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Research article

Open top chamber: an innovative screening technique for temperature stress tolerance of morpho-physiological and fodder yield traits in forage cowpea varieties

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Abstract

A proper screening technique was needed to identify fodder crops with high green fodder yield suitable for elevated temperature. Accordingly, the changes in morpho-physiological characteristics and green fodder yield of forage cowpea [*Vigna unguiculata* (L.) Walp.] varieties (Kohinoor, BL-1, BL-2, BL-4 and EC-4216), were investigated under ambient as well as elevated (2 °C higher than ambient) temperatures at vegetative to maturity stage in open-top chambers. Elevated temperature decreased plant height, leaf length, leaf width, shoot fresh weight, leaf fresh and dry weights. Leaf length reduction (15.89 to 23.81%) was more than leaf width reduction (8.57 to 11.67%) and the highest leaf fresh weight reduction was recorded as (28.6%). Highest shoot dry weight reduction was observed in variety BL-2 (22%) followed by BL-4 (21%), EC-4216 (16%), BL-1 (15%) and least reduction was recorded in Kohinoor (13%). The long duration varieties' life cycles were shortened under elevated temperatures compared to ambient conditions, the highest percentage of reduction was observed in BL-2 (24%) and the least was recorded in BL-4 (17%). Significant interactions were found between variety and elevated temperature in leaf length and leaf width, total chlorophyll, relative water content and membrane stability index. Shoot biomass showed a highly positive significant correlation with elevated temperature, leaf fresh weight and ambient leaf width and a positive significant correlation with ambient leaf fresh weight, leaf length and elevated temperature leaf width. Kohinoor and BL-1 varieties were found relatively tolerant to temperature stress than EC-4216, BL-2 and BL-4.

Keywords: Dry weight, Elevated temperature, Forage cowpea varieties, Open top chamber

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Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] is a diploid and has 2n = 2x = 22 chromosomes. It is a warm season (C_3) legume crop grown in arid and semi-arid regions of India. In Asia, cowpea is grown in an area of 0.15 million hectares with an annual production of 0.14 MT (FAOSTAT, 2016). In India, cowpea is grown throughout India including considerable areas in Rajasthan, Gujarat, Maharashtra, Karnataka and Tamil Nadu. As per the Ministry of Agriculture Report (GOI), India accounts for about 15.06% of global cowpea area and 8.45% of global production (Kaur *et al*., 2018). It has a great potential for sustainable agriculture in marginal lands and semi-arid regions (Dixit *et al*., 2017; Nguyen *et al*., 2017; Nguyen *et al*., 2019; Panchta *et al*., 2021). It is estimated that about 6.5 lakhs hectare land is under different forms of cowpea and the share of fodder cowpea is 3.0 lakhs ha (Pandey and Roy, 2011). Average green fodder yield (GFY) of cowpea is 40-45 t/ha, which is rich with quality nutrients for livestock (Harveen *et al*., 2018). Cowpea crop thrives well between 21 to 35°C (Pandey and Roy, 2011) and proliferates better in optimum temperature lies between 27 to 35°C (Bisikwa *et al.,* 2014; Spriggs *et al.,* 2018). The climatic conditions like temperature and relative humidity have a great impact on crop development. The rate of plant growth and yield would be affected by changes in climatic conditions (Diaz-Ambrona *et al.,* 2013). The photosynthesis, stomatal conductance, leaf transpiration and enzymatic activities were significantly influenced by high temperature (Rafaele *et al*., 2021). Thermal stress had a negative impact on physiological parameters,

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and drastically reduced the growth rate and yield. This was due to leaf sensitivity to high temperature. Photosynthesis was inhibited as a result of chlorophyll loss and reduced carbon fixation and assimilation (Yuan *et al.*, 2017).

As the global human, as well as livestock population continue to increase, crop yields (both grains and forages) must increase proportionally to meet the future demand for food and fodder (Myers *et al.,* 2017). Considering the adverse effects of temperature stress on crop growth and productivity, the development of temperature stress-tolerant crop varieties/ genotypes/ lines and particularly temperature stress tolerant forage cowpea could play a major role in sustaining livestock production in the semi-arid area of India and would also be helpful in future breeding programs. But little information is available on forage cowpea tolerant to temperature stress. Therefore, the present investigation was carried out to study the performance of forage cowpea morpho-physiological parameters under open top chambers for temperature stress tolerance.

Materials and Methods

Study site and experimental design: The experiment was conducted under artificially created open top chambers (OTC) at Technology Demonstration Block, Seed Technology Division, ICAR-Indian Grassland and Fodder Research Institute, Jhansi (25°29′48.4″ N, 78°33′35.6″ E, and 233 m above the mean sea level) during *kharif* in 2021. Forage cowpea was grown in soil inside open top chambers. The experiment consisted of five forage cowpea varieties *viz.,* Kohinoor, BL-1, BL-2, BL-4 (IL-1177) and EC-4216 and seeds were surface sterilized with 0.1% mercuric chloride (w/v) for 10 min to avoid fungal invasion, followed by washing with distilled water. Treated seeds were sown in a randomized block design (RBD) and replicated thrice. Excess seedlings were thinned, and wooden sticks were inserted into the soil for plant support and climbing. Farmyard manure at the rate of 15 t ha^{-1} along with 25 kg N ha^{-1} and 55 kg P_2O_5 ha^{-1} were mixed in the soil before sowing and irrigation was given as and when required.

Open top chambers design: The following simulated environment was created for the experiment *i.e.,* first treatment, T_1-control was ambient temperature and the second treatment was T_2- elevated temperature (>2°C than ambient). The OTC (3 m diameter, 3 m length and 4 m height)] lined with multi-layered UV-protected polycarbonate (6 mm) sheets of more than 85% transparency level were used to grow cowpea varieties under natural conditions. Temperature sensor (Pt100 element) with RTD transmitter from H K Tempson (Sensography) (India) and humidity sensors (Rotronic Switzerland) were fitted inside both OTC chambers along with Ceramic infrared heaters. Temperature enrichment was done with an artificially induced temperature inside OTC. The system simulated warming in a small ecosystem of limited height (162 cm height and it was adjustable up to 100 cm up and down) with uniformity of thermal radiation and canopy temperature across the plot. Galvanized iron (GI) structure with infrared heating systems and controller were used to enhance from ambient temperature up to 2 to 4.5 ºC. IR heating system was comprised of electromagnetic radiation with wavelength between 780 nm to 1000 nm. It could increase air temperature >2°C than ambient as it emits IR electromagnetic waves. Air temperature >2°C than ambient was set in elevated temperature treatment OTC. Monitoring and control of temperature in OTC was fully automatic and desired level of temperature was maintained throughout the experimental period in OTC with the help of supervisory control and data received from data scanner was recorded by SCADA system in Excel sheet. The system monitored desired parameters and control based on the output options of various sensors. Instruments/ tools like temperature, RH sensors, relay card, relay module, data logger were also used for control strategy. Signals from each sensor were obtained and transmitted to control room through four core shielded cable for data logging and control (entire system was built by Genesis Technologies, Mumbai, India). Elevated temperature (Air temperature >2°C than ambient) was imposed everyday morning 8 am to 2.0 pm with help of the control unit monitor and was maintained 30 days after crop sown and maintained up to physiological maturity stage. Temperature (Max) and relative humidity (RH-2) were recorded every day at 2.00 pm in ambient OTC and elevated temperature OTCs, respectively. Weekly averaged data is shown in Fig 1.

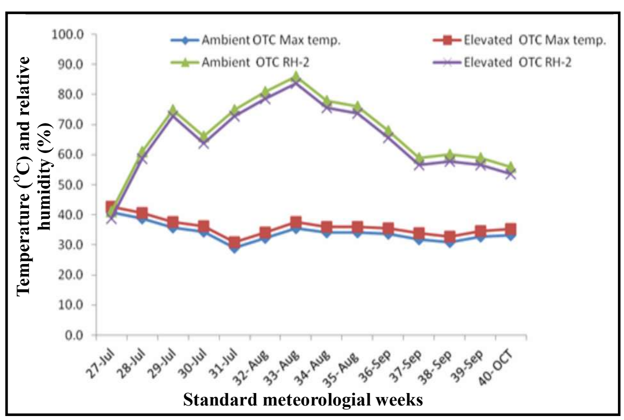

Fig 1. Maximum temperature and relative humidity in OTC

Measurements of growth parameters: The observations on morphological traits like plant height, leaf fresh (15 leaves randomly collected from each treatment and each variety) and dry weight, shoot fresh (5 plants randomly selected from each treatment and each variety) and dry weight was recorded at 65 days after imposition of elevated temperature. The aboveground material was removed, placed in paper bags and oven-dried at 80 °C until a constant weight was reached after about 72 hours to determine the shoot dry weight (SDW).

Physiological parameters: The acetone method was applied to green leaf samples (200 mg fresh weight) from the 5th leaf from top portion to extract chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Total Chl) and carotenoid (Car) and 100 mg leaf (green leaf) samples were used to determine membrane stability index (MSI) (5th leaf from top portion) according to the method of Premachandra *et al.* (1990). The relative water content (RWC) of 100 mg leaf samples (5th leaf from top portion) was analyzed by the method of Weatherley (1950). Chlorophyll stability index (CHSI) was calculated following the method described by Sairam *et al.* (1997) using the formula: Total chlorophyll in elevated temperature plants/Total chlorophyll in ambient plants) × 100; while carotenoid stability index (CARSI) was calculated following the method described by Dheeravathu *et al.* (2018) using the formula: Total carotenoid in elevated temperature plants/Total carotenoid in ambient plants) × 100; Percent reduction to controls (% ROC) was calculated as follows:

$$\%ROC = \frac{\text{Value for control - Value for stressed plants}}{\text{Value for control}} \times 100$$

Statistical analysis: Whole data were subjected to ANOVA (factorial CRD with three replications) using the PROC GLIMMIX procedure in SAS (V 9.3 SAS Institute Inc, Cary, NC, USA) and Microsoft Excel. The correlation study was performed using XL STAT BASIC^{+} at 5% level of significance. The treatment means were separated using Fisher's least significance difference (LSD) test (P<0.05).

Results and Discussion

Stress is an altered physiological response of living organisms caused by physical, chemical or biotic environmental factors that tend to shift their equilibrium away from its optimal thermodynamic state (Gaspar *et al.*, 2002; Strasser, 1988; Antony *et al.*, 2021). Soil or water pollution, climate change or other anthropogenic effects can cause severe abiotic or biotic stress for both crop and forage crop plants and natural vegetation. Our findings showed that active warming OTC facility resulted in enhanced warming of 2°C than ambient (Fig 1). The warming effect varied over time (every day morning to evening and from the date of sowing to harvesting). It elevated temperature was reduced than optimum temperature requirement to cowpea due to rain and also partly influenced by air temperature and wind. Relative air humidity in general showed opposite patterns compared to temperature changes. The main effects of elevated temperature on varieties and the interaction effects among varieties and treatments were found significant in plant height, shoot fresh and dry weights, leaf fresh and dry weights, leaf length (LL) and leaf width (LW), total Chl, RWC, MSI, 50% flowering and maturity (Table 1).

Morphological traits: Reduced plant height (PH), leaf length (LL) and leaf width (LW) were observed for all varieties at elevated temperatures compared to ambient conditions (Table 1). In plant height, the highest percentage reductions were observed in EC-4216 (6.6) followed by BL-4 (6.5), Kohinoor (6.0), BL-2 (3.8) and the least was recorded in BL-1 (3.6) (Table 2). The highest leaf length (11.67 cm) and leaf width (7.0 cm) were observed in Kohinoor and the lowest leaf length (9.60 cm) and leaf width (6.03 cm) were recorded in EC-4216 at ambient condition. At elevated temperature stress, leaf length reduction was more (15.89 to 23.81%) than leaf width (8.57 to 11.67%) (Table 2). The highest leaf length percentage reduction was observed in BL-2 (23.81%) and least was noted in variety BL-1 (15.89%), while the highest leaf width percentage reduction was observed in variety BL-1 (11.67%) and least was observed in variety Kohinoor (8.57%) compared to control and other varieties. The leaf fresh, dry weight, leaf fresh weight to dry weight ratio and shoot biomass of all five cowpea varieties were strongly affected at elevated temperature compared to ambient condition. The highest leaf fresh weight was observed in BL-1 (19 g) and the lowest was recorded in variety EC-4216 (13 g), while the lowest leaf dry weight was recorded in variety EC-4216 (1.68 g) and the highest leaf dry weight was observed in BL-2 (3.69 g) at ambient condition. The highest reduction (28.6%) was observed in leaf fresh weight than leaf dry weight reduction (18.86%). Least leaf fresh and shoot biomass weight reduction was recorded in Kohinoor and BL-2 (12.5% and 29%) and the highest leaf fresh and shoot biomass weight reductions were observed in varieties BL-4 (28.6%) and EC-4216 (37%). Higher leaf dry weight reduction was recorded in BL-4 (18.86%) and the lowest percentage reduction (14.20) was observed in Kohinoor. The highest shoot dry weight was recorded in Kohinoor (36.3 g) and least was observed in BL-2 (22.1 g) at

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Table 1. Effect of high temperature on morphological traits and 50% flowering and physiological maturity

Varieties	AC	ET	AC	ET	AC	ET
	Plant height (cm)		**Leaf length (cm)**		**Leaf width (cm)**	
Kohinoor	250±4.7	235±2.4	11.67±0.72	10.00±0.47	7.00±0.24	3.62±0.09
BL-2	356±4.9	342±4.7	10.50±0.24	8.03±0.21	6.12±0.05	3.09±0.14
BL-1	280±2.4	270±4.7	10.70±0.14	9.23±0.37	6.70±0.05	3.37±0.24
BL-4	310±4.7	290±3.5	10.37±0.31	8.67±0.07	6.07±0.07	3.07±0.09
EC-4216	298±3.1	278±5.0	9.60±0.14	7.97±0.26	6.03±0.17	3.10±0.14
Varieties	**Leaf fresh weight**		**Leaf dry weight**		**Shoot fresh weight**	
Kohinoor	16±0.72	14±0.50	3.45±0.07	3.23±0.02	160±2.36	111±4.24
BL-2	14±0.47	11±0.48	3.69±0.02	3.00±0.01	156±5.66	110±4.71
BL-1	19±0.94	14±0.47	2.56±0.02	2.12±0.01	158±2.83	104±1.19
BL-4	14±0.47	10±0.24	2.28±0.03	1.85±0.01	118±2.83	79±2.36
EC-4216	13±0.49	10±0.49	1.68±0.02	1.42±0.02	113±1.78	71±3.30
Varieties	**Shoot dry weight**		**RWC (%)**		**MSI (%)**	
Kohinoor	36.3±1.36	31.50±0.7	91.5±3.24	85.2±2.30	89±0.73	71±1.19
BL-2	22.1±0.99	17.26±0.6	90.4±4.5	71.9±3.56	87±0.74	64±0.94
BL-1	29.3±0.95	25.00±0.9	83.5±0.57	81.9±1.23	90±0.94	82±1.19
BL-4	31.8±0.87	25.00±0.5	88.1±0.66	84.8±5.47	88±1.67	68±1.67
EC-4216	30.0±0.94	25.08±0.5	93.3±1.47	83.6±4.25	87±1.19	68±1.89
Varieties	**Total chl (mg/g FW)**		**Days to flowering stage**		**Days to maturity stage**	
Kohinoor	2.37±0.043	1.61±0.020	56±0.94	45±1.41	112±1.41	86±1.66
BL-2	1.38±0.087	1.21±0.068	68±1.66	52±1.65	122±1.41	90±1.41
BL-1	1.48±0.148	0.69±0.958	50±1.41	40±0.71	116±1.18	91±1.66
BL-4	2.51±1.152	1.02±0.100	58±3.54	48±1.41	124±1.55	92±1.50
EC-4216	1.76±0.212	0.78±0.083	52±1.89	42±0.72	118±1.89	92±1.19

Mean ± SE (n=3); AC: Ambient condition; ET: Elevated temperature; RWC: Relative water content; MSI: Membrane stability index; Total chl: Total chlorophyll

ambient. Highest shoot dry weight reduction was observed in BL-2 (22%) followed by BL-4 (21%), EC-4216 (16%) BL-1 (15%) and least was recorded in Kohinoor (13%) (Table 2). Leaf fresh weight to dry weight reduction was observed more at ambient temperature (73.64 to 87.08%) than elevated (72.62 to 85.80%) temperature (Table 2), while shoot fresh weight to dry weight reduction was ranged from 73 to 86% and 65 to 84% under ambient and elevated temperature, respectively. Shoot height, leaf area and number of leaves in sensitive genotypes under drought/saline conditions might be due to their leaves having lower relative water content and membrane stability index (Dheeravathu *et al*., 2021b; Antony *et al*., 2021; Singh *et al*., 2020; Dheeravathu *et al.,* 2017a; 2017b). These results corroborated with other studies that decreased fresh and dry weights in berseem and sweet william (*Dianthus barbatus*) (Azizi *et al.,* 2011; Dheeravathu *et al*., 2021a). Global warming is predicted to have a negative effect on plant growth due to the damaging effect of high temperatures on plant growth and development. Our results agreed with Rafaele *et al*. (2021), who reported that shoot dry weight was reduced at high temperature in cowpea genotypes.

Physiological traits: The reduction in relative water content (RWC; %) and membrane stability index (MSI; %) was observed in all five varieties at elevated temperature compared to ambient conditions. Relative water content and membrane stability index were considered reliable parameters to assess the temperature stress tolerance of crop species. RWC of leaf declined in all varieties under elevated temperature with percentage reduction relative to control at the elevated temperature (Table 1). MSI for all varieties also declined under elevated temperatures. RWC percentage reduction range was (1.9-20.4) more than MSI (9.3-26.6) (Table 2). RWC

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and MSI are good indicators of leaf water status and stability of membranes and these have been successfully used to determine stress resistance or tolerance in many crop plants (Bangar *et al.*, 2019; Rahimi *et al.,* 2021). Many reports revealed that RWC and MSI were reduced under drought and salinity and those plants that maintain high RWC and MSI under extreme stress were regarded as more stress-tolerant (Bangar *et al*., 2019; Rahimi *et al.,* 2021).

Biochemical traits: Total chlorophyll (Table 1), chlorophyll a, chlorophyll b, and carotenoid concentrations (Table 3) were decreased under elevated temperature. Highest total chlorophyll content (mg/g FW) was recorded in BL-4 (2.51) followed by Kohinoor (2.37), EC-4216 (1.76), BL-1 (1.48) and least was observed in BL-2 (1.38) (Table 1). Highest carotenoid content (mg/g FW) was observed in BL-4 variety (0.15) followed by Kohinoor variety (0.13) and lowest was recorded in BL-2 (0.08) at ambient condition as compared to elevated temperature (Table 3). Chlorophyll stability index (114 to 246) and carotenoid stability index (106 to 267) also declined under elevated temperature compared to ambient condition. Chlorophyll content is considered as a useful biochemical indicator of temperature stress tolerance in different plants (Akram and Ashraf, 2011) as chlorophyll and carotenoids are involved in the primary step concerning energy production during photosynthesis. Since temperature affects chlorophyll and carotenoid levels, it is not surprising that growth of plants is inhibited when grown in temperature situations. Temperature stress increases the activity of chlorophyllase, which promotes degradation of chlorophyll and reduces chlorophyll concentration in plants (Yang *et al*., 2011). Although elevated temperature can reduce chlorophyll concentration, the extent of reduction depends on temperature tolerance of the particular plant species. Differences in reductions in chlorophyll concentrations between varieties in our study suggested that the degree of tolerance to temperature by the various varieties was relatively similar, although BL-1 and Kohinoor did display lower reductions relative to control than other varieties as temperature level increased. Carotenoids play an important role as a precursor in signaling during plant development under abiotic stress as they protect the membranes from oxidative damage (Verma and Mishra, 2005). While all varieties demonstrated reductions in carotenoid concentrations relative to control at elevated temperature, but Kohinoor showed comparatively lower reduction than other varieties. These results corroborated other studies which indicated that

Table 2. Effect of high temperature on morphological traits (ROC%)

Varieties	PH	LL	LW	LFW	LDW	SFW	SDW	LFW to LDW		SFW to SDW		RWC	MSI	DF	DM
								AC	ET	AC	ET				
Kohinoor	6.0	16.67	8.57	12.5	14.20	31	13	78.44	76.93	77	71	6.9	19.6	20	23
BL-2	3.8	23.81	10.81	21.4	18.37	29	22	73.64	72.62	86	84	20.4	26.6	24	26
BL-1	3.6	15.89	11.67	26.3	17.19	34	15	86.53	84.86	81	76	1.9	9.3	19	22
BL-4	6.5	16.35	10.91	28.6	18.86	33	21	83.71	81.50	73	68	3.8	22.7	17	26
EC-4216	6.6	16.67	8.83	23.1	15.48	37	16	87.08	85.80	73	65	10.4	21.5	19	22

Mean (n=3); PH: Plant height; LL: Leaf length; LW: Leaf width; LFW: Leaf fresh weight; LDW: Leaf dry weight; SFW: Shoot fresh weight: SDW: Shoot dry weight; LFW to LDW: Leaf fresh weight to dry weight reduction; SFW to SDW: Shoot fresh weight to shoot dry weight reduction, AC: Ambient condition; ET: Elevated temperature; RWC: Relative water content; MSI: Membrane stability indexed; DF: Days to flowering; DM: Days to physiological maturity

Table 3. Effect of elevated temperature on photosynthetic pigments and Chlorophyll stability index and Carotenoid stability index

Varieties	AC				ET				CHSI	CARSI
	Chl a (mg/g FW)	Chl b (mg/g FW)	Chl a/b ratio	Car (mg/g FW)	Chl a (mg/g FW)	Chl b (mg/g FW)	Chl a/b	Car (mg/g FW)		
Kohinoor	0.30±0.00	0.07±0.04	0.15±0.07	0.13±0.00	0.26±0.01	1.35±0.00	0.19±1.81	0.11+0.00	147	116
BL-2	0.16±0.00	1.22±0.07	0.14±0.10	0.08±0.00	0.15±0.01	1.06±0.05	0.14±0.27	0.07+0.00	114	106
BL-1	0.21±0.01	1.28±0.13	0.16±0.14	0.09±0.00	0.10±0.13	0.59±0.82	0.17±0.17	0.05+0.06	216	198
BL-4	0.36±0.19	2.15±0.95	0.17±0.20	0.15±0.07	0.14±0.01	0.88±0.08	0.16±0.21	0.07+0.00	246	230
EC-4216	0.26±0.03	1.50±0.18	0.18±0.18	0.12±0.01	0.09±0.01	0.69±0.06	0.13±0.27	0.04+0.00	225	267

Mean ± SE (n=3); AC: Ambient condition; ET: Elevated temperature; FW: Fresh weight; Chl a: Chlorophyll a; Chl b: Chlorophyll b; Car: Carotenoids; Chl a/b ratio: Chlorophyll a/ chlorophyll b ratio; CHSI: Chlorophyll stability index; CARSI-Carotenoid stability index

Table 4. Correlations among different parameters in forage cowpea varieties subjected to ambient and elevated temperature stress

Parameters		PH		SFW		LFW		LDW		LL		LW	
		AC	ET	AC	ET	AC	ET	AC	ET	AC	ET	AC	ET
PH	AC	1											
	ET	0.965**	1										
SFW	AC	-0.157	-0.049	1									
	ET	-0.077	0.030	0.983**	1								
LFW	AC	-0.414	-0.255	0.597*	0.498	1							
	ET	-0.629*	-0.513	0.783**	0.700**	0.772**	1						
LDW	AC	0.137	0.208	0.833**	0.904**	0.151	0.441	1					
	ET	-0.163	-0.216	0.231	0.234	0.097	0.254	0.222	1				
LL	AC	-0.462	-0.281	0.576*	0.609*	0.497	0.619*	0.570*	0.046	1			
	ET	-0.665**	-0.542*	0.516*	0.489	0.698**	0.766**	0.327	0.247	0.792**	1		
LW	AC	-0.720**	-0.596*	0.652**	0.601*	0.637*	0.934**	0.409	0.209	0.761**	0.800**	1	
	ET	-0.632*	-0.510	0.612*	0.555*	0.711**	0.824**	0.372	0.273	0.674**	0.922**	0.761**	1

*(P<0.05); **(P<0.01); PH: Plant height, SFW: Shoot fresh weight/biomass; LFW: Leaf fresh weight; LDW: Leaf dry weight; LL: Leaf length; LW: Leaf width; AC: Ambient condition; ET: Elevated temperature

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plants subjected to elevated temperature recorded decreased photosynthetic pigments (Aghaleh *et al.,* 2009; Jampeetong and Brix, 2009; Al-Ghumaiz *et al.*, 2017).

Days to 50% flowering and physiological maturity: Days to 50% flowering and physiological maturity varied significantly (P<0.05) with elevated temperature and varieties (Table 1). Among the varieties, BL-2 took maximum number of days to 50% flowering (68 days) and maturity (122 days) followed by BL-4 (58 and 124 days, respectively). Under elevated temperature varieties completed its vegetative and reproductive life cycle at an accelerated pace and leading to a shortening of days taken to 50% flowering and physiological maturity. A similar trend was also observed with the physiological maturity period. Long duration verities shortened the life cycle under elevated temperature compared to ambient condition; the highest reduction was observed in BL-2 (24%) and the least was recorded in BL-4 (17%) (Table 2) at 50% flowering and the highest reduction was observed in BL-2 (26%) and BL-4 (26%) and lowest was recorded in BL-1 (22%) and EC-4216 (22%) at physiological maturity. These findings were in conformity with Rafaele *et al*. (2021), who observed that high-temperature stress shortened days taken to 50% flowering and physiological maturity in cowpea genotypes. Indeed, the plant growth rate throughout the life cycle is affected by temperature extremes (Hatfield and Prueger, 2015) and the range differs among species (Lafta and Lorenzen, 1995). In the present study, long duration varieties recorded shortened life cycle under elevated temperature compared to ambient condition; and the highest percentage of reduction was observed in BL-2 (24) and least was recorded in BL-4 (17).

Plant fresh weight, leaf fresh weight, leaf length and leaf width showed (Table 4) significant positive correlation in plants under ambient condition compared to elevated temperature. However, plant height showed significant negative correlation under elevated temperature compared to ambient condition.

Conclusion

The results of this study showed that plant height, leaf length, leaf width, leaf fresh and dry weigh, and shoot fresh and dry weight, RWC, MSI, decreased significantly in forage cowpea and the plant's life cycle was shortened under elevated temperature compared to ambient condition. It was concluded that the Kohinoor and BL-1 varieties showed better temperature tolerance than other tested varieties to elevated temperature. It was also found that long duration varieties showed shorter life cycle under elevated temperature compared to ambient condition. The open top chamber based innovative screening technique was found useful for identifying temperature stress tolerant forage cowpea varieties. This technique could also be used to screen more number of crops in a calendar year under tropical and sub-tropical conditions.

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