 0.74[d]	36.73 ± 0.59[adefg]
ISe- 365	65.40 ± 1.39[cdefh]	54.82 ± 2.04[cdfgh]	29.91 ± 0.74[d]	38.43 ± 0.59[abfg]
ISe- 26	62.40 ± 1.39[fgh]	58.57 ± 2.04[befgh]	31.31 ± 0.74[abcd]	38.53 ± 0.59[abg]
IC0403440	60.66 ± 1.39[ghi]	45.76 ± 2.04[c]	33.69 ± 0.74[abc]	36.23 ± 0.59[cdefg]
ISe- 23	69.42 ± 1.39[abcde]	61.98 ± 2.04[abdfg]	32.89 ± 0.74[abcd]	35.67 ± 0.59[cdef]
IC0479455	70.16 ± 1.39[abcd]	65.94 ± 2.04[abe]	33.89 ± 0.74[ab]	34.53 ± 0.59[ce]
ISe- 57/A	64.16 ± 1.39[defh]	63.16 ± 2.04[abef]	34.49 ± 0.74[a]	35.67 ± 0.59[cdef]
ISe- 128/1	69.04 ± 1.39[abcde]	66.24 ± 2.04[abe]	33.79 ± 0.74[ab]	34.77 ± 0.59[ce]
EC0529793	68.27 ± 1.39[bcdef]	63.72 ± 2.04[abef]	32.19 ± 0.74[abcd]	35.64 ± 0.59[cdef]
ISe- 183/1	71.58 ± 1.39[abc]	63.67 ± 2.04[abef]	33.99 ± 0.74[a]	35.57 ± 0.59[cde]
ISe- 213/1	74.87 ± 1.39[a]	68.92 ± 2.04[a]	33.79 ± 0.74[ab]	33.57 ± 0.59[c]
IC0479804	72.06 ± 1.39[ab]	67.86 ± 2.04[ab]	32.89 ± 0.74[abcd]	33.77 ± 0.59[c]
ISe- 15	75.07 ± 1.39[a]	69.51 ± 2.04[a]	33.69 ± 0.74[abc]	34.57 ± 0.59[ce]
SIA 326 (Check 1)	79.03 ± 0.92[C]	76.52 ± 1.36[B]	31.95 ± 0.5[AB]	34.60 ± 0.39[AB]
Co (Te 7) (Check 2)	81.56 ± 0.92[BC]	79.71 ± 1.36[B]	32.85 ± 0.5[AB]	33.60 ± 0.39[B]
Suryanandi (Check 3)	83.32 ± 0.92[B]	77.84 ± 1.36[B]	31.00 ± 0.5[B]	35.56 ± 0.39[A]
ATL 1 (Check 4)	89.00 ± 0.92[A]	86.35 ± 1.36[A]	33.95 ± 0.5[A]	34.80 ± 0.39[AB]
	CD	**CD**	**CD**	**CD**
Checks	4.160*	6.118*	NS	NS
Genotypes	6.578*	9.674*	NS	2.810*
Checks Vs Genotypes	5.696***	8.378***	NS	2.433**

Values are adjusted mean ± SE (standard error). Significant differences are highlighted by asterisk (*****). *P<0.05; ****** P<0.01; ******* P<0.001; **NS** - non significant, **CD** - Critical difference. Adjusted means not sharing common letters are statistically different at P<0.05. Upper-case letters are for checks and lower-case letters are for genotypes.

The chlorophyll index differed significantly for checks, genotypes and checks Vs genotypes under irrigated and stress treatments. The genotypes ISe- 15 and ISe- 213/1 recorded higher chlorophyll index of 45.76 and 45.49 respectively. Among the checks, ATL 1 recorded a higher value of 48.55 under the combined stress condition. The genotype ISe- 26 recorded a lower chlorophyll index (31.04) than the other genotypes (Fig. 3a, 3b). F_v/F_m ratio showed a significant difference for checks Vs genotypes for interactive drought and high-temperature stress. The genotype IC0479804 recorded the highest F_v/F_m ratio of 0.71 under combined stress (Fig. 4a, 4b).

drought and high temperature was observed in ISe- 23 (22.78 μmol m^{-2}s^{-1}). Checks were statistically non-significant for photosynthetic rate under combined stress conditions (Fig. 5a, 5b). A significant difference was observed for checks Vs genotypes under interactive drought and high-temperature stress for stomatal conductance. The genotypes differed significantly for stomatal conductance under stress. Among the genotypes, ISe- 15 and ISe- 213/1 recorded the highest stomatal conductance of 0.35 mol m^{-2}s^{-1} and 0.33 mol m^{-2}s^{-1} respectively. The least stomatal conductance was recorded by ISe 254 and Tenai 2201 (0.1 mol m^{-2}s^{-1}). Checks had a similar trend for stomatal conductance as photosynthetic rate. They were
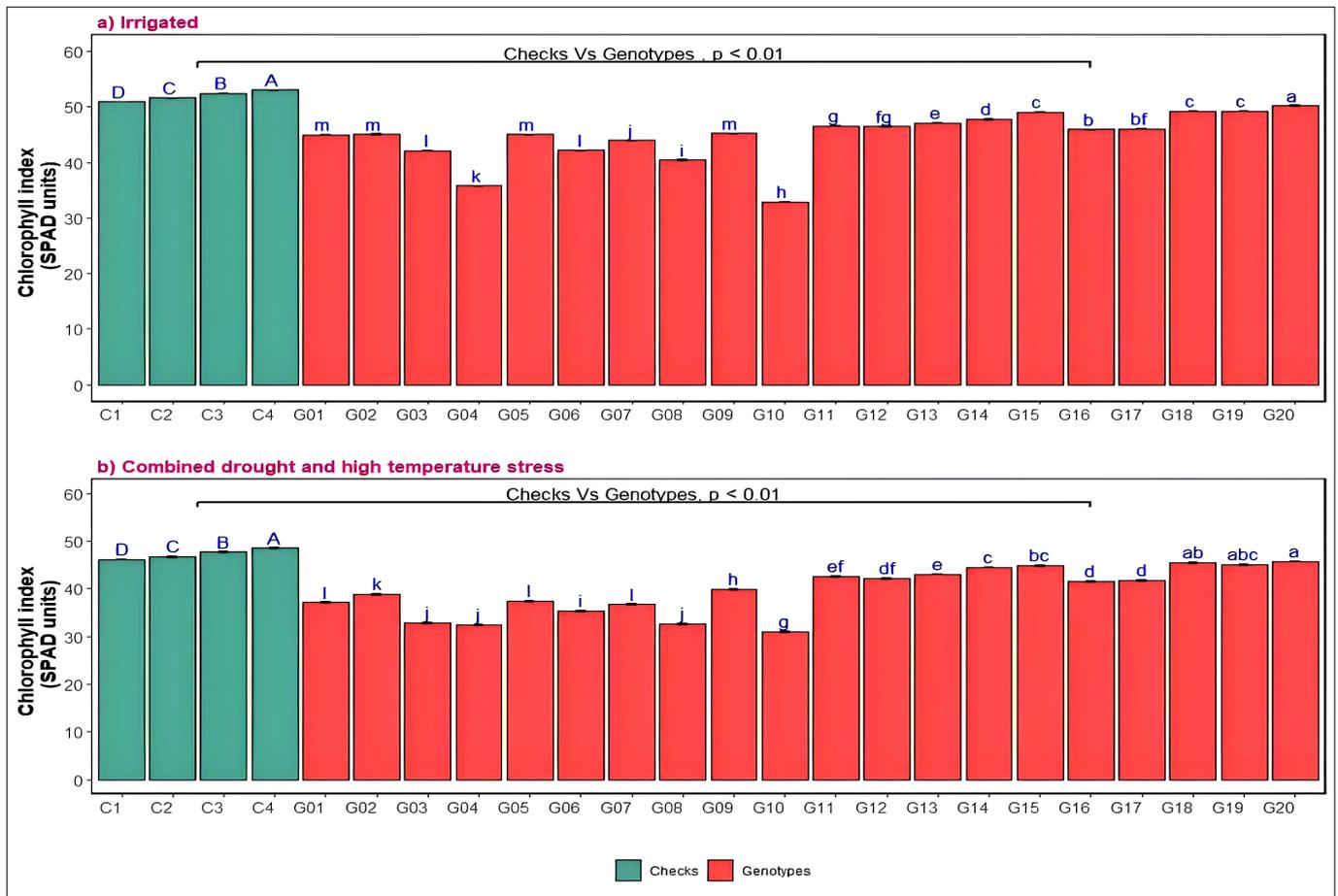
Fig. 3. Chlorophyll index (SPAD units) of foxtail millet genotypes under **a**) Irrigated **b**) combined drought and high-temperature stress treatments recorded during the 4th week of stress imposition. Vertical bars denote ± SE of the adjusted mean. Within a row, values not sharing common letters are statistically different at P < 0.05. Values with higher case letters are checks and values with lower case letters are genotypes. The line inside the graph indicates a significant difference in checks Vs genotypes.
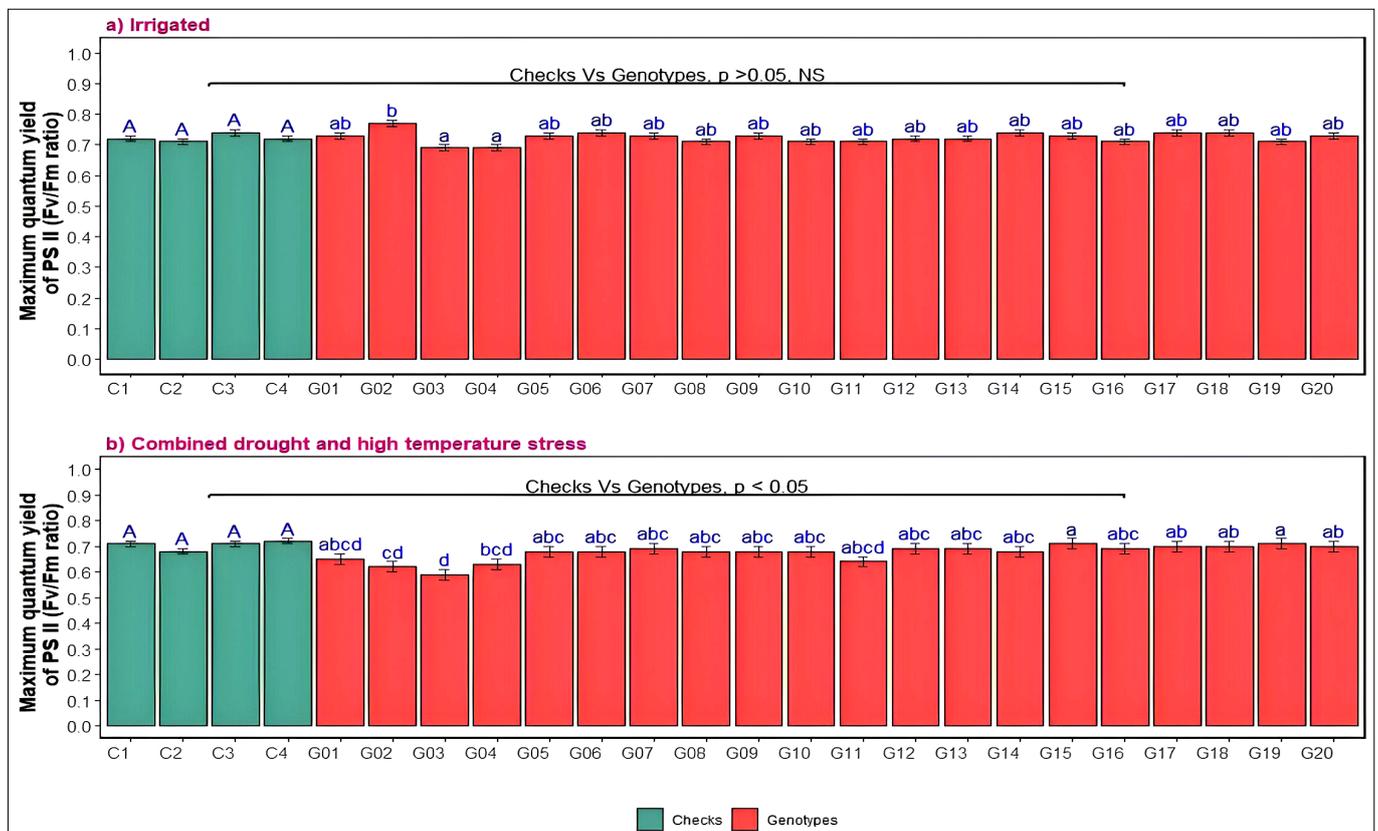
Fig. 4. The maximum quantum yield of PS II (F_v/F_m ratio) in foxtail millet genotypes recorded in **a**) Irrigated **b**) combined drought and high-temperature stress treatments recorded during the 4th week of stress imposition. Vertical bars denote ± SE of the adjusted mean. Within a row, values not sharing common letters are statistically different at P < 0.05. Values with higher case letters are checks and values with lower case letters are genotypes. The line inside the graph indicates a significant difference in checks Vs genotypes.
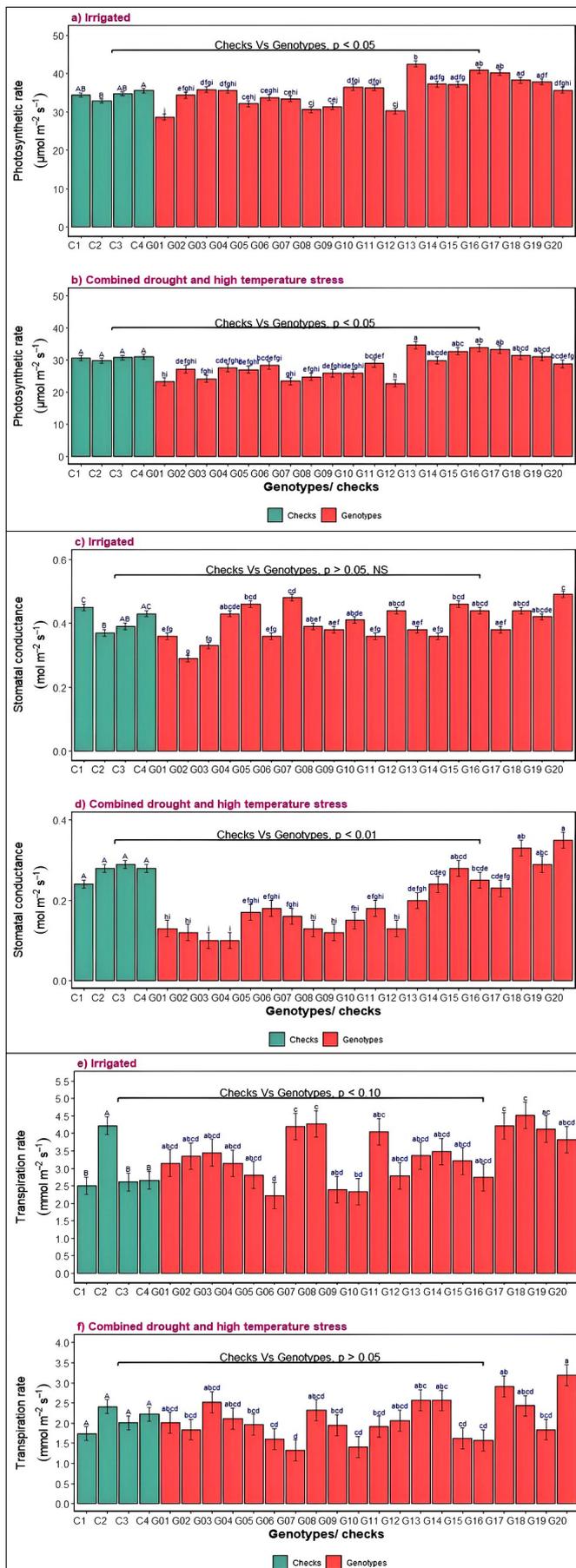
Fig. 5. Photosynthetic rate (μmol m^{-2}s^{-1}) (**a**, **b**), stomatal conductance (mol m^{-2}s^{-1}) (**c**, **d**) and transpiration rate (mmol m^{-2}s^{-1}) (**e**, **f**) in foxtail millet genotypes exposed to irrigated and combined drought and high-temperature stress recorded during the 4th week of stress imposition. Vertical bars denote ± SE of the adjusted mean. Within a row, values not sharing common letters are statistically different at P < 0.05. Values with higher case letters are checks and values with lower case letters are genotypes. The line inside the graph indicates a significant difference in checks Vs genotypes.

statistically non-significant under combined stress (Fig. 5c, 5d). The transpiration rate was statistically non-significant at 5% under both control and combined stress conditions (Fig. 5e, 5f).

Among the twenty-four genotypes studied, it was observed that the Athiyandhal genotypes ISe- 15 and ISe- 213/1 had better performance for physiological traits *viz.,* RWC, chlorophyll index, leaf temperature and gas exchange traits when exposed to combined drought and high-temperature stress. In contrast, the genotypes ISe- 254 and Tenai 2201 recorded less RWC, chlorophyll index, F_v/F_m ratio and gas exchange traits coupled with high leaf temperature indicating the susceptibility of the genotypes to combined drought and high-temperature stress.

Influence of combined drought and high temperature stress on yield and yield components of S. italica genotypes

Significant differences were recorded for panicle weight for both irrigated and combined drought and high-temperature stress. The genotypes ISe- 15, ISe- 213/1 and the check ATL 1 recorded higher panicle weights of 9.7, 9.7 and 9.3 g panicle^{-1} respectively under combined stress. Lower panicle weight was recorded in IC0403470 (5.5 g panicle^{-1}) (Table 3). Significant differences were observed in checks Vs genotypes for 1000 seed weight. The genotypes ISe- 15 and ISe- 213/1 recorded the highest 1000 seed weights of 2.97 and 2.77 g respectively when subjected to drought and high-temperature stress in combination (Table 3). Grain yield showed a significant difference (P<0.01) for checks Vs genotypes under irrigated and combined stress treatments. The genotypes ISe- 15 and IC0479455 recorded the highest grain yield of 19.59 and 19.56 g plant^{-1} respectively under combined stress. Similarly, among the checks, ATL 1 recorded the highest grain yield of 23.58 g plant^{-1} (Table 3). There was a significant difference among the genotypes (P<0.05) under combined stress for TDMP. Checks Vs genotypes varied significantly (P<0.001) under irrigated and combined drought and high-temperature stress. The genotypes EC0529793 and ISe- 57/A recorded the highest TDMP of 28.15 g plant^{-1} and 27.89 g plant^{-1} under the interactive stress treatment (Table 3).

The results concluded that the Athiyandhal genotype ISe-15, which showed better physiological efficiency, recorded comparable yield with the check varieties under stress. It was found to have heavier panicles, along with higher 1000 seed weight, grain yield and TDMP.

Principal Component Analysis (PCA)

The principal component analysis indicated that the first two principal component vectors [Principal component (PC) 1 and PC 2] accounted for 78.5% of the total variation (Fig. 6a). Among the various traits in PC 1, maximum variation was explained by grain yield (11.87 %), stomatal conductance (11.26 %) and panicle weight (11.07 %). In PC 2 the maximum variation was observed for transpiration rate (77.24 %) and F_v/F_m ratio (17.81 %). Among the foxtail millet genotypes, 8 genotypes *viz.,* ISe-57/A, ISe- 128/1, EC0529793, IC0479455, ISe- 183/1, ISe-213/1, IC0479804,

Table 3. Yield components and yield of foxtail millet genotypes exposed to combined drought and high temperature stress.

Genotypes/ checks	Panicle weight (g panicle⁻¹)		1000 seed weight (g)		Grain yield (g plant⁻¹)		TDMP (g plant⁻¹)	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
SEA 12	06.8 ± 0.17^i	6.6 ± 0.21^{fghi}	2.45 ± 0.19^{cde}	2.22 ± 0.22^{abc}	19.15 ± 0.8^d	10.44 ± 0.27^f	21.09 ± 0.43^{ghi}	16.02 ± 1.16^{cde}
IC0403470	06.0 ± 0.17^{gh}	5.5 ± 0.21^e	2.15 ± 0.19^e	1.63 ± 0.22^c	18.61 ± 0.8^d	10.4 ± 0.27^f	19.88 ± 0.43^{fi}	16.24 ± 1.16^{cde}
ISe- 254	07.0 ± 0.17^i	6.3 ± 0.21^{eghi}	2.45 ± 0.19^{cde}	1.92 ± 0.22^{abc}	19.91 ± 0.8^{cd}	10.54 ± 0.27^f	16.53 ± 0.43^e	14.21 ± 1.16^e
Tenai 2201	05.8 ± 0.17^h	5.6 ± 0.21^{ei}	2.25 ± 0.19^{de}	1.82 ± 0.23^{bc}	19.34 ± 0.8^{cd}	11.84 ± 0.27^e	20.15 ± 0.43^{fhi}	19.86 ± 1.16^{bcd}
Tenai 2202	06.9 ± 0.17^i	6.7 ± 0.21^{fgh}	2.55 ± 0.19^{bcde}	2.22 ± 0.22^{abc}	21.13 ± 0.8^{bcd}	12.44 ± 0.27^{de}	21.64 ± 0.43^{ghi}	16.56 ± 1.16^{cde}
IC0403487	07.5 ± 0.17^{fi}	7.3 ± 0.21^{fh}	2.85 ± 0.19^{abcde}	2.42 ± 0.22^{abc}	20.10 ± 0.8^{cd}	13.53 ± 0.27^{cd}	23.08 ± 0.43^g	15.05 ± 1.16^{de}
ISe- 2/3	07.9 ± 0.17^f	7.3 ± 0.21^{fh}	2.55 ± 0.19^{bcde}	2.32 ± 0.22^{abc}	20.28 ± 0.8^{cd}	14.9 ± 0.27^b	22.10 ± 0.43^{gh}	16.91 ± 1.16^{cde}
IC0479711	06.9 ± 0.17^i	6.6 ± 0.21^{fghi}	2.55 ± 0.19^{bcde}	2.22 ± 0.22^{abc}	18.10 ± 0.8^d	11.6 ± 0.27^{ef}	18.55 ± 0.43^{ef}	15.08 ± 1.16^{de}
ISe- 365	08.0 ± 0.17^f	7.6 ± 0.21^{dh}	3.05 ± 0.19^{abcde}	2.72 ± 0.22^{ab}	20.10 ± 0.8^{cd}	14.4 ± 0.27^{bc}	20.37 ± 0.43^{fhi}	16.92 ± 1.16^{cde}
ISe- 26	07.0 ± 0.17^i	6.5 ± 0.21^{eghi}	2.55 ± 0.19^{bcde}	2.13 ± 0.22^{abc}	18.32 ± 0.8^d	10.44 ± 0.27^f	22.72 ± 0.43^g	15.39 ± 1.16^{cde}
IC0403440	06.7 ± 0.17^{gi}	6.0 ± 0.21^{egi}	3.05 ± 0.19^{abcde}	2.57 ± 0.22^{abc}	22.96 ± 0.8^{abe}	18.36 ± 0.27^a	30.33 ± 0.43^{cd}	24.26 ± 1.16^{ab}
ISe- 23	09.2 ± 0.17^{de}	8.4 ± 0.21^{cd}	2.25 ± 0.19^{de}	1.78 ± 0.22^{bc}	20.75 ± 0.8^{bcd}	11.44 ± 0.27^{ef}	33.27 ± 0.43^b	20.2 ± 1.16^{bcd}
IC0479455	09.0 ± 0.17^e	8.5 ± 0.21^{bcd}	2.85 ± 0.19^{abcde}	2.47 ± 0.22^{abc}	26.05 ± 0.8^a	19.56 ± 0.27^a	28.54 ± 0.43^{ab}	20.6 ± 1.16^{bc}
ISe- 57/A	09.5 ± 0.17^{cde}	8.8 ± 0.21^{abc}	3.15 ± 0.19^{abcd}	2.37 ± 0.22^{abc}	25.18 ± 0.8^a	19.26 ± 0.27^a	30.28 ± 0.43^{cd}	27.89 ± 1.16^a
ISe- 128/1	09.8 ± 0.17^{bcd}	9.4 ± 0.21^{abc}	3.25 ± 0.19^{abc}	2.47 ± 0.22^{abc}	24.51 ± 0.8^{ab}	18.31 ± 0.27^a	30.59 ± 0.43^c	20.77 ± 1.16^{bc}
EC0529793	09.8 ± 0.17^{bcd}	9.3 ± 0.21^{abc}	3.55 ± 0.19^a	2.57 ± 0.22^{abc}	25.93 ± 0.8^a	18.67 ± 0.27^a	30.73 ± 0.43^c	28.15 ± 1.16^a
ISe- 183/1	09.8 ± 0.17^{bcd}	9.5 ± 0.21^{ab}	3.65 ± 0.19^a	2.57 ± 0.22^{abc}	24.16 ± 0.8^{ab}	19.17 ± 0.27^a	30.23 ± 0.43^{cd}	23.64 ± 1.16^{ab}
ISe- 213/1	10.4 ± 0.17^{ab}	9.7 ± 0.21^a	3.15 ± 0.19^{abcd}	2.77 ± 0.23^{ab}	25.81 ± 0.8^a	19.37 ± 0.27^a	29.48 ± 0.43^{acd}	26.6 ± 1.16^a
IC0479804	10.2 ± 0.17^{abc}	9.5 ± 0.21^{ab}	3.15 ± 0.19^{abcd}	2.47 ± 0.22^{abc}	25.51 ± 0.8^a	19.36 ± 0.27^a	28.1 ± 0.43^a	20.9 ± 1.16^{bc}
ISe- 15	10.6 ± 0.17^a	9.7 ± 0.21^a	3.45 ± 0.19^{ab}	2.97 ± 0.22^a	25.96 ± 0.8^a	19.59 ± 0.27^a	29.72 ± 0.43^{acd}	4.23 ± 1.16^{ab}
SIA 326 (Check 1)	09.5 ± 0.11^A	8.9 ± 0.14^A	3.60 ± 0.13^{AB}	2.80 ± 0.15^A	25.64 ± 0.53^A	22.34 ± 0.18^B	31.75 ± 0.29^B	28.95 ± 0.77^A
Co (Te 7) (Check 2)	09.6 ± 0.11^A	8.9 ± 0.14^A	3.20 ± 0.13^B	2.70 ± 0.15^A	25.72 ± 0.53^A	22.46 ± 0.18^B	32.65 ± 0.29^B	27.02 ± 0.77^A
Suryanandi (Check 3)	09.7 ± 0.11^A	9.1 ± 0.14^A	3.80 ± 0.13^A	3.00 ± 0.15^A	24.83 ± 0.53^A	22.76 ± 0.18^B	32.52 ± 0.29^B	28.13 ± 0.77^A
ATL 1 (Check 4)	09.8 ± 0.11^A	9.3 ± 0.14^A	3.70 ± 0.13^{AB}	3.20 ± 0.15^A	26.27 ± 0.53^A	23.58 ± 0.18^A	34.49 ± 0.29^A	29.71 ± 0.77^A
	CD	CD	CD	CD	CD	CD	CD	CD
Checks	NS	NS	NS	NS	NS	0.812*	1.285*	NS
Genotypes	0.789**	1.006**	NS	NS	3.774*	1.284***	2.031**	5.51*
Checks Vs Geno-	0.683***	0.872***	0.796**	0.924**	3.268**	1.112***	1.759***	4.77***

Values are adjusted mean ± SE (standard error). Significant differences are highlighted by asterisk (*****). *P<0.05; ** P<0.01; *** P<0.001; **NS** - non significant, **CD** - Critical difference. Adjusted means not sharing common letters are statistically different at P<0.05. Upper-case letters are for checks and lower-case letters are for genotypes.
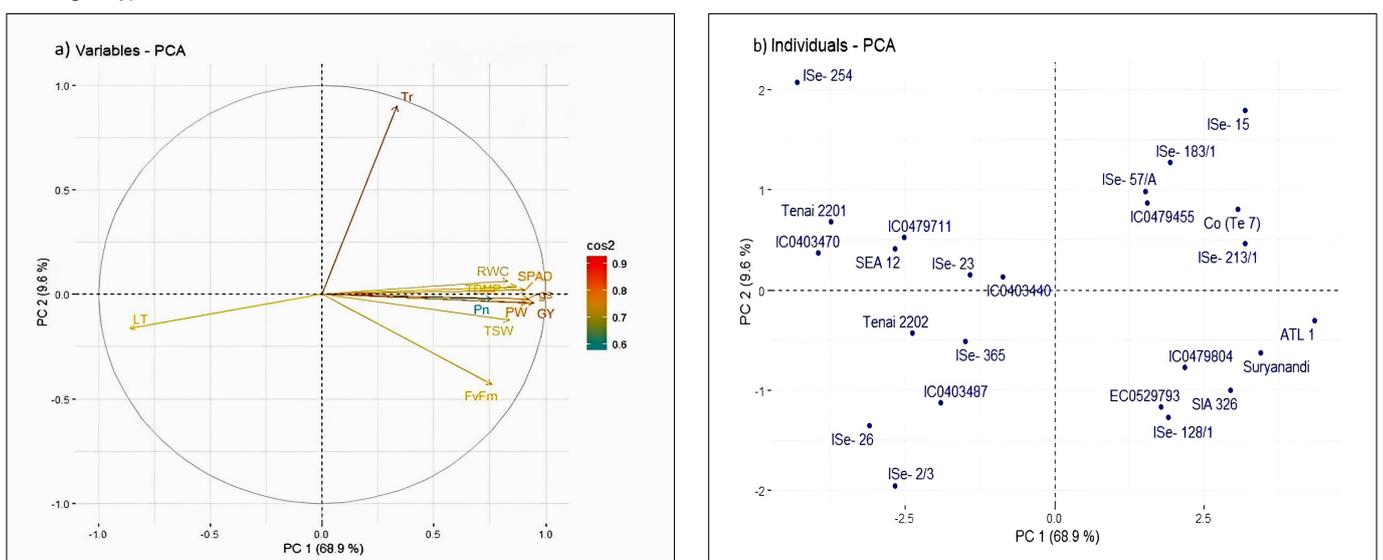
Fig. 6. First and second principal component scores (**PC 1** and **PC 2**) for identifying traits conferring combined drought and high-temperature stress tolerance in foxtail millet (**a**) the factor loading value for traits is indicated by thick lines radiating from the centre showing the direction (angle) and magnitude (length) of the trait's contribution to the principal component and (**b**) classification of 24 foxtail millet genotype based on the factor scores of PC 1 and PC 2. The principal components are shown in the axis, and the variance contributed by each principal component is indicated inside the parentheses. **RWC** - Relative water content; **SPAD** - Chlorophyll index; **LT** - Leaf temperature; **FvFm** - Maximum quantum yield of PS II; **Pn**-Photosynthetic rate; **gs**-Stomatal conductance; **Tr**- Transpiration rate; **PW** - Panicle weight; **TSW** - 1000 seed weight; **GY** - Grain Yield; **TDMP** - Total dry matter production

ISe-15 and 4 checks *viz.,* SIA 326, Co Te (7), Suryanandi and

ATL 1 were classified as tolerant genotypes [quadrant I (+, +) and quadrant II (+, -)]. The other 12 genotypes *viz.,* Sea 12, IC0403470, ISe- 254, Tenai 2201, Tenai 2202, IC0403487, ISe- 2/3, IC0479711, ISe- 365, ISe- 26, IC0403440 and ISe- 23 were classified as susceptible genotypes [quadrant III (-, +) and quadrant IV (-, -)] (Fig. 6b). As expected, grain yield had positive relationship with photosynthetic rate, F_v/F_m, RWC and 1000 seed weight. In contrast, leaf temperature had a negative relationship with transpiration rate and grain yield (Fig. 6a). The study identified that 1000 seed weight, photosynthetic rate, SPAD and RWC as key physiological traits governing combined drought and high-temperature stress tolerance in foxtail millet genotypes, as these traits are positively correlated with the tolerant genotypes.

Discussion

Foxtail millet is a versatile C_4 crop and a staple food crop in arid and semi-arid regions of the world. Previous reports on foxtail millet confirm that the crop is sensitive to drought stress at inflorescence and spikelet developmental stage (7). However, reports on combined drought and high-temperature stress in foxtail millet are lacking. Hence this study was conducted to evaluate the response of foxtail millet genotypes to combined drought and high-temperature stress at the reproductive stage.

RWC was identified as an indicator of drought stress tolerance in foxtail millet (25) and a widely accepted index to determine the water status of the plant (8). Tolerant genotypes maintained higher RWC than the susceptible genotypes under drought stress in foxtail millet (6), and finger millet (26). Reduced RWC under combined drought and high-temperature stress altered the gas exchange traits and reduced the grain yield. Similar results were reported under combined drought and high-temperature stress in maize (12) and desert grass (27).

The chlorophyll index significantly decreased in all the foxtail millet genotypes exposed to combined drought and high-temperature stress. In line with the above findings decrease in chlorophyll content was observed in foxtail millet exposed to drought at the reproductive stage (10), combined drought and high-temperature stress in maize (12). The tolerant genotypes were able to maintain a considerable amount of green pigment compared to susceptible ones. The pigment degradation in susceptible genotypes might be the reason for the lesser photosynthetic rate and other yield traits (28).

The photosynthetic rate was found to be decreased under combined drought and high-temperature stress in the present study. A similar result of reduced photosynthetic rate was observed under combined drought and high-temperature stress (12), individual drought in foxtail millet (10, 29) and high-temperature stress in pearl millet (30). Similar to the photosynthetic rate the other gas exchange traits namely transpiration rate and stomatal conductance decreased drastically under combined drought and high-temperature stress. In line with the above findings, the gas exchange traits were reduced under drought stress in sorghum genotypes (11). A severe decrease in stomatal conductance was observed under combined drought and high-temperature stress than under individual stress in maize hybrids (12). Transpiration rate was found to be reduced under drought stress in barnyard and pearl millet (31) and in foxtail millet (29). However, the eight tolerant foxtail millet genotypes maintained a moderate transpiration rate to lower the leaf temperature to mitigate the negative impacts of combined drought and high temperature. Similar to the above findings, (12) reported a higher transpiration rate in tolerant maize hybrids than in the susceptible lines. The study also found that the stomatal conductance was comparatively higher in the tolerant genotypes as against the susceptible genotypes. Concomitant with the above findings reproductive stage heat (17) and drought (29) reduced the conductance and chlorophyll in foxtail millet germplasm.

The damage to PS II photochemistry was higher in susceptible genotypes. Maximum quantum yield of PS II (F_v/F_m ratio) was reduced under water deficit and heat stress and also in combination stress in pearl millet (32). The reason may be reduced charge separation in the oxygen-evolving complex under combined drought and high-temperature stress (32) leading to lesser panicle weight and grain yield.

The yield and yield components proved the classification of foxtail millet germplasm for tolerance / susceptibility to combined drought and high-temperature stress. The susceptible genotypes had decreased grain yield due to reduced 1000 seed weight and panicle weight. In line with the above results, combined drought and high-temperature stress reduced the ear weight, 1000 seed weight and grain yield in maize (12). A 17% reduction in grain yield was recorded in the tolerant genotype (ISe-15) as against 60% in the susceptible genotype (IC0403470). The decrease in grain yield might have been due to decreased floret fertility and seed set under combined stress as in pearl millet and sorghum (30, 33). The tolerant genotypes were able to maintain better yield due to maintenance of RWC, green pigment, gas exchange traits and PS II photochemistry.

Conclusion

Among the twenty-four foxtail millet genotypes taken for the study, Athiyandhal genotypes *viz.,* ISe- 15 and ISe- 213/1 were found to be tolerant to combined drought and high-temperature stress. The tolerance of these genotypes was due to higher chlorophyll index, stomatal conductance, transpiration rate and maintenance of plant water status which ultimately resulted in higher 1000 seed weight and grain yield than the other genotypes. These tolerant genotypes for combined drought and high-temperature stress need to be further validated in the target environment (Athiyandhal). The major limitation is the occurrence of drought scenarios combined with natural high-temperature stresses in the target environment. Hence, the other way to confirm the research findings is to explore the genes and enzymes contributing to combined drought and high-temperature stress tolerance. This will

help in further breeding programs to use these genotypes as donors to develop tolerant lines for combined drought and high-temperature stress.

Authors' contributions

GS conduct of experiment, formal analysis, investigation, data visualization; writing-original draft; VD conceptualization, reviewed and edited, SGP - data visualization; SS, SR, IK, KE, SU reviewed and edited.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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