Ind J Plant Physiol. (April–June 2017) 22(2):164–177
DOI 10.1007/s40502-017-0301-4

ORIGINAL ARTICLE

Evaluation of chickpea (*Cicer arietinum* L.) genotypes for heat tolerance: a physiological assessment

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Received: 22 December 2016 / Accepted: 26 June 2017 / Published online: 13 July 2017

Abstract A field experiment was carried out in factorial randomized block design to evaluate 56 chickpea (*Cicer arietinum* L.) genotypes for high temperature tolerance. High temperature was imposed by delaying sowing dates i.e. normal (9th November) and late sowing (19th December). Under late sown condition, high temperature was experienced by crop starting from flowering stage to crop maturity (during this period maximum temperature ranged from 25 to 40 °C). Chickpea genotypes were assessed based on various physiological tests. A significant genotypic variability was recorded in relative water content, membrane stability index, canopy temperature depression (CTD), photosynthetic pigments, photosynthetic rate (P_N), canopy photosynthesis, growth, and yield based indices. In general, late sown high temperature stress condition significantly reduced all the physiological, growth and yield parameters except CTD. For each trait promising genotypes under late sown (high temperature) condition were identified. Furthermore, photosynthetic pigment profile under late sown high temperature condition at podding stage was analyzed using thin layer chromatography and that also revealed the genotypic variations. Tolerant genotypes in general maintained darker bands and also showed more number of photosynthetic pigments than relatively sensitive ones. In addition to this, total carotenoids content, under late sown condition at podding stage exhibited significant positive association with heat tolerance index (HTI), CTD, rate of photosynthesis and total chlorophyll content. That in turn indicated that higher level of total carotenoids played important role to maintain heat tolerance under late sown high temperature condition by protecting the photosynthetic machinery. In general, genotypes identified for high temperature tolerance based on HTI, heat susceptibility index (HSI) and heat yield stability index (HYSI), also had better physiological performance as evident from higher values of almost all physiological parameters recorded during the present study. Further, on the basis of all over performance, eight genotypes Pusa 1103, Pusa 1003, KWR 108, BGM 408, BG 240, PG 95333, JG 14, BG 1077 proved to be high temperature tolerant (HSI $\leq$ 0.9, HTI $\geq$ 0.59 and HYSI $\geq$ 50%).

Keywords Chickpea · High temperature · Heat tolerance · Physiological traits · Stress indices

Introduction

Chickpea being a winter season crop, often experiences abnormally high temperature (>35 °C) during its reproductive phase. Terminal drought and high temperature stress are major constraints to chickpea production in warmer short season environments. Chickpea area under late sown high temperature condition is increasing particularly in Northern and Central region due to inclusion of chickpea in new cropping system and intense sequential cropping practice leading to prolonged exposure of chickpea to high temperature stress (Krishnamurthy et al. 2011). According to an estimate approximately 11.7 million ha area in India, presently remains fallow due to late harvest of rice (Subbarao et al. 2001). These aforesaid fallow lands may be utilized to expand chickpea cultivation, provided the genotypes are capable of standing in heat stress

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condition. Flowering and podding are the sensitive stages to high temperature in chickpea. Drastic reduction in chickpea yield has been observed when plants were exposed to high temperature (30–35 °C) at flowering and pod development stages (Summerfield et al. 1984; Wang et al. 2006). In north India, chickpea yield decreased by 53 kg/ha in Utter Pradesh and 301 kg/ha in Haryana per 1 °C increase in seasonal temperature (Kalra et al. 2008).

High temperature is highly injurious to leguminous crop as it directly affects the physiological processes and indirectly grain yield (Stoddard et al. 2006). Therefore, physiological trait-based breeding approach is gaining the importance as it raises the probability of crosses resulting in additive gene action (Reynolds and Trethowan 2007; Wasson et al. 2012). A number of physiological traits and yield based indices are associated with genotypic performance under high temperature condition and can be used as screening techniques under field conditions (Porch 2006; Wahid et al. 2007; Devasirvatham et al. 2012; Kumar et al. 2013). In order to minimize yield losses in chickpea caused by late sowing, there is need to search chickpea genotypes that have increased heat tolerance under late sown condition. Therefore, present study was carried out with an objective to select genotypes among the existing chickpea germplasm with in-built tolerance to terminal heat stress with late planting so as to utilize them for understanding the physiological basis of heat tolerance in subsequent studies and of course for the chickpea yield improvement programme under late sown conditions.

Materials and methods

A field trial using 56 diverse genotypes of chickpea was conducted in factorial randomized block design with three replicates at IARI research farm, New Delhi. Their seeds were obtained from Division of Genetics, IARI, New Delhi. High temperature was imposed during reproductive/terminal phase of crop by altering the sowing dates i.e. normal sowing (9th November) and over 1 month late to normal sown (19th December). Plot size having six rows for each entry was kept 10 m^2. All the standard agronomic packages of practice were used to raise healthy crop. Daily metrological data were recorded for the entire crop duration. During the present study the physiological traits were recorded at flowering and podding stages while biomass and yield traits at harvest.

Relative water content

Relative water content was estimated following the method given by Weatherly (1950). Leaf related water content (RWC) was estimated using fully expanded third or fourth leaf from the top by recording the turgid weight of 0.5 g fresh leaf sample by keeping in distilled water for 4 h, followed by drying in hot air oven at 70 °C until constant weight was achieved. RWC (%) was calculated by using the following formula:

$$\text{RWC}\,(\%) = (\text{FW} - \text{DW}/\text{TW} - \text{DW}) \times 100$$

where, FW = Fresh weight (g); TW = Turgid weight (g); DW = Dry weight (g).

Membrane stability index (MSI)

For estimating temperature tolerance, conductivity tests were carried out by using the method as described earlier (Blum and Ebercon 1981). 100 mg leaf tissue of fully expanded fourth leaf from the top was weighed in three replicates and placed in a test tube containing 10 ml of double distilled de-ionized water. These tubes were incubated at 40 °C for half an hour in a water bath. Then initial electrical conductivity (C_1) of this solution was measured with the help of conductivity meter. These test tubes were kept in boiling water at 100 °C for 10 min and cooled at room temperature and final electrical conductivity (C_2) was measured again. Percent conductivity was used to calculate membrane stability index using following formula:

$$\text{Membrane Stability Index}\,(\%) = 1 - (C_1/C_2) \times 100$$

where, C_1 = Initial electrical conductivity (μS) at test temperature (40 °C); C_2 = Final electrical conductivity (μS) at 100 °C

Canopy temperature depression

Canopy temperature was recorded by using Infrared thermometer (model infrared and K-type, IR 10 model China). Canopy temperature depression (CTD) was calculated by using the following formula:

$$\text{CTD} = \text{Ambient atmospheric temperature}\,(^\circ\text{C}) - \text{Canopy temperature}\,(^\circ\text{C})$$

Measurement of the rate of photosynthesis

Observations on leaf photosynthesis were recorded on fully developed 4th leaf from the top by using LI-COR portable photosynthesis system (IRGA LI-6400 model, LI-COR, Nebaraska, USA) between 10.00 AM to 11.30 AM during clear day by providing artificial light source 1000 μmol m^{-2} s^{-1}. The rate of photosynthesis (μmol CO_2 m^{-2} s^{-1}) and stomatal conductance (mmol H_2O m^{-2} s^{-1}) were recorded by operating the Infrared gas analyzer (IRGA) in the closed mode. Canopy photosynthesis (CP_N) was estimating by multiplying photosynthesis rate (P_N) with leaf area index (LAI) i.e. $CP_N = P_N \times LAI$.

Estimation of chlorophyll and carotenoids content

Chlorophyll and carotenoids content were measured as per the method described by Hiscox and Israelstam 1979. The procedure for estimation of chlorophyll content in plants is based on the absorption of light by chlorophyll extracts prepared by incubating the leaf tissues in DMSO (dimethyl sulfoxide). DMSO renders plasmalemma permeable thereby, causing the leaching of the pigments (Hiscox and Israelstam 1979). The absorbance of the known volume of solution containing known quantity of leaf tissue at two respective wavelengths (663 and 645) was determined for chlorophyll content and at 480 nm for total carotenoid contents. Chlorophyll a, chlorophyll b and total chlorophyll content were estimated using the formula given by Arnon (1949) while carotenoid content was determined by following the formula given by Lichtenthaler and Welburn 1983. Thirty mg fresh leaf samples were added to the test tubes containing 4 ml DMSO. Tubes were kept in dark for 4 h at 65 °C. Then the samples were taken out cooled at room temperature and the absorbance was recorded at 663, 645 and 480 nm using DMSO as blank and was expressed as mg g^{-1} dry wt.

$$\begin{aligned}
\text{Chlorophyll } 'a' &= (12.7 \times A_{663} - 2.69 \times A_{645}) \times V/W \times 1000 \\
\text{Chlorophyll } 'b' &= (22.9 \times A_{645} - 4.68 \times A_{663}) \times V/W \times 1000 \\
\text{Total chlorophyll} &= (20.2 \times A_{645} + 8.02 \times A_{663}) \times V/W \times 1000\,. \\
\text{Total carotenoids} &= (A_{480} + (0.114 \times A_{663}) - (0.638 - A_{645})) \\
&\quad \times V/W \times 1000
\end{aligned}$$

where,

A_{663} = Absorbance at 663 nm W = Weight of the sample in g
A_{645} = Absorbance at 645 nm V = Volume of the solvent used (ml)
A_{480} = Absorbance at 480 nm

Photosynthetic pigments profiling using thin layer chromatography (TLC)

Separation of pigments by TLC was done according to the method described by Pocock et al. (2004) with minor modifications.

Extraction of leaf pigments

Fresh leaves (5 g) were grinded in a mortar and pestle with 20 ml acetone, 3 ml petroleum ether and little quantity of calcium carbonate. The homogenate was filtered through Whatman filter paper No. 1. Then this filtrate was transferred to a separating funnel and 5% NaCl and 5 ml petroleum ether were added to it. The mixture in a separating funnel was shaken carefully and was partitioned using separating funnel. The upper layer was collected and washed three to four times with double distilled water. The final extract was evaporated in a cool and dark place and the volume was made up to 2 ml using acetone.

Application of extract to the TLC plate

The TLC plate was kept in the oven for 3–4 h at 90–100 °C in order to remove any traces of moisture present in it. A line was drawn on the plate with pencil (1.5 cm above bottom) and the extract was applied on it. The spot was dried thoroughly. Then the plate was kept in the TLC chamber consisting of petroleum ether, acetone and distilled water based solvent system as described elsewhere (Pocock et al. 2004) with little modifications. The chromatogram was removed when the solvent went 15 cm above from the origin and it was immediately photographed. For the identification of the different photosynthetic pigments, bands of pigments from TLC plate were scratched and eluted using acetone by centrifuging at 3000 g for 10 min. Then the spectra of each band were drawn with the help of UV–Visible spectrophotometer. Thus, based on the spectra and R_f value photosynthetic pigments were identified.

Leaf area and leaf are index

Leaf area was measured by using leaf area meter (model LI-3100, USA) and was expressed as cm^2 $plant^{-1}$ while leaf area index (LAI) was calculated by using the following formula:

$$\text{LAI} = \text{Leaf area}/\text{Ground area}$$

Yield and yield indices

Yield and yield attributes were recorded at harvest. Heat susceptibility index (HSI), heat tolerance index (HTI) and heat intensity index (HII) was calculated using the formula described earlier by Porch 2006.

$$HSI = \left\{1 - \left(\frac{Y_s}{Y_p}\right)/1 - \left(\frac{X_s}{X_p}\right)\right\}$$

$$HTI = \left(Y_p \times Y_s\right)/X_p^2$$

$$HII = 1 - \left(\frac{X_s}{X_p}\right)$$

where, Ys and Yp indicate genotypic yield under stress and non-stressed conditions respectively and Xs and Xp are the mean yield of all genotypes per trial under stress and non stress condition.

Heat yield stability index (HYSI) was calculated using the following formula given by (Bouslama and Schapaugh 1984).

$$\text{HYSI} = (\text{Grain yield under stress}/\text{Grain yield under normal condition}) \times 100$$

Results and discussion

Variation in weather

Under late sown condition, high temperature coincided with crop from its flowering stage onwards. Under normal sown condition maximum temperature (Fig. 1A) from flowering to crop maturity was recorded in the range of 20–30 °C while under late sown condition maximum temperature was recorded from 25–40 °C. Under normal sown condition mean temperature (Fig. 1B) from flowering stage to maturity was recorded in the range of 13–25° C while under late sown condition it was recorded in the range of 17–30 °C. Similarly, under normal sown condition minimum temperature (Fig. 1C) from flowering to maturity was recorded in the range of 5–15° C while under late sown condition minimum temperature varied from 10–22 °C.

Variation in physiological traits

All the physiological traits showed enormous variation under both normal and late sown high temperature conditions. Physiological and yield performance of chickpea genotypes recorded under normal and late sown high temperature conditions are given in subsequent paragraphs.

Relative water content [RWC (%)]

Significant genotypic variation in RWC (%) was recorded under normal and late sown conditions at both flowering and podding stages and late sown condition reduced RWC (%). Generally, high temperature stress is frequently associated with reduced water availability under field conditions (Simoes-Araujo et al. 2003). RWC (%) decreased due to increase in transpiration under late sown high temperature condition (Tsukaguchi et al. 2003; Wahid and Close 2007).

The overall distribution of accessions for their RWC (%) at flowering stage was normal with maximum frequency of accessions at RWC (%) class intervals between 70 and 75 under normal sown condition while under late sown high temperature condition it was shifted between 65 and 70 (Fig. 2A). However, at podding stage, the overall distribution of accessions for their RWC (%) had maximum frequency of accessions was recorded at class intervals between 65 and 70 under normal sown and late sown condition with shifting of accessions frequency towards lower class intervals under late sown conditions (Fig. 2A) because high temperature enhances transpiration rate that results decrease in RWC (Wahid and Close 2007).

Membrane stability index [MSI (%)]

Similarly, significant genotypic variation in MSI (%) was recorded under both normal and late sown conditions at flowering and podding stages and late sown high temperature condition significantly reduced MSI (%).Similar, genotypic variability in MSI was also reported earlier in chickpea (Srinivasan et al. 1996; Tongden et al. 2006).

The overall distribution of accessions for their MSI (%) at flowering stage was normal with maximum frequency of accessions at MSI (%) class intervals between 85 and 90 under normal sown condition while under late sown condition it was shifted between 80 and 85 (Fig. 2B). However, at podding stage, the overall distribution of accessions for their MSI (%) showed maximum frequency of accessions was recorded at class intervals between 80

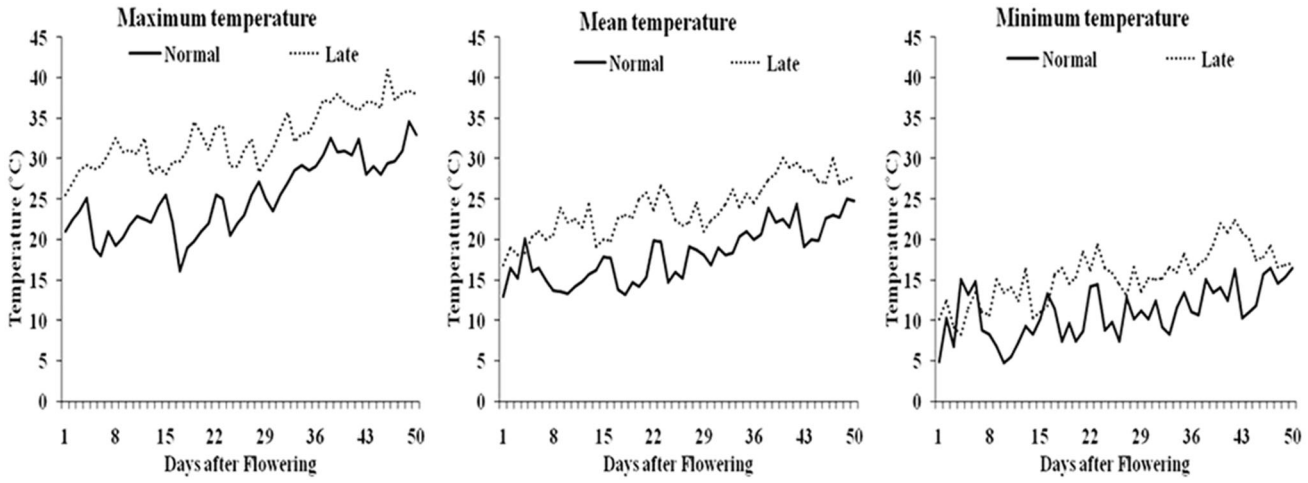

Fig. 1 Maximum, mean and minimum temperature recorded from flowering stage to crop maturity under both normal and late sown conditions

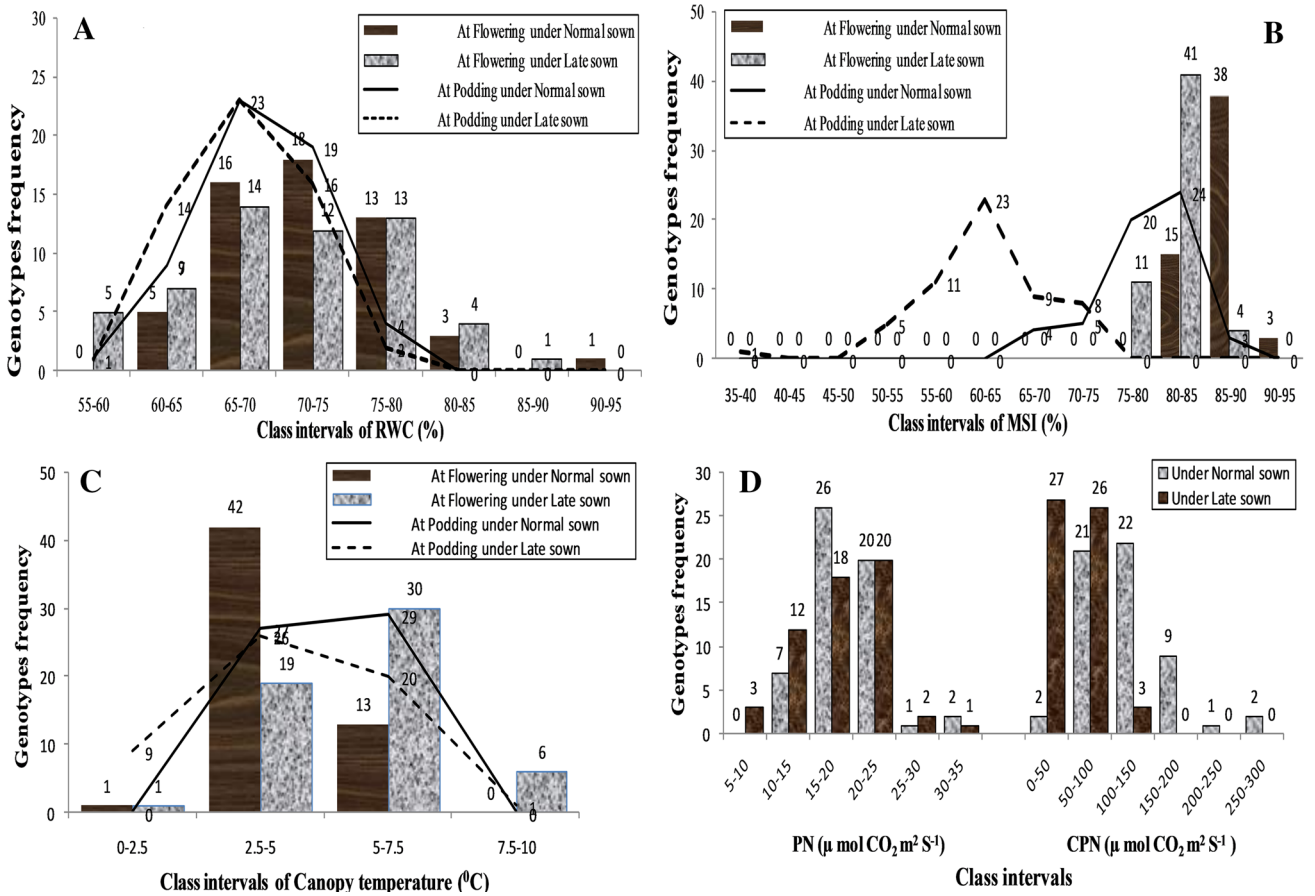

Fig. 2 Frequency distribution of the RWC (%) (**A**), MSI (**B**), CTD (**C**) and rate of photosynthesis (P_N) & canopy photosynthesis (CP_N) (**D**) by chickpea genotypes under normal sown and late sown high temperature conditions

and 85 under normal sown and late sown condition with shifting of accessions maximum frequency towards lower class intervals 60–65 under high temperature conditions (Fig. 2B). Under late sown condition, shifting of accessions frequency towards lower side was due to damage in membranes due to exposure to high temperature. The increased solute leakage, as an indication of decreased cell membrane thermostability (CMT), has long been used as an indirect measure of heat-stress tolerance in diverse plant species, including soybean (Martineau et al. 1979), potato and tomato (Chen et al. 1982), wheat (Blum et al. 2001), maize (Ashraf and Hafeez 2004) and barley (Wahid and Shabbir 2005).

Canopy temperature depression (CTD)

Significant genotypic variations in canopy temperature depression were recorded under both normal and late sown conditions. By and large distribution of accessions for their CTD at flowering stage was normal with maximum frequency of accessions at CTD class intervals between 2.5 and 5.0 under normal sown condition while under late sown condition it was shifted between 5.0 and 7.5 (Fig. 2C). Under late sown condition, shifting of accessions frequency towards higher side was because of higher transpiration due to exposure of crop to high temperature. However, at podding stage, the overall distribution of accessions for their CTD exhibited maximum frequency of accessions at class intervals between 5.0 and 7.5 under normal sown and late sown condition with shifting of accessions maximum frequency towards lower class intervals 2.5–5.0 under high temperature conditions (Fig. 2C). Similar observations were recorded earlier in chickpea (Purushothamana et al. 2015).

Photosynthetic rate

Genotypic variation in photosynthetic rate among chickpea genotypes was recorded. Late sowing reduced photosynthetic rate of genotypes. Heat stress directly affects photosynthesis including photosystem II in chickpea (Srinivasan et al. 1996). Photochemical reactions in thylakoid lamellae and carbon metabolism in the stroma of chloroplast have been suggested as the primary sites of

injury at high temperatures (Wise et al. 2004). Plants grown under high temperature stress condition have a lower stomatal conductance in order to conserve water. Consequently, CO_2 fixation is reduced and photosynthetic rate decreases, resulting in less assimilate production for growth and yield of plants (Wahid et al. 2007). The overall distribution of accessions was normal for their photosynthesis rate at flowering stage under both normal and late sown condition and maximum frequency of accessions lies between 15 and 20 class intervals of photosynthetic rate (Fig. 2D).

Canopy photosynthesis rate

Similarly, genotypic variation in canopy photosynthesis rate was recorded. Reduction in canopy photosynthesis under late planting was due to decrease in leaf area under heat stress. By and large distribution of accessions was normal for their canopy photosynthesis rate at flowering stage under normal sown condition and maximum frequency of accessions lies between 100 and 150 class intervals of canopy photosynthetic rate. However, under late sown condition distribution of accessions was positively skewed for their canopy photosynthesis rate as maximum frequency of accessions lies between 0 and 50 class intervals of canopy photosynthetic rate because of reduction in leaf area under late sown high temperature condition (Fig. 2D).

Chlorophyll a (Chl_a) content

Under late sown high temperature condition, significant genotypic variation and reduction in chl_a was recorded particularly at podding stage. Reduction in chl_a under late sowing was due to destruction in photosynthetic machinery (Xu et al. 1995). In general distribution of accessions for their Chl_a content at both flowering and podding stage was positively skewed with maximum frequency of accessions at class intervals between 5 and 10 under normal sown condition while under late sown condition distribution of accessions for their Chl_a content at both flowering and podding stage was negatively skewed with maximum frequency of accessions at class interval of 5–10 (Fig. 3A).

Chlorophyll b (Chl_b) content

Similarly, chickpea genotypes showed significant genotypic variation in Chl_b. Significant reduction in Chl_b content under late sown high temperature condition particularly at podding stage was probably due to adverse change/destruction in photosynthetic machinery (Xu et al. 1995).Under normal sown condition, distribution of accessions for their Chl_b content at flowering was positively skewed with maximum frequency of accessions at class intervals between 1.0 and 1.5 while under late sown condition distribution of accessions was normal for their Chl_a content at flowering stage with maximum frequency of accessions at class intervals between 1.5 and 2.0. At podding stage, under normal and late sown conditions distribution of accessions was normal for their Chl_b content with maximum frequency of accessions at class intervals between 1.5–2.0 and 1.0–1.5 respectively (Fig. 3B).

Total chlorophyll content

Genotypic variation in total chlorophyll was recorded and a significant reduction in its content was observed under late sown high temperature condition particularly at podding stage. Genotypic variation in photosynthetic pigments have also been reported in wheat (Kumar et al. 2013).The overall distribution of accessions was normal for their total chlorophyll content at flowering stage under both normal and late sown condition with maximum frequency of accessions at class intervals between 10 and 15 while at podding stage distribution of accessions for their total chlorophyll content under normal sown was positively skewed shifted and under late sown condition it was negatively skewed with maximum frequency of accessions at class intervals between 5 and 10 (Fig. 3C). Chlorophylls are reduced under heat stress due the inhibition of their synthesis and oxidation caused by reactive oxygen species (Van Hasselt and Strikwerda (1976).

Total carotenoids content

Similarly, genotypic variations in total carotenoids and its reduction under late planting were noted in chickpea genotypes. The overall distribution of accessions was normal for their total carotenoids content at flowering under normal sown and late sown condition with maximum frequency of accessions at class interval 2–3. At podding stage distribution of accessions for their total carotenoids content under normal sown condition was it was positively skewed under late sown condition it was negatively skewed with the shifting towards lower side with maximum frequency of accessions at class interval between 1 and 2 (Fig. 3D).

Chl_a/chl_b ratio

Under normal sown condition, Chl_a/chl_b ratio at flowering stage varied from 1.43 (Pusa 261) to 9.47 (Pusa 1103) while under late sown condition it varied from 4.07 (BGD 72) to 7.18 (ICSN K34). Similarly Chl_a/chl_b at podding stage under normal condition varied from 2.15 (PDG

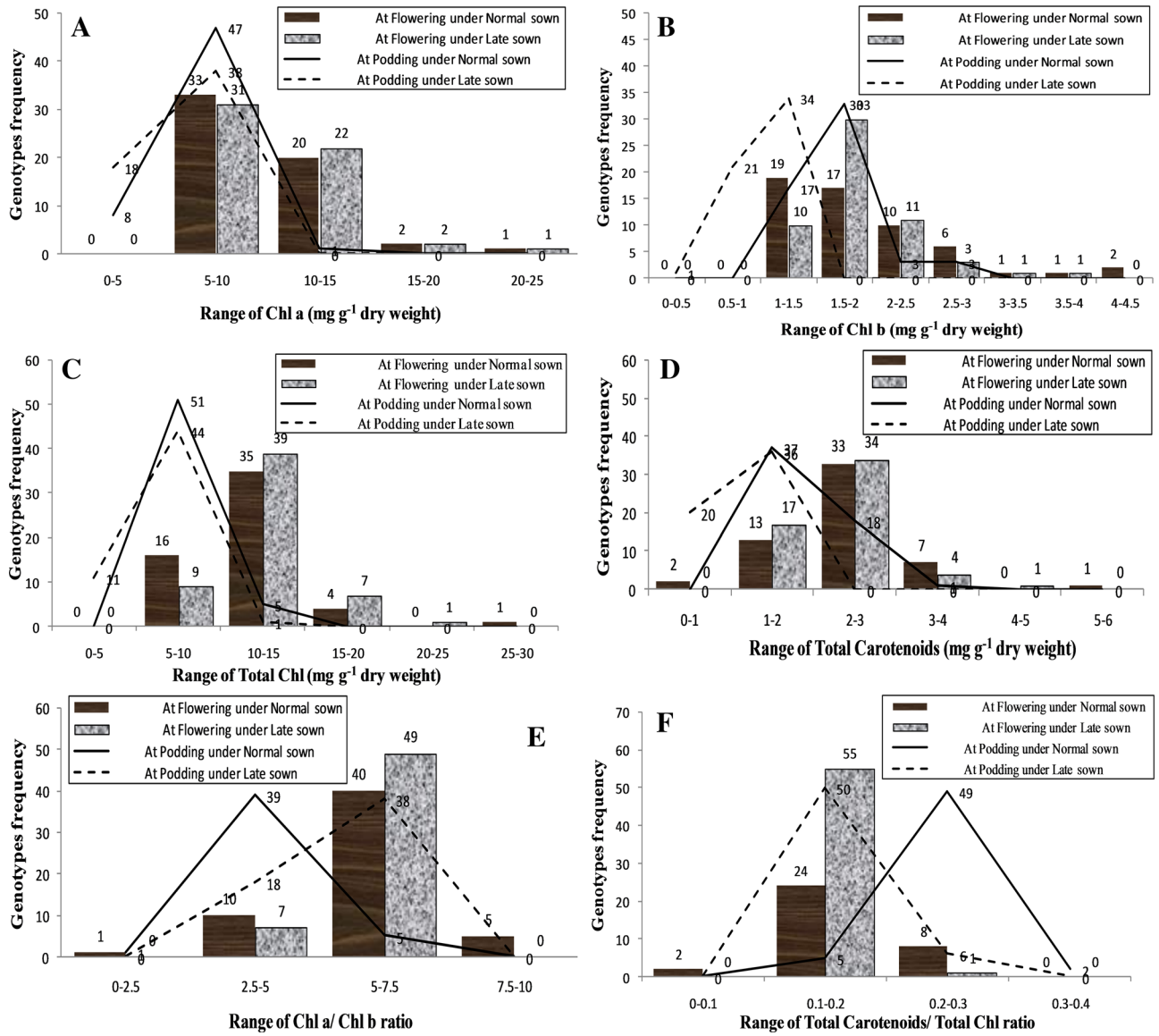

Fig. 3 Frequency distribution of the chlorophyll a (**A**), chlorophyll b (**B**), total chlorophyll (**C**), total carotenoids (**D**) contents and the ratio of Chla/Chlb (**E**) and total chlorophyll/total carotenoids (**F**) by chickpea genotypes under normal sown and late sown high temperature conditions

84-16) to 6.67 (RSG 888) while under late sown condition, it ranged from 4.29 (PG 96006) to 6.45 (Pusa 1003). In contrasting genotypes of tomato and sugar cane an increased chlorophyll *a*:*b* ratio were observed in the tolerant genotypes under high temperatures, indicating that these changes were related to thermotolerance of tomato (Camejo et al. 2005; Wahid and Ghazanfar 2006). The overall distribution of accessions was normal for their chl_a/chl_b ratio at both flowering and podding stages under normal sown condition with maximum frequency of accessions at class interval between 5.0–7.5 and 2.5–5.0 respectively. However, under late sown condition at both flowering and podding stages distribution of accessions was positively skewed for their chl_a/chl_b ratio with maximum frequency of accessions at class interval 5.0–7.5(Fig. 3E).

Total carotenoids/total chlorophylls ratio

Total carotenoids/total chlorophylls ratio showed genotypic variability and appreciable reduction under late sown condition due to high temperature exposure. Higher ratio of chlorophyll:carotenoids were made in the tolerant genotypes of tomato and sugar cane under high temperatures which indicated that these changes were related to thermotolerance (Camejo et al. 2005; Wahid and Ghazanfar 2006). The overall distribution of accessions was normal for their total carotenoids/total chlorophylls ratio at

flowering stage and positively skewed at podding stage under normal sown condition with maximum frequency of accessions at class interval between 0.1–0.2 and 0.2–0.3 respectively. However, under late sown condition at both flowering and podding stages distribution of accessions was negatively skewed for their ratio with maximum frequency of accessions at class interval between 0.1 and 0.2 (Fig. 3F).

Photosynthetic pigment profile

Under late sown high temperature condition during podding photosynthetic pigment profiling was done using thin layer chromatography (TLC). Chlorophyll a, chlorophyll b, pheophytin a, pheophytin b, β carotene, lutein, zeaxanthin, neaxanthin were seen in pigments profile. In addition to this intermediates of chlorophyll a and chlorophyll b were also seen which have not been marked on TLC plates. Genotypic variation in photosynthetic pigment profile was also observed among chickpea genotypes. In general, tolerant genotypes maintained darker and higher number of bands as compared to sensitive ones. Amongst the fifty six genotypes under study, Pusa 256, Pusa 5028, Pusa 362, Pusa 1053, Pusa 1105, GNG 469, C 235, PG 96006, Pusa 261, BGM 408, BG 240, JG 14, HK-00-299, HK-94-134, H-00-108, BG 1077, ICC 4993 showed darker bands particularly of carotenoids (Fig. 4). Carotenoids protect cellular structures in various plant species under abiotic stress (Havaux 1998; Wahid and Ghazanfar 2006; Wahid 2007).

Plant height

Genotypic variation in plant height was observed under both normal and late sown conditions. Heat stress significantly reduced plant height under late sowing. High temperatures main effect on shoot growth is a severe reduction in the first internode length of plants (Hall 1992). The overall distribution of accessions was normal for their plant height and maximum frequency of accessions for plant height at flowering stage and podding stage under normal sown condition lies between 50 and 60 while under late sown condition for both flowering stage and podding stage highest frequency of accessions for plant height were recorded between 40 and 50 class intervals due reduction in plant height of accessions under high temperature condition (Fig. 5A).

Total dry matter

Genotypes showed genotypic variation in total dry matter and significantly reduced total dry matter under late sown high temperature condition particularly at podding stage.-High temperatures causes significant reduction in shoot dry mass, relative growth rate and net assimilation rate in maize, pearl millet and sugarcane (Ashraf and Hafeez 2004; Wahid 2007). By and large distribution of accessions was normal for their total dry matter and maximum frequency of accessions for their total dry matter at flowering stage under normal sown and late sown condition lies between 2.5 and 5.0 while at podding stage under normal sown maximum frequency of accessions for their total dry matter were recorded between 7.5 and 10.0 and under late sown condition, maximum frequency was shifted between 5.0 and 7.5 as crop coincided to high temperature condition (Fig. 5B).

Leaf area index

Genotypic variation in leaf area index (LAI) was recorded under both normal and late sown conditions. Late planting significantly reduced LAI particularly at podding stage. In general distribution of accessions was normal for LAI and maximum frequency of accessions for LAI at flowering stage under normal sown and late sown condition lies between 5.0–7.5 and 2.5–5.0 while at podding stage under both normal and late sown condition maximum frequency of accessions for LAI were recorded between 2.5 and 5.0 (Fig. 5C).

Yield and its associated traits

Chickpea genotypes showed significant genotypic variations in total dry matter, grain yield, harvest index, and test weight. Grain yield, total dry matter and test weight were significantly reduced under late sown high temperature stress condition.

Total dry matter (g m^{-2})

Under normal sown condition, TDM varied from 364.37 (BDG 132) to 1129.0 (Pusa 5023) and mean value was 765.21 while under late sown condition TDM varied from 156.97 (PDG 84-16) to 719.65 (BG 240) and mean value was 336.12. Craufurd et al. 2002 also found that high temperature reduced total dry weight by 20–35% in peanut genotypes.

Grain yield (g m^{-2})

Under normal sown condition grain yield *(g m^{-2})* was recorded to vary from 115.71 (PDG 84-16) to 396.67 (RSG 143-1) and mean yield was 259.50, while under late sown condition grain yield varied from 25.15 (ICC 4993) to 244.47 (JG 14) and mean yield was 119.12. High temperature stress can reduce crop yield by affecting both source and sink for assimilates (Mendham and Salisbury 1995; Devasirvatham et al. 2012). The decrease in grain length and width was found to be associated with a reduction in

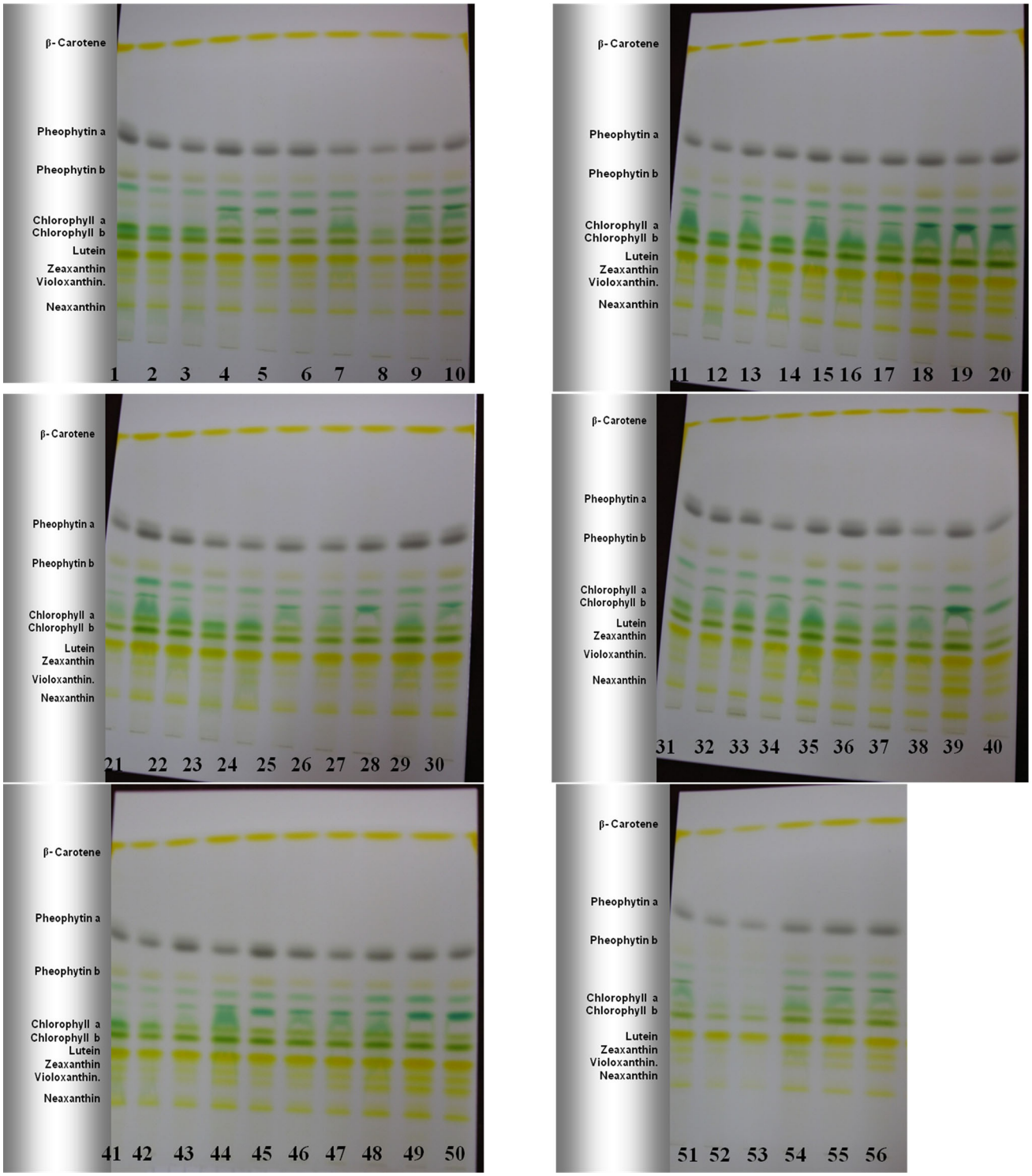

Fig. 4 Photosynthetic pigment profile of chickpea genotypes (lane *1–56* consist of following genotypes. *1* Pusa 112, *2* Pusa 1103, *3* RSG 991, *4* RSG 807, *5* Pusa 2024, *6* RSG 973, *7* Pusa 1108, *8* ICC 1882, *9* Pusa 256, *10* Pusa 372, *11* BGD 72, *12* BG 1088, *13* Pusa 5023, *14* Pusa 391, *15* BGD 1005, *16* BGM 547, *17* Pusa 5028, *18* Pusa 362, *19* Pusa 1053, *20* Pusa 1105, *21* Pusa 1003, *22* KWR 108, *23* RSG 888, *24* JG 11, *25* ICCV 10, *26* Pusa 3004, *27* GNG 469, *28* CSJD 884, *29* C 235, *30* PG 96006, *31* RSG 963, *32* Vijay, *33* Chaffa, *34* RSG 931, *35* RSG 143-1, *36* Pusa 212, *37* Pusa 261, *38* BGM 408, *39* BG 240, *40* ICSN K(34). *41* SBD 377, *42* IPC 92-1, *43* IG 20314-2, *44* PG 95333, *45* WR 315, *46* PDG 84-16, *47* JG 14, *48* HK-00-299, *49* HK-94-134, *50* H-00-108, *51* AKG-10, *52* BDG 132, *53* BDG 9812, *54* BG 1077, *55* ICC 4993, *56* Flip 87-82 C

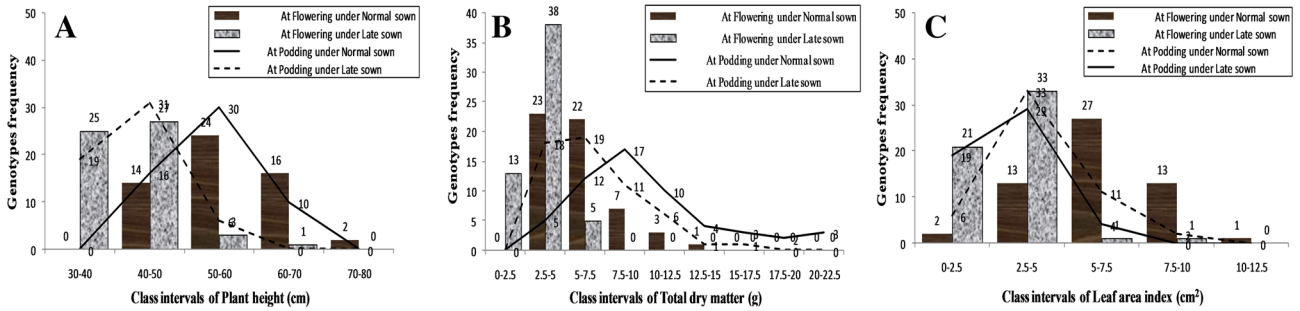

Fig. 5 Frequency distribution of the plant height (**A**), total dry matter (**B**) and leaf area index (**C**) by chickpea genotypes under normal sown and late sown high temperature conditions

the average endosperm cell area observed under high night temperature (Morita et al. 2005). Plants grown under high temperature stress condition have a lower stomatal conductance in order to conserve water. Consequently, CO_2 fixation is reduced and photosynthetic rate decreases, resulting in less assimilate production for growth and yield of plants (Wahid et al. 2007). The overall distribution of accessions was normal for grain yield and maximum frequency of accessions for grain yield at harvest under normal sown and late sown condition were recorded between 250–300 and 100–150 respectively (Fig. 6A). The shifting of the highest frequency of accessions for grain yield towards lower side under late sown high temperature condition was due to reduction in grain yield of sensitive genotypes (Fig. 6A).

Test weight (g)

Under normal sown condition test weight was recorded from 106.35 (ICCV 10) to 301.15 (ICSN K (34)) and mean value was 196.52. Under late sown condition test weight ranged from 65.94 (ICSN K (34)) to 262.48 (BG 1077) and mean test weight was 158.06. Similarly, in peanut genotypes, high temperature reduced seed dry weight by 23–78% (Craufurd et al. 2002). The overall distribution of accessions was normal for test weight and maximum frequency of accessions for test weight under normal sown and late sown condition were recorded between 200–250 and 100–150 respectively (Fig. 6A). The shifting of the highest frequency of accessions for test weight towards lower side under late sown condition was due to reduction in seed weight under heat stress (Fig. 6A).

Harvest index (HI)

The overall distribution of accessions was normal for HI (%) and maximum frequency of accessions for HI (%) at harvest under normal sown and late sown condition were recorded between 30 and 40 (Fig. 6B). The shifting of the distribution frequency of accessions for HI towards higher side under late sown high temperature condition was due to severe reduction in biomass of sensitive genotypes (Fig. 6B). Reduction in seed harvest index by 0– 65% was also estimated in peanut genotypes under high temperature condition (Craufurd et al. 2002).

Promising genotypes

Based on the performance of chickpea genotypes under late sown high temperature conditions promising genotypes were identified for physiological and yield traits are listed in Table 1

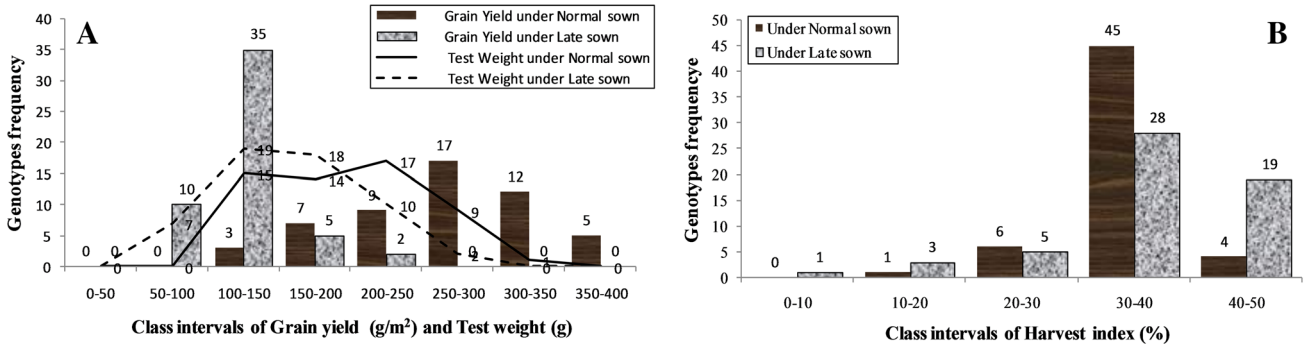

Fig. 6 Frequency distribution of the grain yield & test weight (**A**) and harvest index (**B**) by chickpea genotypes under normal sown and late sown high temperature conditions

Table 1 Promising genotypes of chickpea identified for different physiological and yield traits under late sown high temperature condition

S. no.	Traits	Promising genotypes
1.	RWC (%) (above 65)	Pusa 1103, RSG 991, Pusa 2024, RSG 973, ICC 1882, Pusa 256, Pusa 372, Pusa 391, BGD 1005, KWR 108, ICCV 10, C 235, RSG 963, Vijay, Chaffa, BGM 408, BG 240, ICSN K (34), SBD 377, IG 20314-2, PG 95333, WR 315, PDG 84-16, BG 1077, JG 14, BG 1077, Pusa 112, BG 1077, Pusa 391
2.	MSI (%) (above 70)	Pusa 1103, BG 1088, Pusa 5023, Pusa 362, Pusa 1105, Pusa 1003, RSG 888, Pusa 3004, IG 20314-2, AKG-10, Pusa 1003, KWR 108, BGM 408, BG 240, PG 95333, JG 14,, BG 1077,
3.	CTD (^{o}C) (above 5.0)	Pusa 112, Pusa 1103, Pusa 1108, BGD 1005, BGM 547, Pusa 5028, Pusa 362, Pusa 1105, Pusa 1003, KWR 108, RSG 888,, BGM 408, BG 240, IG 20314-2, PG 95333, WR 315, JG 14, H-00-108, BDG 9812, BG 1077
4.	Total Chlorophyll (mg g^{-1} d wt) (above 7.0)	Pusa 112, Pusa 1103, SBD 377, RSG 991, BGD 1005, Pusa 5028, Pusa 256, Pusa 362, Pusa 1053, Pusa 1105, Pusa 1003, JG 11, Pusa 3004, Vijay, Chaffa, Pusa 212, Pusa 261,BG 240, SBD 377, IG 20314-2, PG 95333, WR 315, JG 14, HK-00-299, HK-94-134, Pusa 1003, KWR 108, PG 96006, HK-94-134, H-00-108, BG 1077, ICC 4993, GNG 469, Pusa 1105, BGM 408, C 235,
5.	Total Carotenoids (mg g^{-1} d wt) (above 1.25)	Pusa 1003, BGM 408, BG 240, JG 14, HK-00-299, HK-94-134, BG 1077, ICC 4993, Pusa 112, Pusa 1103, RSG 991, RSG 807, Pusa 391, BGD 1005, Pusa 362, KWR 108, Vijay, RSG 143-1, Pusa 212, PG 95333, PG 96006, H-00-108, ICC 4993, C 235
6.	Leaf area index (LAI) (above 3.0)	Pusa 112, Pusa 1103, Pusa 2024, Pusa 256, BGD 72, BG 1088, Pusa 391, BGD 1005, BGM 547, Pusa 5028,Pusa 362, Pusa 1053, KWR 108, BGM 408, BG 240, PG 95333, Pusa 1105, RSG 888, JG 11, ICCV 10, Pusa 3004, PG 96006, RSG 963, Vijay, Chaffa, RSG 143-1, Pusa 261, Pusa 1003, Pusa 1108
7.	P_N (μmol CO_2 $m^{-1}S^{-1}$) (above 20.0)	Pusa 1103, RSG 991, Pusa 1108, Pusa 256, BGD 72, BG 1088, JG 11, ICCV 10, RSG 963, Vijay, Chaffa, RSG 143-1, Pusa 212, Pusa 261, BGM 408, BG 240, IPC 92-1, IG 20314-2, PG 95333, JG 14, Pusa 1003
8.	CP_N (μmol CO_2 $m^{-1}S^{-1}$) (above 60.0)	Pusa 112, RSG 991, Pusa 2024, Pusa 256, BGD 72, BG 1088, BGD 1005, Pusa 5028, Pusa 362, Pusa 1053, KWR 108, JG 11, ICCV 10, PG 96006, Chaffa, BGM 408, BG 240, PG 95333, JG 14, Pusa 1003
9.	TDM (g m^{-2}) (above 400)	RSG 807, KWR 108, Pusa 212, Pusa 261, BGM 408, BG 240, ICSN K (34), PG 95333, JG 14, H-00-108
10.	Grain yield (g m^{-2}) (above 150)	RSG 807, Pusa 1003, KWR 108, BG 240, PG 95333, JG 14, BG 1077
11.	HI (%) (above 40)	Pusa 1103, Pusa 2024, RSG 973, Pusa 372, BGD 72, Pusa 362, Pusa 1105, Pusa 1003,RSG 888,ICCV 10, Pusa 3004, C 235, Chaffa, RSG 931, IG 20314-2, WR 315, JG 14, BDG 9812, BG 1077
12.	Test wt. (g) (above 200)	Pusa 2024, Pusa 1108, BG 1088, Pusa 5023, Pusa 5028, Pusa 1105, Pusa 1003, IPC 92-1,IG 20314-2, PG 95333, WR 315, BDG 132, BG 1077

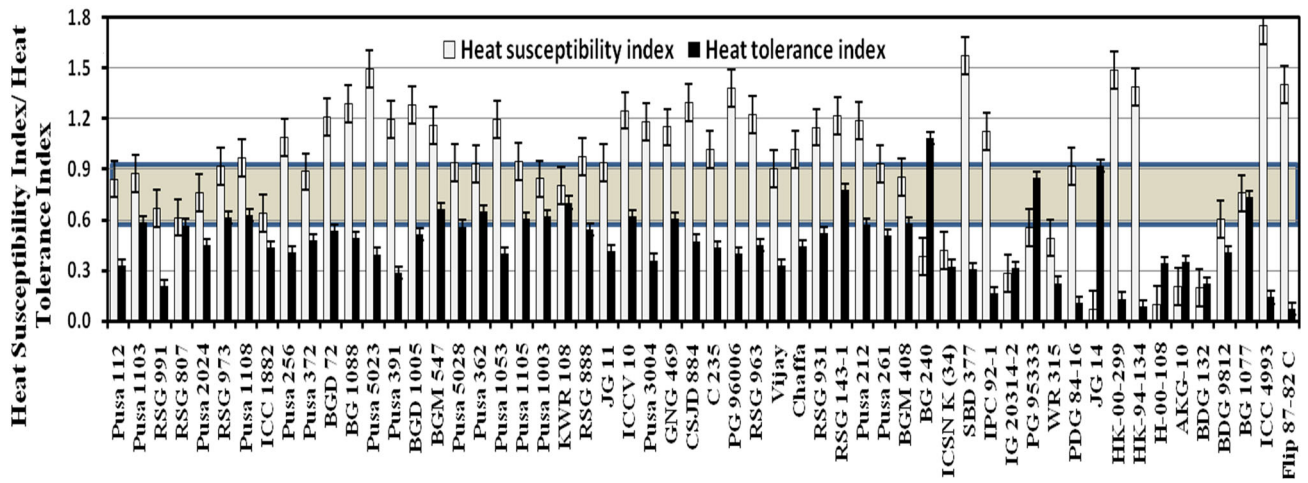

Fig. 7 Genotypic variability in heat susceptibility index and heat tolerance index

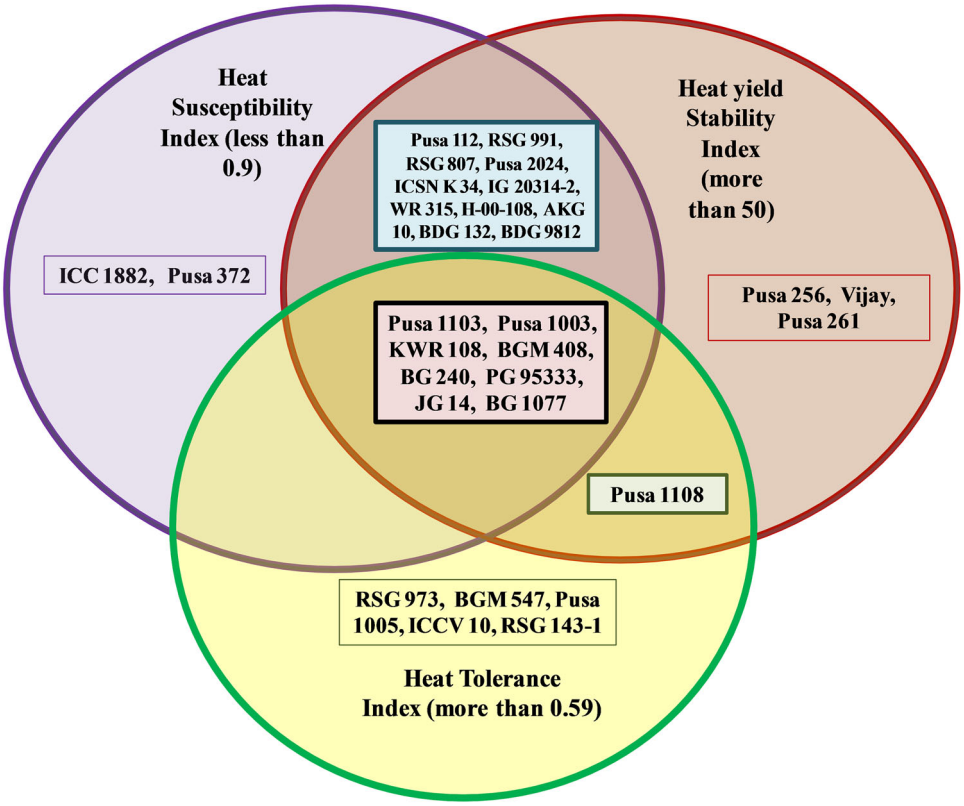

Fig. 8 Promising genotypes identified on basis of heat susceptibility index, heat tolerance index and heat yield stability index

Stress indices

Significant genotypic variation was recorded in heat susceptibility index (HSI), heat tolerance index (HTI) and heat yield stability index (HYSI) under late sown condition. Under late sown condition heat intensity index (HII) was estimated to be 0.54. HSI was recorded to vary from 0.10 (H-00-108) to 1.75 (ICC 4993) and the mean value was 0.95. HTI was recorded from 0.07 (Flip 87-82C) to 1.08 (BG 240) and mean value was 0.46 (Fig. 7). Heat susceptibility is responsible for yield loss in chickpea (Wang et al. 2006).

HYSI was recorded to vary from 6.71 (ICC 4993) to 96.21 (JG 14) and mean value was 49.56. Heat tolerant genotypes were selected based on their performance in terms of HSI (less than 0.9) and HTI (more than 0.59) and thus following genotypes were identified promising for heat tolerance, Pusa 1103, Pusa 1003, KWR 108, BGM 408, BG 240, PG 95333, JG 14, BG 1077 (Fig. 8). In addition, aforementioned genotypes also maintained higher level of HYSI under late sown high temperature condition.

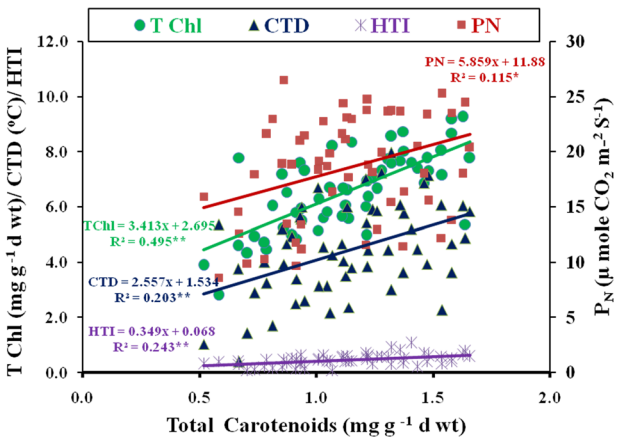

Fig. 9 Association of total carotenoids with total chlorophyll (TChl), canopy temperature depression (CTD), heat tolerance index (HTI) and photosynthesis rate (P_N)

Association of total carotenoids with physiological and yield traits

Total carotenoids relationship with photosynthetic rate, total chlorophyll content, canopy temperature depression

and heat tolerance index was analyzed that showed significant positive association with the rate of photosynthesis, total chlorophyll content, canopy temperature depression and heat tolerance index (Fig. 9). Significant positive associations in turn indicated the protective role of carotenoids in heat tolerance by protecting the photosynthetic machinery and maintaining better physiological adaptability of plants under high temperature stress condition (late sown) because carotenoids protect cellular structures under abiotic stress (Havaux 1998; Wahid and Ghazanfar 2006; Wahid 2007).

Conclusion

A appreciable genotypic variability was recorded in all physiological, growth, and yield based indices. Due to heat stress, late planting significantly reduced all the physiological, growth and yield parameters except canopy temperature depression due to heat stress. Further, genotypes identified for high temperature tolerance based on heat tolerance index, heat susceptibility index and heat yield stability index, also had better physiological performance as evident from higher values of almost all physiological parameters recorded during the present study. On the basis of all over performance, eight genotypes Pusa 1103, Pusa 1003, KWR 108, BGM 408, BG 240, PG 95333, JG 14, BG 1077 proved to be heat tolerant.

Acknowledgements The authors thank the IARI, New Delhi for providing necessary facility and Council of Scientific and Industrial Research, New Delhi for financial support (CSIR Project No.: 38(1335)/12/EMR).

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