

Morpho-physiological Changes in Chilli under Drought and Heat Stress

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Authors' contributions

This work was carried out in collaboration among all authors. Authors PJ and DV designed the study. Authors VR and SS performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors RS and SV managed the analyses and literature searches of the study. All authors read and approved the final manuscript.

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ABSTRACT

Drought spells and heat stress have become quite common and agricultural production would experience a lag in near future. The combined effect of heat and drought stress is expected to cause negative impact on crop growth. Hence, an experiment was framed to assess the morphological and photosynthetic characters of chilli under combined drought and heat stress. Three different genotypes of chilli viz., K1, TNAU chilli hybrid CO 1, Ramanathapuram gundu were subjected to seven drought and temperature treatments. The experiment was designed in factorial completely randomized design (FCRD) at temperature controlled Open Top Chambers (OTC) and drought stress was gravimetrically assessed. The results showed that, morphology and photosynthetic characters were affected irrespective of genotypes. The maximum reduction in plant height and leaf area was observed when plants were grown under 40% pot capacity and temperature of + 5°C from the ambient condition. The study also revealed that, the reduction of gas exchange parameters at

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40% PC and A + 5°C with yield reduction of almost 76 per cent irrespective of genotypes. Stress treatments reduced the fruit length, fruit diameter compared to control in all genotypes. Stress Tolerance Index was calculated to study the physiological basis under combined drought and heat stress. The optimum level of stress by STI of 0.501 in 60% PC and A+ 3°C was standardized to study the basic physiological functions of chilli.

Keywords: Chilli; photosynthesis; drought; heat stress; stress tolerant index; yield.

1. INTRODUCTION

In the era of global warming, occurrence of one or more abiotic stresses together is quite common. Increasing temperature, delayed monsoon and water scarcity are threatening the agricultural production Rizhsky et al. [1]. Increasing population and changing climatic conditions setback to agriculture for food security and quality. *Capsicum annum* (L.) a solanaceous crop fetches high economic value for its pungency and colour. It is an important vegetable as well as a spice crop with high nutritional value and pigment content. Besides its importance is found susceptible to heat and drought stress. Reproductive stage of chilli is much critical to heat and drought stress resulting in yield loss [2].

Drought and heat are most common abiotic stresses affecting normal plant growth, development and production [3]. Global average temperature would experience an increase upto 4°C at the end of this century. It accounts for about 17% yield reduction per degree rise in temperature [4]. Drought and heat stress for short periods would expected to occur frequently in future climatic scenarios [5] and had direct effect on yield and quality of chilli [6]. Drought and heat stress alters the basic physiological and biochemical process including the photosynthetic metabolism, downregulating the functions of stomata, enzymatic activities etc. Adjustability of leaf area and plant height of chilli found as a sensitive adaptation for abiotic stress tolerance [7]. Interaction of water scarcity and heat stress negatively correlates with plant height and hampers overall growth, number of leaves; leaf expansion resulted in early maturity of chilli [8]. Photosynthetic process should maintain the carbon balance under stress condition to tolerate the stress effects [9] whereas reduction of photosynthesis decreases the yield [4]. Heat stress on leaves with low transpirational rate have experienced fluctuating leaf temperature and energy balance. Increase in critical temperature alters photosynthesis with reduced activity of photosystem I, low chlorophyll content, and chlorophyll a/b ratio in wheat [10]. Stomatal

closure restricts CO₂ intake by leaves, reduces photophosphorylation, lowers Rubisco activity and decrease in water potential are drought induced physiological changes in crop plants [11]. Reduced photosynthetic assimilation and transpiration rate were seen under water limited condition coupled with high temperature stress during anthesis in wheat [12]. The physiological and molecular basis in chilles are related to combined drought and heat stress not yet clearly understood. Hence, the present study was framed to study the morpho-physiological traits under combined drought and heat stress in chilli along with the yield attributes.

2. MATERIALS AND METHODS

2.1 Chilli Genotypes

Chilli genotypes (varieties/ hybrid) having varied phenology and preference among farmers were selected for the study. The study include two varieties (Kovilpatti 1 (K1) and Ramanathapuram gundu (Gundu) and a hybrid (TNAU Chilli Hybrid CO1). The fruits of the genotypes were morphologically different with elongated (K1, Hybrid CO1) and rounded (GUNDU) one. Nursery of chilli were raised with seeds sown in portrays and managed with all recommended practices. Two seedlings per pot were transplanted after 35DAS to 18kg capacity pot with 15 kg of soil. The pots were maintained under normal condition until flowering stage.

2.2 Drought and High Temperature Treatments

An experiment on combined stress was carried out during 2019-2020 in Open Top Chamber (OTC) facility located at Department of Crop Physiology, TNAU, Coimbatore. OTC was 4 m × 4 m in dimension and fabricated with polycarbonate sheet. Three OTCs with temperature control were used for the study. One chamber was maintained as control and other two were temperature elevated chambers by +3°C and +5°C compared to ambient temperature. The drought treatments were

imposed by dry down gravimetric method [13]. Different heat and drought levels were imposed based on pot capacity under two temperature elevated chambers. During the flowering stage, the pots were shifted to the respective chambers and temperature elevation of 3°C and 5°C were given for 2 weeks. The treatments include T1- Control (100% Pot Capacity (PC) + Ambient Temperature), T2- 80 % PC and A+ 3°C, T3- 80% PC and A+ 5°C, T4- 60% PC and A+ 3°C, T5- 60% PC and A+ 5°C, T6- 40% PC and A+ 3°C and T7- 40% PC and A+ 5°C. During flowering stage, the morphological, gas exchange parameters were recorded and yield data were collected from the tagged plants and flowers that are exposed to stress treatments. After treatment the plants were subjected to stress recovery by placing the pots under natural condition. Maximum temperature and relative humidity during the period of stress treatments was given in Fig. 1.

2.3 Morphological Parameters

Height of the plant was measured from the ground level to the tip of the plant. It was expressed in cm plant⁻¹. The leaves from the tagged plant were detached from the shoots. The leaves were placed over the moving belt of leaf area meter (LICOR Model 3100) where the camera captures the leaf area and the readings were digitalised. It was expressed as cm² plant⁻¹.

2.4 Leaf Gas Exchange Parameters

Gas exchange parameters viz., photosynthetic rate, transpiration rate and stomatal conductance were recorded using an advanced portable photosynthesis system (LI-6400 XT, LicorInc, Nebraska, USA). The readings were recorded on a clear sunny day when the photo synthetically active radiation was more than 1000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ also without photo-inhibition. Fully expanded leaf from the top was clamped inside the leaf chamber and held perpendicular to incident light and computed values were recorded. The instrument maintained a constant CO₂ flux to leaf chamber, which was maintained at ambient concentration. Relative humidity was maintained at a steady level equal to the ambient relative humidity to simulate a condition similar to that of ambient air. The photosynthetic rate expressed as $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, stomatal conductance expressed as $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ and transpiration rate expressed as $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$.

2.5 Yield Parameters

The yield parameters like fruit length, fruit diameter and total weight of the fruit at 1st picking were taken. The measurements and weights were recorded with five replication each with five fruits and the readings were statistically analyzed. The length and diameter of the fruit was expressed in cm and the weight was expressed in g plant⁻¹.

2.6 Stress Tolerance Index

Stress tolerance index (STI) was calculated based on the yield of chilli. $STI = (Y_p \times Y_s) / Y_p^2$ where Y_p is the yield under control condition, Y_s is the yield under stress condition [14].

2.7 Statistical Analysis

The replicated data collected for this study was analyzed using the software IBM SPSS statistics 21 by univariate analysis of variance. Experiments were arranged in a Factorial completely randomized design (FCRD), with four replications. The two factors taken for the study, one was genotype and other was stress treatments. The collected data were presented with the respective standard errors of means and the least significant difference (LSD 0.05).

3. RESULTS AND DISCUSSION

3.1 Plant Height

The treatments and genotypes showed significant variations ($P = 0.05$) on plant height, leaf area and gas exchange parameters in chilli. Plants exposed to PC 40% + 5°C (T7) recorded the lowest plant height and the genotype TNAU chilli hybrid CO1 with a height of 52.7 cm had highest decrease percentage over control. (Fig. 2A and 2B). Additive effect of drought and heat stress had negative impact on the plant height compared to its individual effects. [15]. As an adaptation strategy, the plant reduces its growth and decreases its normal metabolic reactions [16]. In line with above findings, Gunawardena and De Silva [8], reported that, drought and heat stress reduced the plant height, number of leaves and leaf expansion in capsicum. Heat stress of 5°C with 40% PC had higher reduction over control. Under stress condition in order to cope up with the adverse effects, the plants limits the growth character by limiting the assimilation [9]. Variety K1 was found to be have drought tolerant nature with minimal reduction in plant height of 27.49% at 40% PC and A+ 5°C stress condition.

3.2. Leaf Area

The drought combined heat stress significantly reduced the leaf area of chilli and showed significant difference ($P = 0.05$) between stress treatments and genotypes. Under control (100% PC + ambient air temperature) condition TNAU chilli hybrid CO1 had higher leaf area followed by K1 (Fig. 3A and 3B). Stress condition the leaf area showed decreasing trend compared to control. Under stress conditions at 2WAS the TNAU chilli hybrid CO1 had higher leaf area reduction (23.89, 25.54%) followed by gundu variety (19.21, 27.11%) under 40% PC +3°C and

40% PC +5°C respectively. The drought combined with high temperature of +5°C had higher impact on than +3°C. Overall, K1 variety had higher leaf area to withstand heat and drought stress conditions. Heat stress reduced the leaf area expansion, leaf area and leaf morphogenesis with reduced sink strength [17]. Capsicum with its wide transpiring leaf surface and elevated stomatal opening became susceptible to the drought and heat stress [6]. Results conclude leaf area reduction in chilli had tolerance to drought and heat stress [7].

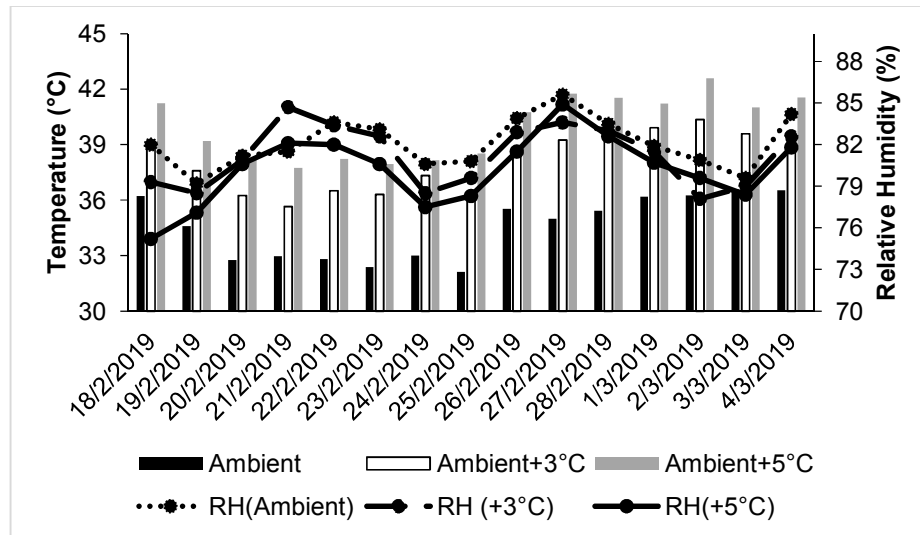
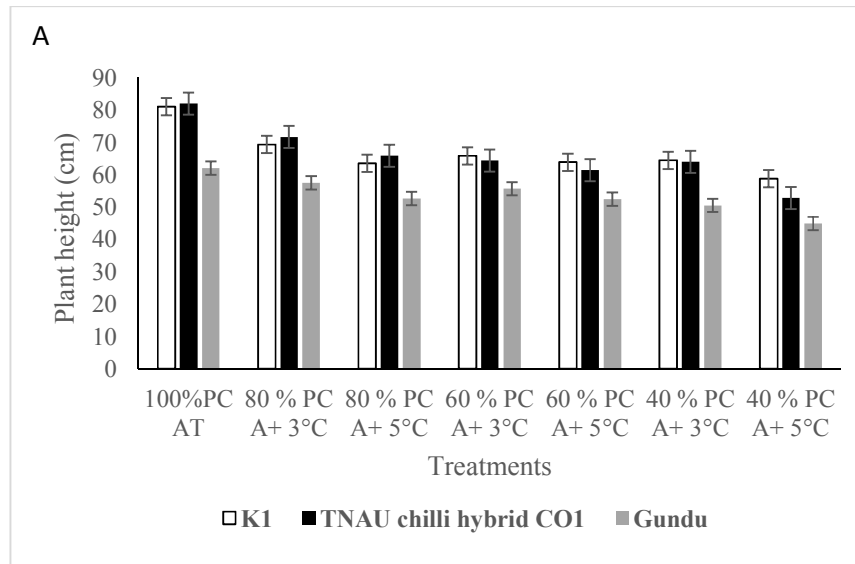


Fig. 1. Temperature and relative humidity recorded during the experimental period



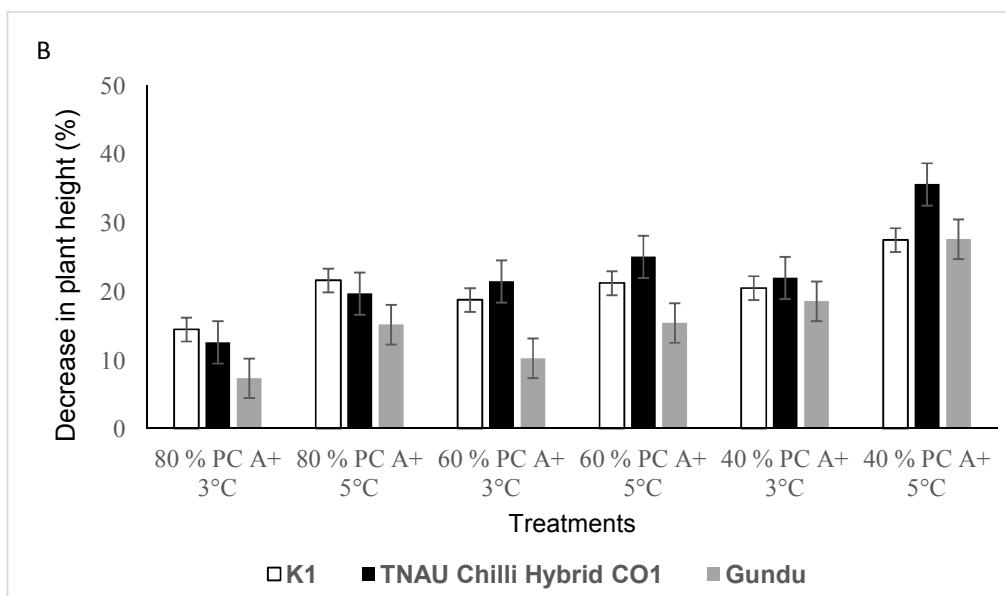


Fig. 2. Effect of combined drought and heat stress on plant height at A) Two week after stress B) Percent reduction over control. (Factorial completely randomized design, significance at $P < 0.05$)

3.3 Leaf Gas Exchange Parameters

The data on gas exchange parameters (Table 1) showed significant difference between genotypes and treatments. The photosynthetic rate, transpiration and stomatal conductance showed decreasing trend among the treatments compared with control. Control plant recorded higher photosynthetic rate in all genotypes (33.95 , 32.20 and $34.56 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and lowest was recorded in T7 (25.61 , 21.47 and $23.56 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) in K1, TNAU chilli hybrid CO1 and gundu respectively. The maximum photosynthetic rate under stress conditions was recorded by K1 variety compared to other two. Significant interaction effect was observed in transpiration rate between the genotypes and among the treatments. The transpiration rate of chilli showed variations among the treatments. The drought stress with different field capacity (80%, 60%, 40%) and heat with A+ 5°C (T3, T5, T7) had showed decreased transpiration rate irrespective of genotypes compared with A+ 3°C (T2, T4, T6). The transpiration rate of combined treatments found lower on comparing with normal condition of 100% pot capacity + Ambient Temperature (K1: 12.26 , TNAU chilli hybrid CO1: 11.21 and Gundu: $13.39 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$). Variety K1 had maintained higher transpiration rate and higher assimilative capacity under moderate and intense stress treatments. Among

the three genotypes taken for the study K1 maintained lower transpiration rate even when subjected to combined stress with minimum yield reduction. Stomatal conductance of the combined stress ranges between (0.60 to $0.20 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$). Control experiment had normal rate of conductance whether other treatment had reduced stomatal conductance (Table 1). The treatment of 40% PC and A+ 5°C had minimum level of conductance irrespective of genotypes (TNAU chilli hybrid CO1: $0.24 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$, Gundu: 0.28) while the K1 type chilli had higher conductance under stress condition with higher tolerance ($0.29 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$). Stomatal conductance followed the same trend with photosynthesis. The result showed that the K1 found to have tolerance under combined stress of drought and high temperature stress.

The rate of decrease in gas exchange had minimum amount of carbon assimilation, synthesis and translocation [18]. According to Gargallo-Garriga et al. [19] combined drought and heat stress reduced the overall growth, development, metabolism and productivity. In line with the above findings the present study on combined effect on drought and heat reduced plant height and leaf area expansion in chilli decreased photosynthetic gas exchange, carbon fixation and allocation and finally the yield loss.

Among the genotypes, TNAU chilli hybrid CO1 had higher photosynthetic transpiration rate and stomatal conductance under stress condition while the K1 recorded the minimum. Maintenance of lower transpirational and stomatal changes had higher level of tolerance to stress and reduced the yield loss in chilli. The above result was in accordance with the result of Malika et al., [20] who reported a maximum stomatal conductance under water stress and negative effect to photosynthesis and yield in

chilli. Drought stress experienced partial stomatal closure by restricting CO₂ influx and production of limited photo-assimilates [21]. Temperature stress had direct effect on chlorophyll membrane, enzyme denaturation and reduced the carboxylation efficiency [22]. The stomatal closure under stress condition in turns affects CO₂ and H₂O exchange retarding the photosynthetic process for want of CO₂ and increase in leaf temperature because of reduced transpiration [23].

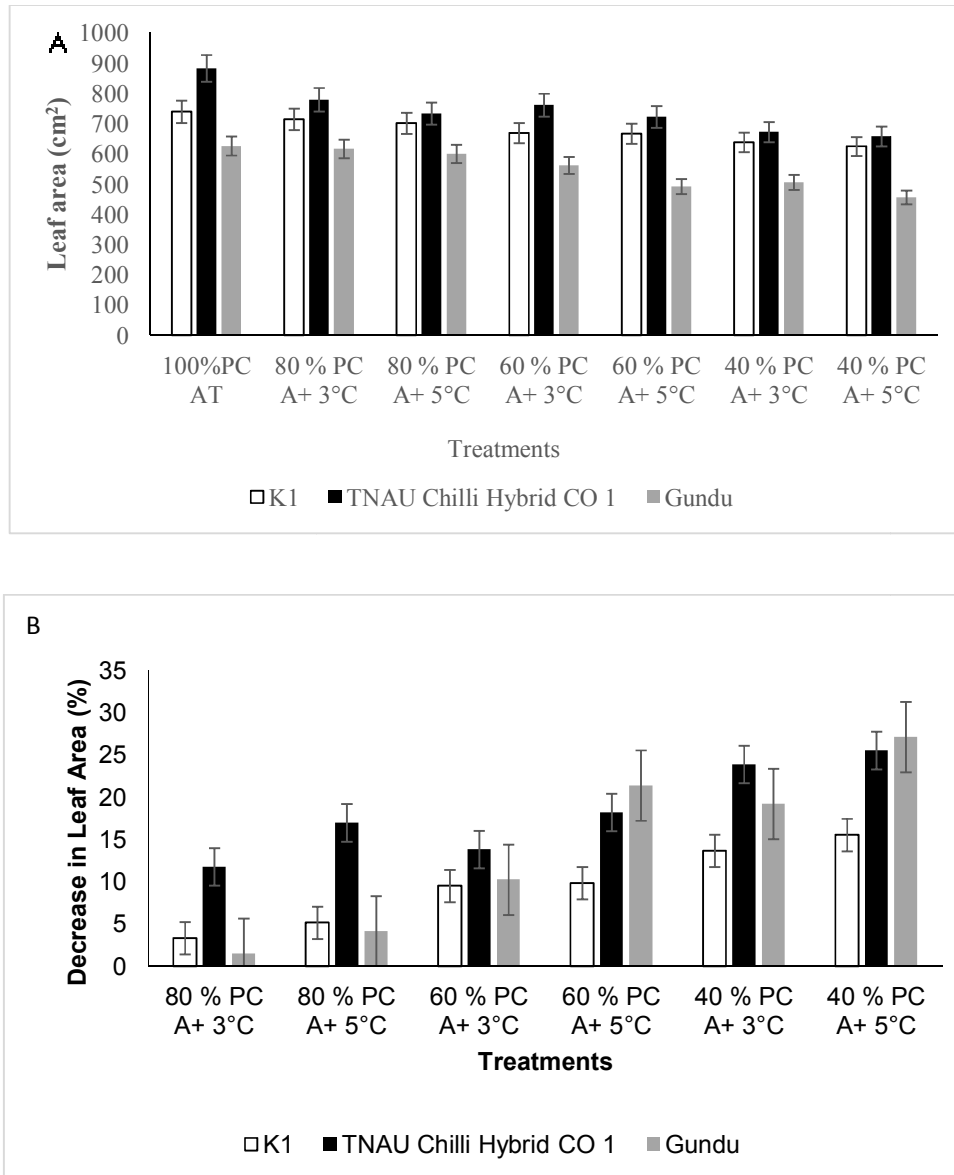


Fig. 3. Effect of combined drought and heat stress on leaf area A) Two week after stress and stress recovery B) Percent reduction over control (Factorial completely randomized design, significance at (P<0.05))

3.4 Yield Parameters

Yield characters of chilli showed significant variation ($P=0.05$) between treatments and genotypes (Table 2). Occurrence of stress gradually decreased fruit length, fruit diameter, total weight at first picking in chilli irrespective of genotypes and the lowest was recorded in treatment of 40% PC and A+ 5°C compared to normal condition. The drought of chilli with three different pot capacities (80%, 60%, 40%) combined with high temperature of (+ 3°C and + 5°C) had direct reduction on growth of chilli fruits. Under ambient condition, the length and diameter of TNAU chilli hybrid CO1 (9.90, 1.73 cm) was found higher followed by K1 (8.73, 1.50

cm) while under T7 (40% PC and A+ 5°C) stress condition the length and diameter was reduced to 6.20, 0.70 cm and 5.77, 0.53 cm respectively. Gundu type chilli normally had higher diameter of 4.00 cm but under stress, it was reduced to 2.73 cm (Table 2). Total weight of chilli at first picking had higher degree of reduction nearly 76% over control irrespective of genotypes in T7. The result of the present study supported by the findings of Praba et al., [24] drought stress showed reduction in growth, water relation, photosynthesis, assimilate partitioning, while high temperature stress affects the membrane stability, pollination, fertilization and finally a noticeable reduction in the yield of tomato [25].

Table 1. Leaf gas exchange parameters of chilli during combined drought and heat stresses

Traits	Photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)			Transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)			Stomatal conductance ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)			
	Treatments	K1	CO1	Gundu	K1	CO1	Gundu	K1	CO1	Gundu
T1		33.95	32.20	34.56	12.26	11.21	13.39	0.56	0.52	0.54
T2		29.09	26.55	29.93	10.39	9.90	7.70	0.35	0.38	0.36
T3		26.89	24.92	24.59	9.08	8.72	7.01	0.34	0.31	0.32
T4		27.91	27.33	25.64	9.46	10.38	8.66	0.37	0.34	0.33
T5		27.40	24.38	24.43	7.89	7.44	7.83	0.35	0.27	0.23
T6		27.45	22.19	26.06	9.12	7.73	7.77	0.34	0.27	0.33
T7		25.61	21.47	23.56	8.03	6.81	7.85	0.29	0.24	0.28
		T	G	T×G	T	G	T×G	T	G	T×G
Se(d)		0.16	0.07	0.48	0.09	0.04	0.28	0.002	0.002	0.008
Cd		0.32**	0.14**	0.96**	0.19**	0.08**	0.57**	0.006**	0.04**	0.018**

NS- Non significant *-significant **-Highly significant; T1- Control (100% Pot Capacity (PC) + Ambient Temperature), T2- 80 % PC and A+ 3°C, T3- 80% PC and A+ 5°C, T4- 60% PC and A+ 3°C, T5- 60% PC and A+ 5°C, T6- 40% PC and A+ 3°C and T7- 40% PC and A+ 5°C

Table 2. Yield characters of chilli under combined drought and heat stresses

Treatments	Fruit diameter (cm)			Fruit length (cm)			Total weight (g)		
	K1	CO1	Gundu	K1	CO1	Gundu	K1	CO1	Gundu
T1	1.50	1.73	4.00	8.73	9.90	1.33	263.82	321.24	186.12
T2	1.33	1.40	3.60	8.07	9.43	1.37	226.30	269.04	163.95
T3	1.13	1.27	3.40	7.43	8.73	1.13	186.15	234.21	136.28
T4	1.00	1.13	3.63	7.80	8.30	1.00	203.48	208.46	127.56
T5	0.80	0.93	3.40	6.60	7.57	0.83	163.17	154.00	90.91
T6	0.70	0.73	2.90	6.10	7.70	0.73	112.50	113.81	58.21
T7	0.53	0.70	2.73	5.77	6.20	0.67	61.52	74.58	43.94
		T	G	T×G	T	G	T×G	T	V×T
Se(d)	0.063	0.027	0.189	0.138	0.059	0.414	1.652	0.708	4.955
Cd	0.12**	0.05**	0.38*	0.27**	0.11**	0.83**	3.33**	1.43**	10.01**

($P < 0.05$)
NS- Non significant *-significant **-Highly significant; T1- Control (100% Pot Capacity (PC) + Ambient Temperature), T2- 80 % PC and A+ 3°C, T3- 80% PC and A+ 5°C, T4- 60% PC and A+ 3°C, T5- 60% PC and A+ 5°C, T6- 40% PC and A+ 3°C and T7- 40% PC and A+ 5°C

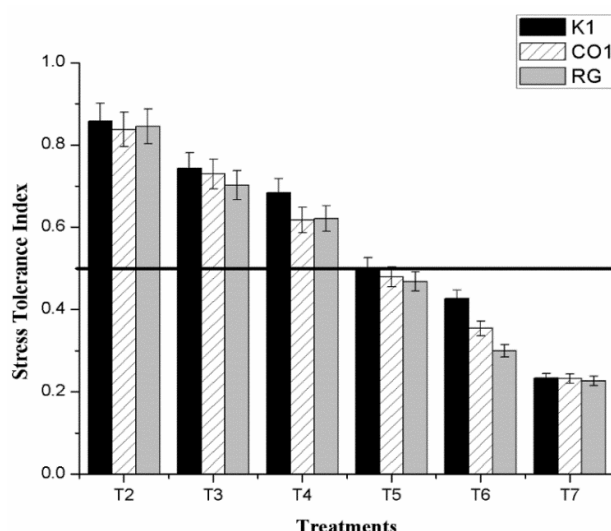


Fig. 4. Stress tolerant index of chilli under combined stress treatments

3.5 Stress Tolerance Index

With the yield of combined treatments, the stress tolerance index was calculated to determine the stress tolerance level in chilli. Based on the stress tolerance index the combined drought and heat treatment of 60% PC and A+ 3°C with 0.501 STI were standardized to study the further physiological studies (Fig. 4). T2, T3, T4 had better stress tolerance with above 50% STI, while other treatments had lower STI. Yield loss in chilli during stress is associated with reduced flower and fruit numbers and fruits per plant due to pollen abortion, reduced fertilization and malformation of the floral characters [26]. Reduction in number of fruits per plant, fruit length and diameter, individual fruit weight, fruit yield per plant and fruit dry weight per plant were seen under water deficit condition in chilli [27].

4. CONCLUSION

K1 variety had higher photosynthetic gas exchange under stress condition and registered lower yield reduction. TNAU chilli hybrid CO 1 had higher yield reduction over control and other genotypes, which had lower photosynthetic activity under all the stress treatments. Drought and heat stress treatment reduced the yield by decreased morphological and leaf gas exchange parameters. Stress tolerant index was found to be a reliable index to categorize the stress treatments based on the yield.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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