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High temperature tolerance in chickpea (*Cicer arietinum*) genotypes as evaluated by membrane integrity, heat susceptibility index and chlorophyll fluorescence techniques

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In India, chickpea (*Cicer arietinum* L.) is mainly grown in arid and semi-arid zones and is subjected to drought and high temperature stresses at terminal stages particularly during active pod-filling stage. In dryland area of the country, the recommended sowing time of chickpea is the middle of October and first week of November in semi-arid zones. Delay in sowing results in drastic reduction in the potential yield as it results in exposure of post anthesis phase to high temperature. Yield reduction have been observed at a temperature of 30 °C at 50% flowering and >30 °C for 3-4 days at 100% flowering (Summerfield *et al.* 1984). van Rheenen *et al* (1997) reported that such conditions reduced the duration of flowering and pod filling, resulting in large yield losses. Wang *et al.* (2006) reported that the heat stress during pod development decreased seed yield by more than 53%.

Global mean temperature will rise to 0.3 °C per decade reaching to approximately 1 and 3 °C above the present values by years 2025 and 2100, respectively (Jones *et al.* 1999). Rising temperature may lead to altered geographical distribution and growing season of agricultural crops by allowing the threshold temperature for start of the season and crop maturity to reach earlier. At very high temperature severe cellular injury and even cell death occur within minutes, which could be attributed to a catastrophic collapse of cellular organization (Schoffl *et al.* 1999). At moderately high temperatures, injuries or death may occur only after long-term exposure (Haworth 2005). Heat killing temperature in chickpea was found to be 44.3 °C for 41 minutes (Srinivasan *et al.* 1996). Plant responses to high temperature are diverse. Although resistance to high temperature involves several complex tolerance and avoidance mechanisms, the membrane is thought to be a site of primary physiological injury by heat (Blum 1988), and measurement of solute leakage from tissue can be used to estimate damage to membranes. Canopy temperature depression itself is a mechanism of heat escape as suggested by Cornish *et al*. (1991). Very little information is available regarding the effect of heat stress on photochemical efficiency/quantum yield of photosystem II (PS II) in chickpea. Measurement of chlorophyll fluorescence can give quantitative assessment of inhibition or damage to electron transfer. The technique is rapid, sensitive, non-destructive, relatively cheap and able to detect the injury even before visible symptoms appear (Baker and Rosenqvist 2004).

Information on tolerance to high temperature stress in chickpea genotypes is still not well understood. The performance of chickpea under heat stress depends on their inherent genetic capacity and on the whole agro ecosystem in which they are managed. Improvement of heat tolerance can contribute to sustainability and provides a mean of extending chickpea cultivation to previously unsuitable regions and season. Therefore, to adopt successfully to climate change will need chickpea varieties with greater tolerance to stresses such as high temperature and with this objective 30 chickpea genotypes obtained from Indian Institute of Pulses Research (IIPR), Kanpur, India were screened against high temperature tolerance by studying various morpho-physiological traits and evaluating tolerance to heat stress via heat susceptibility index (HSI), i e yield reduction under the said environment.

The field experiment was conducted at CCS Haryana Agricultural University, Hisar, Haryana, (about 29° 10' N, 75° 46' E, 215.2 m above sea level) during *rabi* season of 2008–09. The experimental plot consisted of two rows of 4 m length, 45 cm apart. Chickpea genotypes were grown in a randomized complete block design with three replications. High temperature stress was given by manipulation of sowing dates, i e normal sown (11 November 2008) and late sown (3 January 2009) conditions.

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The experimental areas were fertilized @ 15 kg N and 40 kg P_2O_5/ha as basal dose before sowing. The seeds were inoculated with *Rhizobium* culture, Ca-181. Weed control was done by hand hoeing twice, i e seedling stage and prior to flowering. Intensive protection measures were taken against pod borer (*Helicoverpa armigera*). One irrigation was given before the flowering in normal and late sown trials just to avoid any drought during the experiment.

Under late sown conditions, observations for days to flowering, podding, physiological maturity, canopy temperature depression (CTD), membrane integrity in terms of relative stress injury (RSI%), photochemical efficiency/ quantum yield (Fv/Fm) were recorded when the temperature rose to 30 ^{0}C or exceeding above. The relative stress injury in leaves was evaluated by Sullivan test (1972). Transpiration cooling, i e canopy temperature depression (CTD) was measured by using Infra-red thermometer (Model AG-42 Tele-temp Corp.CA). Photochemical efficiency/quantum yield was determined with intact plants in the field with an OS-30P Chlorophyll Flurometer (Opti-Science, Inc., Hudson, USA). Initial (F_0) and maximum (F_m) fluorescence were recorded and variable fluorescence (F_v), derived by subtracting F_o from F_m. Quantum yield/photochemical efficiency which is F_v/F_m ratios were than calculated.

Yield/plant was recorded at harvest under normal and late sown conditions to calculate the heat susceptibility index (HSI) for each genotype by the formula given by Fischer and Maurer (1978).

Heat Susceptibility Index (HSI) = (1-YL/YN)/(1-XL/ XN)

where YL, Mean seed yield of a line under late sown conditions; YN, mean seed yield of a line under normal sown conditions; XL, mean seed yield of all line under late sown conditions; XN, mean seed yield of all line under normal sown conditions. Yield stability ratio (YS) was calculated as per Lewis (1954).

YS = (Seed yield under late/seed yield under normal) × 100

Data were subjected to analysis of variance (ANOVA) using Online Statistical Analsis Package (OPSTAT, Computer Section, CCS Haryana Agricultural University, Hisar) with level of significance at P= 0.05.

The second fortnight of March and first fortnight of April were the critical period to study the effect of high temperature as it exceeded more than 35 ^oC in this region. The minimum and maximum temperature for these periods for the years 2008/09 are given in the Table 1.

The chickpea genotypes differed significantly for days to flowering. Among genotypes, L 550 required minimum 60 days under late sown conditions, while the genotypes GNG 469 took maximum 71 days. Plants grown under late sown flowered and matured early. Pusa 240 took maximum 108 days to maturity, whereas minimum 100 days to maturity was noticed in ICCV 92944 (Table 2).

Table 1 Mean weekly temperature prevailing at the experimental location (*HAU, Hisar) during the year 2008–09

Month /year	Week	Temperature (^{0}C)	
		Maximum	Minimum
February 2009	6 (Feb. 5-11)	23.7	7.0
	7 (Feb. 12-18)	25.7	6.6
	8 (Feb. 19-25)	26.3	8.8
	9 (Feb. 26- Mar. -4)	27.1	8.8
March 2009	10 (Mar. 5-11)	28.5	9.4
	11 (Mar. 12-18)	30.2	10.6
	12 (Mar. 19-25)	31.7	14.6
	13 (Mar. 26- April -1)	28.3	14.0
April 2009	14 (April 2-8)	34.6	16.9
	15 (April 9-15)	34.1	16.1
	16 (April 16-22)	38.9	18.6
	17 (April 23-29)	39.1	17.6
	18 (April 30- May 6)	23.7	7.0

**Source:* Department of Meteorology, CCS HAU, Hisar, Haryana, India

The canopy temperature depression (CTD) exhibited significant differences among the genotypes. Under late sown condition, the maximum value (–2.1 ^oC) was in Pusa 240 and minimum (–4.8 ^oC) was in IPC 98-12. There was large variability among the genotypes for relative stress injury (RSI%). It was highest in GG 2 (69%) and was the lowest in Pusa 240 (45%). Genotypes which showed less RSI % showed better tolerance to high temeperature (Table 2). Photochemical efficiency of photosystem II (PS II) or quantum yield as indicated by F_v/F_m ratios was in the range of 0.157 to 0.352. Genotype, Pusa 240 maintained maximum F_v/F_m ratio, i e 0.352 than others when the temperature was more than 30 ^{0}C however, genotype L 550 was the most sensitive in this respect where F_v/F_m ratio was 0.157.

Genotypic variation in plant height at harvest among the genotypes was recorded with minimum 31 cm (ICCV 37) and maximum 49 cm (C 235). Similarly pod/plant were minimum 19 (PBG 5) and maximum 44 (Annigeri), whereas biological yield/plant was lowest 18 g (CSJD 884) and highest 32 g (Pusa 256). The reduction in seed yield was due to reduction in biomass accumulation and yield attributes in late sown plants. Our result suggests that high temperature stress during the reproductive development affects sink size more than assimilate source in chickpea. It is also possible that stress during reproductive development may have indirectly affected the remobilization of the photosynthates to grain (Wang *et al.* 2006).

Heat susceptibility index (HSI) computed to test the sensitivity of genotypes to high temperature stress ranged from 0.60 (Pusa 240) to 1.34 (IPC 98-12). The values less than 1 was recorded for the genotypes Pusa 240 (0.60) followed by K 850 (0.79), ICCV 92944 (0.80), Vaibhava

Table 2 Phenology, quantum yield (QY), relative stress injury (RSI %) and canopy temperature depression (CTD) under late sown condition of different genotypes of chickpea

Genotypes	Days to flowering	Days to podding	Days to maturity	*QY (Fv/Fm)	*RSI (%)	*CTD (–°C)
ICCV 4958	62	76	102	0.271	50	2.3
ICCV 92944	60	72	100	0.303	49	3.9
RSG 143-1	64	74	104	0.228	46	3.2
ICCV 37	62	77	105	0.260	50	3.4
GG 2	63	74	103	0.259	69	2.9
Annegiri	67	70	103	0.239	52	2.6
L550	60	77	105	0.157	62	3.8
Vaibhava	66	79	103	0.288	51	2.9
Avrodhi	68	80	106	0.225	52	2.4
JG 218	65	74	103	0.206	54	2.6
Dohad yellow	70	81	107	0.222	61	2.8
PG 96006	70	74	104	0.233	56	3.3
C 235	66	83	107	0.213	62	3.2
Pusa 256	70	80	108	0.254	52	2.8
GNG 469	71	76	105	0.263	46	3.4
K 850	70	82	106	0.329	46	2.8
KWR 108	70	80	104	0.208	64	2.6
GNG 663	70	84	106	0.195	59	3.2
PUSA 244	66	84	108	0.248	58	2.6
IPC 98-12	71	80	106	0.166	57	4.8
PUSA 329	68	77	105	0.162	57	3.2
PUSA 240	66	83	108	0.352	45	2.1
Vijay	68	76	105	0.272	53	3.2
RSG 931	68	74	103	0.166	46	2.2
CSJD 884	68	76	104	0.190	52	3.4
PBG 5	68	84	106	0.236	59	3.8
GCP 101	71	78	104	0.272	51	3.6
JG 11	68	79	106	0.192	56	2.4
RSG 888	69	77	104	0.236	52	3.4
PG 5	68	79	108	0.273	46	4.5
CD (P = 0.05)	1.7	1.6	2.2	0.006	2.7	0.4
CV (%)	1.5	1.3	1.4	1.508	1.3	8.8

*Plants exposed to high temperature from 17th March to 25th March 2009 (30-35 °C) and quantum yield (Fv/Fm) recorded on 26th March

(0.83), PG 5 (0.85), ICCV 4958 (0.88), ICCV 37 (0.89), GNG 469 (0.91), GCP 101 (0.92), RSG 888 (0.95), Vijay (0.96), PBG 5 (0.98) and Annegiri (0.99). The genotype, Pusa 240, was identified as the most thermo-insensitive and showed the maximum tolerance against heat stress with HSI value (0.60) and yield stability ratio 72.8% (Table 3). Distinct differences in chlorophyll fluorescence properties were seen and it has been found that the genotypes tolerant to high temperature and having HSI values less than one also maintained high quantum yield/high photochemical efficiency (Fv/Fm) of PS-II under late sown conditions. High values of Fv/Fm of PS-II measured indicate that there was lesser photochemical damage in these genotypes than the other genotypes.

In conclusion, the present study has shown that wide genetic variation exists among chickpea genotypes for heat susceptibility index (HSI). Genotypes Pusa 240 was identified thermo-insensitive and showed the maximum tolerance against high temperature stress with least variation for seed yield and heat susceptibility index (HSI) value was 0.60. This genotype is also found to have high value of quantum yield (Fv/Fm), but low value of RSI and maintained the cooler canopy temperature This genotype as mentioned above can be useful in global warming crop improvement programme at national level.

SUMMARY

Chickpea (*Cicer arietinum* L.) is mainly grown in arid

Table 3 Yield-attributes, yields, heat susceptibility index (HSI), yield stability ratio (YS) of different genotypes of chickpea under normal (N) and late (L) sown conditions

Genotypes	Height (cm)	Pods/plant	Biological yield (g/plant)	Grain yield (g/plant)		HSI	YS (%)
				N	L		
ICCV 4958	41	35	28	13.8	8.3	0.88	60.1
ICCV 92944	38	30	29	14.1	9.0	0.80	63.8
RSG 143-1	32	24	24	13.2	6.8	1.07	51.5
ICCV 37	31	32	26	14.4	8.6	0.89	59.7
GG 2	41	34	25	15.1	8.2	1.01	54.3
Annegiri	35	44	30	16.9	9.3	0.99	55.0
L550	37	25	23	14.5	7.1	1.31	48.9
Vaibhava	39	29	28	13.9	8.7	0.83	62.5
Avrodhi	38	25	31	17.5	9.3	1.04	63.1
JG 218	42	28	22	13.1	6.2	1.17	47.3
Dohad yellow	38	38	24	20.1	10.3	1.08	51.2
PG 96006	39	26	29	12.8	6.9	1.02	52.9
C 235	49	29	24	13.3	7.2	1.01	54.1
Pusa 256	44	33	32	19.0	10.5	0.99	55.2
GNG 469	48	29	21	12.1	7.1	0.91	50.6
K 850	36	28	29	13.9	8.9	0.79	64.0
KWR 108	37	32	30	16.8	8.6	1.08	51.2
GNG 663	39	28	23	15.4	8.1	1.05	52.5
PUSA 244	40	20	27	15.9	8.6	1.02	54.0
IPC 98-12	37	25	24	19.2	7.6	1.34	39.5
PUSA 329	34	21	21	17.0	7.0	1.30	41.1
PUSA 240	36	35	26	11.4	8.3	0.60	72.8
Vijay	33	24	23	13.6	7.7	0.96	56.6
RSG 931	31	28	25	14.3	7.4	1.07	51.7
CSJD 884	36	22	18	13.0	5.7	1.24	43.8
PBG 5	39	19	23	13.6	7.6	0.98	55.8
GCP 101	32	26	20	10.6	6.2	0.92	50.4
JG 11	42	35	28	15.7	8.6	1.00	54.7
RSG 888	39	32	24	14.2	8.1	0.95	57.0
PG 5	44	34	26	13.3	8.2	0.85	61.6
CD (P = 0.05)	7.1	5.5	2.9	1.1	0.7		
CV (%)	11.3	11	7.1	4.5	5.7		

and semi-arid zones and high temperature stress particularly during active pod filling stage proved major limiting factor affecting its productivity. With this objective, plants of 30 chickpea genotypes were grown under normal (11 November 2008) and late (3 January 2009) sown conditions at CCS Haryana Agricultural University, Hisar, Haryana, India to screen their tolerance against high temperature (>30 °C) by studying various morpho-physiological traits. The canopy temperature depression (CTD) and relative stress injury (RSI %) exhibited significant differences among the genotypes under late sown environments. The CTD value was –2.1 °C in Pusa 240 and –4.8°C in IPC 98-12. RSI (%) value was highest in GG 2 (69%) and lowest (45%) in Pusa 240. Quantum yield, as indicated by F_v/F_m ratios was in the range of 0.157 to 0.352 for late sown plants. Genotype Pusa 240 also maintained maximum quantum yield (0.352) than others. Genotype Pusa 240 was found to be thermo-insensitive and showed the maximum tolerance against high temperature stress with least variation for seed yield, and was having heat susceptibility index (HSI) values less than one. This genotype also maintained high photochemical efficiency/quantum yield of PS-II, but low values of RSI (%) and having cooler canopies hence bears some characters which can be beneficial in breeding programme of chickpea for high temperature tolerance.

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