Brazilian Journal of Botany (2018) 41:551–565
https://doi.org/10.1007/s40415-018-0484-6

ORIGINAL ARTICLE

Analysis of thermotolerance behaviour of five chickpea genotypes at early growth stages

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Received: 22 August 2017 / Accepted: 4 July 2018 / Published online: 14 July 2018
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Abstract
Global warming has negative effects on agriculture which affect different crop species around the world. In different geographical conditions, temperature is an important factor for crop growth. Chickpea is a heat-sensitive crop, and heat stress affects chickpea production across the globe. In the present study, effects of heat stress on different chickpea genotypes have been studied. Five different chickpea genotypes viz. C-235, CSJD-884, RSG-888, GNG-1581, and RSG-895 have been used to analyse the heat stress effect and thermotolerance behaviour of these genotypes at early growth stages under three different temperature regimes, i.e. 30, 35 and 40 °C. Different growth parameters were analysed at different time intervals after heat stress followed by 10 days of recovery period at 25 °C. Seedling length in control versus stressed plant was estimated after 48 h and 72 h, while branching, cotyledon colour and shoot colour were observed after 96 h. Root length, shoot length, relative water content (RWC) and photosynthetic pigments (chl *a*, chl *b* and carotenoids) were examined after recovery period. A comparative analysis among all the selected genotypes was carried out to predict the thermotolerance behaviour. Heat stress under 40 °C showed the lethal effect on growth of the plant after 96 h. Seedling length and branching of roots were increased under heat stress as compared to control. Photosynthetic pigments as well as RWC were negatively affected under heat stress as compared to control. In conclusion, genotypes CSJD-884 and RSG-895 showed thermotolerance behaviour with the highest growth rate, RWC and photosynthetic pigments and C-235 variety was the most sensitive genotype under heat stress.

Keywords Abiotic stress · *Cicer arietinum* · Heat stress · Legume · Physio-morphological

1 Introduction

Plant growth and development are affected by several environmental cues, resulting in low productivity. There are several abiotic and biotic stress factors that alter the plant–environmental equilibrium (Epstein et al. 1980). Among the major threats for agriculture and food safety, global warming has negative impact on agriculture and affects different crop species around the world (Hatfield et al. 2011; Lobell et al. 2011). The climate change includes a rise in concentration of greenhouse gases (GHG) and subsequently a temperature rise, which is fatal to crop production. Intergovernmental Panel on Climate Change (IPCC) has projected a temperature increase of 1.8–4 °C by the year 2100 (IPCC 2007, 2014). The gradual increase in global temperature is being experienced by the plant as heat stress. Heat stress may impair morphology and all the vital processes and also affect the functioning of enzymes, proteins and hormones (Wahid et al. 2007; Kumar et al. 2011). Thus, heat stress will be a critical factor for crop production in near future, due to climate change and global warming.

The temperature rise can adversely affect the cool-season crops more, i.e. chickpea (*Cicer arietinum* L.), than the rainy-season crops (Kumar 2006) in northern parts of India including Rajasthan. Generally, chickpea adapts to high

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temperature through an escape mechanism. Chickpea is a high-value pulse crop and widely cultivated under a range of climatic conditions. Its sowing time may vary at different locations depending on the temperature experienced at different stages of crop development. Therefore, temperature is the most important environmental factor for growth, development and adaptation of chickpea. The optimum conditions for the growth of chickpea have been suggested to be 18–26 °C day and 21–29 °C night temperature and an annual rainfall of 600–1000 mm (Duke 1981; Smithson et al. 1985). Berger et al. (2011) gave the global chickpea distribution based on climate analysis, and the production trends that showed the current chickpea grown areas are under threat from high temperature. The effects of heat stress during the vegetative and reproductive growth stages using agronomic, morphological and physiological assessment have been studied in various economically important crops such as wheat (Sharma et al. 2005), rice (Weerakoon et al. 2008) and cotton (Cottee et al. 2010), while only limited research has been conducted in chickpea (Wang et al. 2006). Therefore, heat stress being a critical factor for plant growth challenges the basic and applied plant scientists to identify and characterize the chickpea genotypes that can withstand these unfavourable stress conditions.

Rajasthan is the third important chickpea-growing region in India with over one million hectare cultivated (Ali and Sharma 2003) and also a warm temperature region. A minimum decrease of 53 kg/ha of chickpea yield was observed in India per 1 °C increase in seasonal temperature (Kalra et al. 2008). By 2050, a rise in temperature by at least 2 °C, particularly the night temperature, is being predicted with higher levels of warming in northern parts of India, so it is important to study the effect of heat on different varieties of chickpea. Adaptation to all environmental stresses is associated with different kinds of metabolic adjustments. High temperature adversely affects several morphological and physiological aspects that include seed germination, photosynthesis, morphology, respiration, protoplasmic movement, water transport, membrane stability, seed quality, modulations of hormones and metabolites, etc. (Chen et al. 1982; Wahid et al. 2007; Torabi et al. 2016). Plants use a complex network of metabolic and interconnected signalling pathways to cope with the set of stress factors (Rasmussen et al. 2013). The study of heat stress effects on germination, growth, morphology, physiology, etc. of chickpea varieties will help to explore strategies to improve chickpea breeding for heat tolerance. Before undertaking a thorough molecular and genetic analysis of the traits involved in thermotolerance, the identification of heat-tolerant varieties of chickpea is a prerequisite. Keeping this in view, the present study was undertaken to compare five different genotypes of chickpea for their physio-morphological changes and growth parameters at early stages, under heat stress. In the present study, three different temperature conditions were chosen to examine their effects on growth parameter of five chickpea genotypes and a comparative analysis among these genotypes was done for initial isolation of sensitive or tolerant genotypes under heat stress. During this study, first the seeds were grown under control and stress condition for 5 days and then shifted to 10-day recovery period. The analysed data showed RSG-895 and CSJD-884 were heat tolerant, and C-235 was the most heat-sensitive variety among all the five selected genotypes. In future, more holistic understanding requires integration of data from multiple sources, including molecular studies. Unravelling molecular basis will further shed light on the effect of heat stress in an important economical crop, i.e. chickpea.

2 Materials and methods

Experimental materials – For this study, all the seeds of chickpea genotypes were procured from the Agricultural Research Station (ARS) Durgapura, Jaipur, Rajasthan, India (Table 1). Four different varieties of chickpea (RSG-895, RSG-888, GNG-1581, CSJD-884) were randomly selected that had originating centre in Rajasthan, and the fifth variety (C-235) was also taken from non-Rajasthan region to examine the effect of heat treatment on germination and early growth stages. According to Rathore et al. (2013) annual, seasonal and monthly mean (minimum and maximum) temperature, trends of Punjab and Rajasthan based upon 282 surface meteorological stations for 1951–2010 are shown in Table 2. The seeds were grown in pots and later harvested to maintain proper supply of seeds.

Seed sterilization – Seeds of all the above five varieties were washed twice with deionized water to remove the dirt particles. The seeds were later washed with 70% ethanol for 1 min and subsequently with 0.1% mercuric chloride ($HgCl_2$) solution for 5 min. The seeds were rinsed for 4–5 times in deionized water for 1 min and soaked for overnight (16 h.).

Heat stress treatment – The pre-soaked seeds were then decoated and transferred to sterile petri dishes on Murashige and Skoog (MS) medium with 3% sucrose, about 10 seeds/petri dishes. Seeds of all the genotypes were kept in incubator under three temperature regimes for germination and further growth, i.e. 30, 35 and 40 °C for heat stress treatment. 25 °C temperature was taken as a control throughout the experiment. The experiments were carried out in triplicates.

Table 1 Five different varieties of *Cicer arietinum* used in this study

S. no.	Variety	Common name	Year of release	Geographical location (originating centre)	Lineage
1	RSG-895	Arpita	2006	Durgapura, Rajasthan	RSG 44 × RSG 255
2	RSG-888	Anubhav	2002	Durgapura, Rajasthan	RSG 44 × E 100 Y
3	GNG-1581	Ganguar	2007	Sriganganagar, Rajasthan	GPF 2 × H 82-2
4	CSJD-884	Akash	2003	Durgapura, Rajasthan	RSG 44 × E 100 Y
5	C-235	–	1960	Ludhiana, Punjab	IP 58 × C 1234

Table displays their common name, year of release, originating centre and lineage

Table 2 Annual, seasonal and monthly mean temperature trends of Punjab and Rajasthan based upon 282 surface meteorological stations for 1951–2010

State	Annual	Winter	Summer	Monsoon	Post-monsoon
Mean maximum temperature trends in °C per year					
Punjab	**− 0.01***	**− 0.02***	No trend	**− 0.02***	No trend
Rajasthan	**+ 0.01***	No trend	**+ 0.02***	**+ 0.01***	**+ 0.01***
Mean minimum temperature trends in °C per year					
Punjab	**− 0.01***	No trend	No trend	**− 0.01***	No trend
Rajasthan	**+ 0.01***	**+ 0.02***	**+ 0.02***	No trend	**+ 0.03***
Mean temperature trends in °C per year					
Punjab	− **0.01***	− **0.02***	No trend	− **0.01***	No trend
Rajasthan	**+ 0.01***	**+ 0.01***	**+ 0.02***	**+ 0.01***	**+ 0.02***

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Monthly mean maximum temperature trends in °C per year												
Punjab	− **0.03***	− 0.02	− 0.01	+ 0.02	NT	− **0.05***	− 0.02	NT	− **0.02***	− 0.01	+ 0.01	− 0.01
Rajasthan	+ 0.01	+ 0.01	+ 0.02	**+ 0.03***	+ 0.02	+ 0.01	NT	**+ 0.02***	**+ 0.03***	+ 0.01	+ 0.01	+ 0.01
Monthly mean minimum temperature trends in °C per year												
Punjab	− 0.02	NT	− 0.01	− 0.01	+ 0.01	− **0.02***	NT	NT	− **0.02***	− 0.01	+ 0.01	NT
Rajasthan	+ 0.02	**+ 0.03***	**+ 0.02***	**0.02***	**+ 0.02***	− 0.01	NT	NT	+ 0.01	**+ 0.02***	**+ 0.03***	**0.02***

Increasing (+) and decreasing (−) trends significant at 95% level of significance are shown in bold and marked with '*' sign. No trend is indicated by abbreviation NT

Measurement of seedling length – The germinating seedling length was measured for each seed in all the control plates, and average length was calculated. This was further compared with average seedling length of all the five varieties under stress condition at three temperatures 30, 35 and 40 °C after 48 h and 72 h. A comparative analysis of seedling growth among all the five varieties was also performed under heat stress (30, 35 and 40 °C) after 48 and 72 h.

Evaluation of morphological features – Evaluation of branching was done after 96 h in control seedlings and compared with branching in stressed seedlings of five different chickpea genotypes, under all the three different temperatures (30, 35 and 40 °C). The number of branches was counted in each growing seedling, and average branching was calculated for each variety. Effect of heat stress on branching was also analysed in control and stressed seedlings after 7 days. The data were compared between control and stressed plants, and a comparative account among five chickpea genotypes was also analysed. Heat stress effects on colour of cotyledon and growing shoots were also analysed in control and stressed plates under three different temperatures (30, 35 and 40 °C).

Measurement of root length and shoot length after recovery period – After 5 days of heat stress treatment under three different temperatures (30, 35 and 40 °C), the plants were shifted to recovery period of next 10 days and shifted to 25 °C. At the end of recovery period that is on 15th day, root length and shoot length of plant were measured. The average root and shoot length of all the five varieties were compared between control and stressed plants to evaluate the effect of heat stress under three temperatures (30, 35 and 40 °C).

Determination of relative water content (RWC) – After 10 days of recovery, chickpea tissues were collected and immediately 0.5 g of tissue was weighed as fresh weight (FW). The tissues were then rehydrated in distilled water for 24 h until fully turgid. After that tissue was surface-dried and reweighed as turgid weight (TW) followed by oven drying at 80 °C for 48 h, and reweighed as dry weight (DW). The experiment was carried out in triplicates. The relative water content (RWC) was calculated by the following formula:

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

% RWC was calculated for all the five varieties, and comparative analysis between control versus stressed plant and among all the varieties was performed under heat stress (30, 35 and 40 °C).

Pigment estimation Analysis of pigments chlorophyll *a* (chl *a*), chlorophyll *b* (chl *b*) and carotenoid was performed on 15th day after recovery (5 days of stress and 10 days of recovery period). Chlorophyll (chl *a*, *b* and carotenoid) contents were determined by the method of Lichenthaler (1987). Leaf tissue (50 mg) from all the five varieties under control and stressed plants was homogenized in 10 ml chilled acetone (80%). The homogenate was centrifuged at 4000*g* for 12 min. The supernatant was taken for determination of photosynthetic pigments. Absorbance of the supernatant was recorded at 663, 647 and 470 nm for chl *a*, chl *b* and carotenoids, respectively, using spectrophotometer (Lichenthaler 1987). Finally, the comparative analysis was performed between controls versus stressed plants. Pigment estimation was done using following formula

$$\begin{aligned}
&\mathrm{Chlorophyll}\, a(\mathrm{mg/g}) = ((12.7 \times A_{663} - 2.69 \times A_{645})\ \mathrm{v/w}),\\
&\mathrm{Chlorophyll}\, b(\mathrm{mg/g}) = ((22.9 \times A_{645} - 4.68 \times A_{663})\ \mathrm{v/w}),\\
&\mathrm{Carotenoid}\ (\mathrm{mg/g}) = (((1000 \times A_{470})\\
&\qquad -(3.27 \times \mathrm{Chlorophyll}\, a + 1.04\\
&\qquad \times \mathrm{Chlorophyll}\, b))/227\ \mathrm{v/w}).
\end{aligned}$$

Statistical data analysis – The results were the average values with ± standard errors (SE) for three independent replicates. Statistical significance between mean values was measured by Student *t* test. The data were found to be significant with *P* value < 0.001. For graphical presentation, Sigmaplot version 11.0 (Systat Inc., San Jose, CA, USA) was used.

3 Results

Study of average seedling length of five chickpea genotypes under control and heat stress condition and their comparative analysis after 48 h – The average seedling length under heat stress (30 and 35 °C) was increased in all the five chickpea genotypes in comparison with control (Fig. 1a). The average length was in the range of 1.02 ± 0.06–2.15 ± 0.01 cm under heat stress at 30 °C (Fig. 1b) and 0.52 ± 0.04–1.19 ± 0.01 cm under 35 °C (Fig. 1b). Seedlings of all varieties growing under control conditions exhibited average size from 0.41 ± 0.01 to 0.51 ± 0.03 cm. Negative effect of increasing temperature on seedling length was seen under 40 °C as compared to 30 and 35 °C temperature after 48 h with average length of 0.31 ± 0.01–1.02 ± 0.04 cm (Fig. 1a, b). In comparison with control, heat stress under 40 °C promoted the seedling length in all the chickpea genotypes except C-235 genotype with average length of 0.31 ± 0.01 (Fig. 1a, b).

During comparative analysis performed among all five genotypes for their thermotolerance, CSJD-884 and RSG-895 genotypes showed more tolerance with average length of 1.92 ± 0.02 and 2.15 ± 0.01 cm under heat stress 30 °C, 1.05 ± 0.05 and 1.19 ± 0.01 cm under heat stress 35 °C and 0.81 ± 0.02 and 1.02 ± 0.04 cm under 40 °C (Fig. 1b). Genotype C-235 was found to be the most sensitive variety with average seedling length of 1.02 ± 0.06, 0.52 ± 0.04 and 0.31 ± 0.01 cm under all the three temperature 30, 35 and 40 °C, respectively (Fig. 1b). Genotypes RSG-888 and GNG-1581 were more sensitive to increasing temperature in comparison with CSJD-884 and RSG-895 but more tolerant than C-235 (Fig. 1b).

Heat stress effect on average seedling length of chickpea genotypes after 72 h and their comparative analysis – Control versus stress analysis was done after 72 h also to check the effect of increasing temperature on seedling growth (Fig. 2a). Heat stress under 30 and 35 °C were supporting the growth of the seedling in all the five chickpea genotypes after 72 h also. The average seedling length was in the range of 1.23 ± 0.20–4.25 ± 0.18 cm under heat stress at 30 °C (Fig. 2b). Heat stress at 35 °C showed average seedling length of 1.38 ± 0.05–2.23 ± 0.03 cm (Fig. 2b). After 72 h at 40 °C, there was a steep reduction in seedling length that ranges between 0.53 ± 0.02 and 1.45 ± 0.12 cm as compared to control plant as well as seedlings growing under 30 and 35 °C heat stress (Fig. 2b).

Genotypes CSJD-884 and RSG-895 showed more tolerance during comparative analysis after 72 h with average length of 4.25 ± 0.18 and 1.81 ± 0.06 cm under heat stress 30 °C, 2.03 ± 0.05 and 2.23 ± 0.03 cm under heat

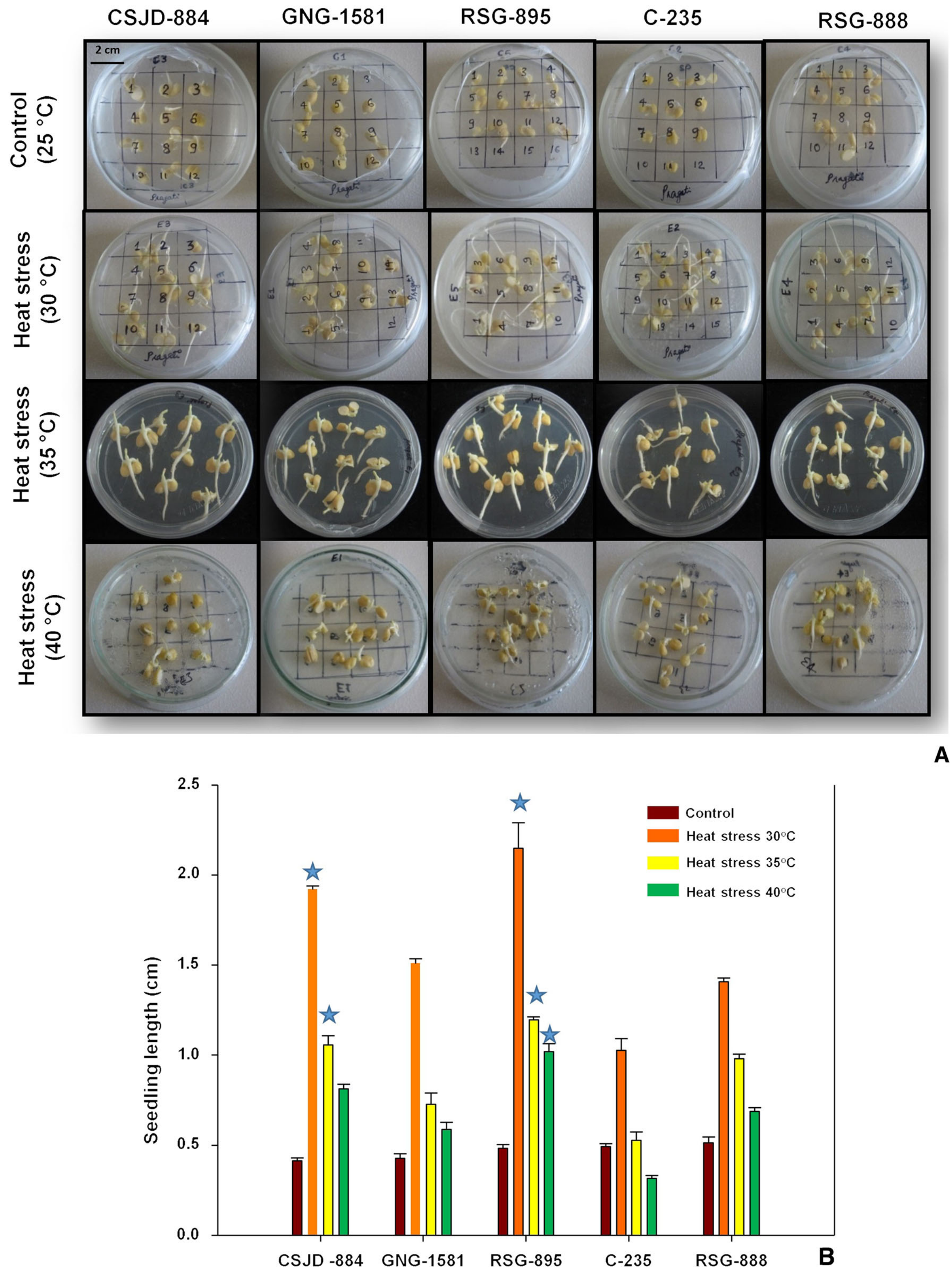


Fig. 1 **a** Evaluation of seedling growth of five different chickpea genotypes, under control and stress condition after 48 h. **b** Evaluation of seedling growth of control versus heat stress-treated seeds of five different chickpea genotypes and comparative analysis of germinating seedlings length of five chickpea genotypes after 48 h. Data represented here is the mean ± SE of three replicates. Star depicts the data to be highly significant with P value < 0.001

stress 35 °C and 1.45 ± 0.12 and 1.04 ± 0.07 cm under 40 °C (Fig. 2b). Genotype C-235 was the most sensitive variety with average seedling length of 1.40 ± 0.02, 1.38 ± 0.05 and 0.53 ± 0.02 cm under all the three

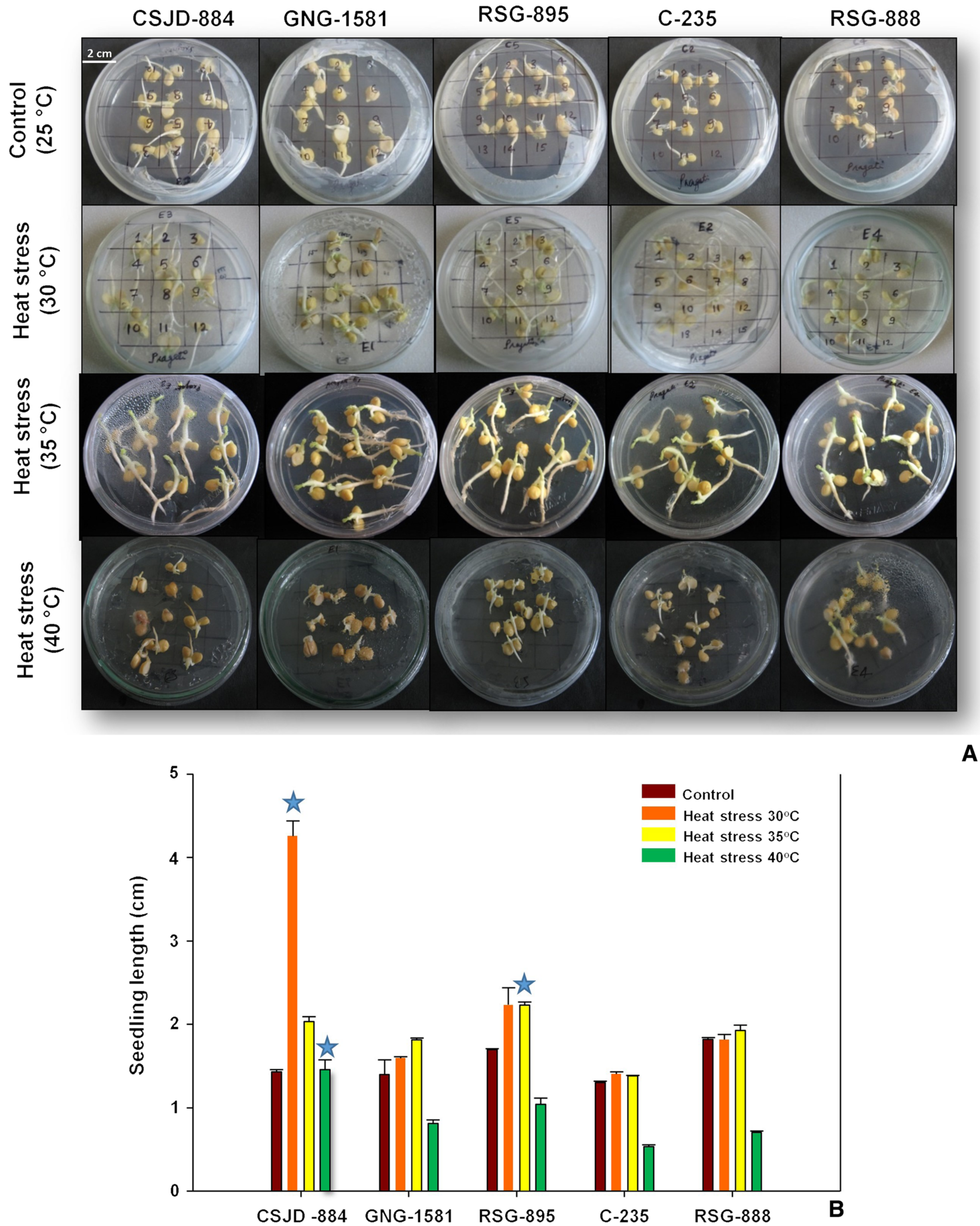


Fig. 2 **a** Evaluation of seedling growth of five different chickpea genotypes, under control and stress condition after 72 h. **b** Evaluation of seedling growth of control versus heat stress-treated seeds of five different chickpea genotypes and comparative analysis of germinating seedlings length of five chickpea genotypes after 72 h. Data represented here are the mean ± SE of three replicates. Star depicts the data to be highly significant with P value < 0.001

temperatures 30, 35 and 40 °C, respectively. RSG-888 and GNG-1581 showed more tolerance than C-235 (Fig. 2b).

Effect of heat stress on root branching, cotyledon and shoot colour after 96 h – In general, the number of root branches per plant was higher in genotypes CSJD-884 and RSG-895 under 30 °C after 96 h and less branching was observed in GNG-1581 genotype (Fig. 3). Heat stress at 35 °C also affected root branching in positive manner, but

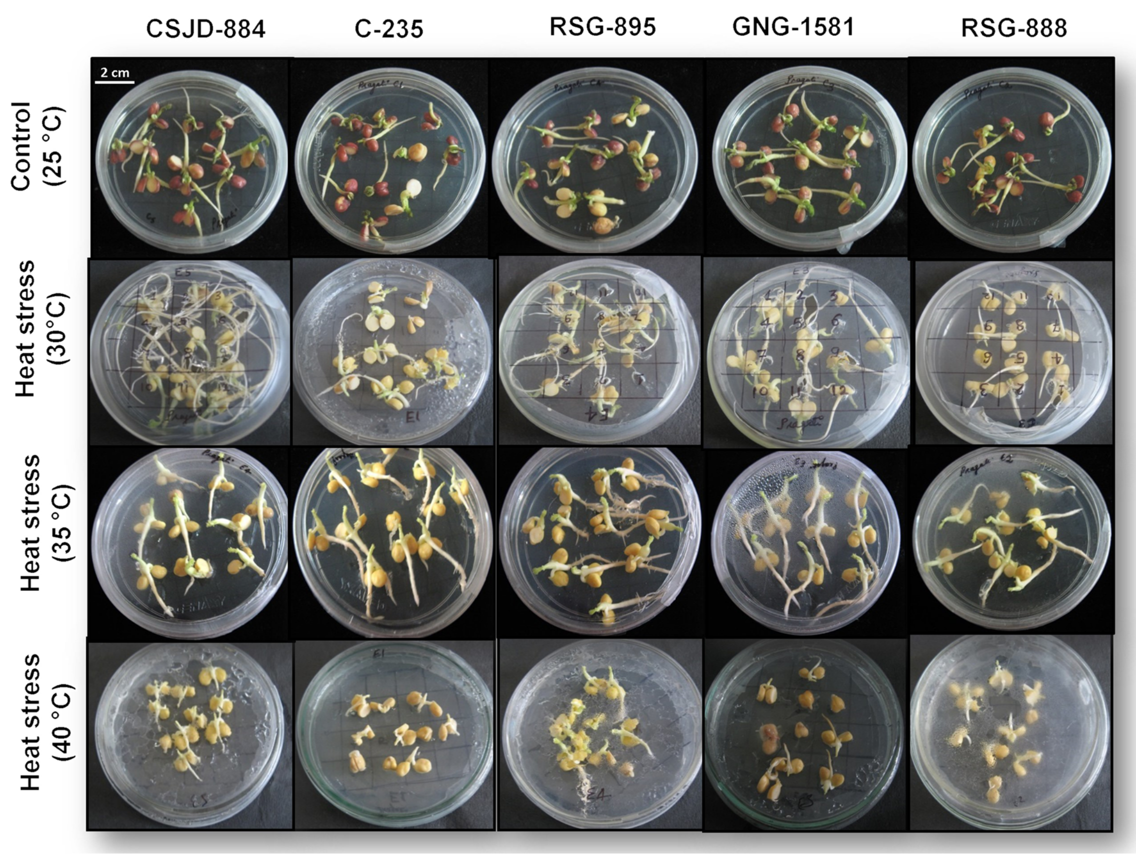


Fig. 3 Evaluation of branching, cotyledon colour and shoot colour in growing seedling of five different chickpea genotypes under control and stress condition after 96 h. (Color figure online)

less branches were seen at 30 °C. No branching was observed in control (25 °C) after 96 h, in all the genotypes. Heat stress at 40 °C for 96 h had negative effect on the growth of all the genotypes mainly branching of roots (Fig. 3). The cotyledon colour was green under control condition, while it was yellow in all the genotypes under heat stress after 96 h (Fig. 3). The dark green colour of shoots was observed in the control plants, but shoots under heat stress showed very light green colour in all the genotypes after 96 h (Fig. 3).

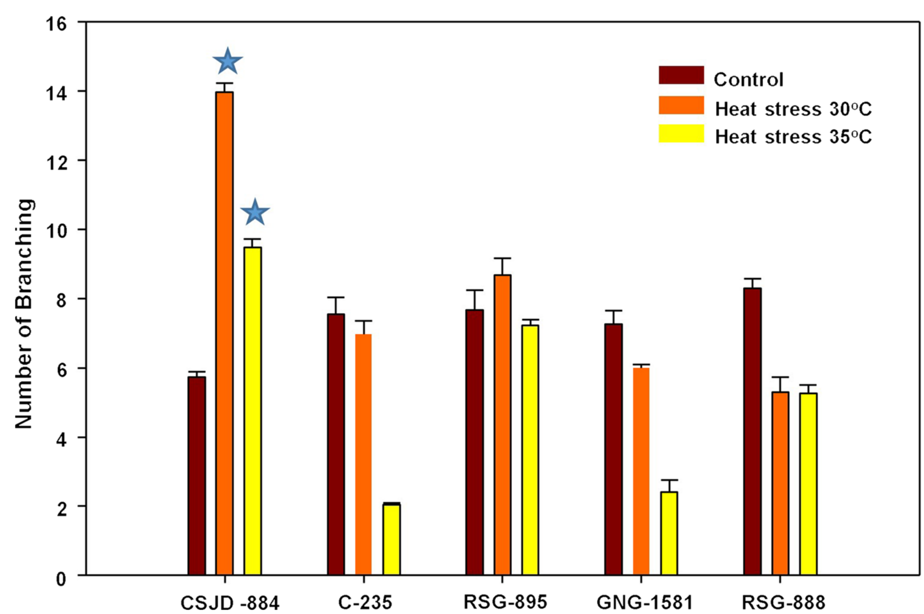


Fig. 4 Comparative analysis of branching of growing seedlings of five chickpea genotypes after 7 days, under control and heat stress conditions (30 and 35 °C). Data represented here are the mean ± SE of three replicates. Star depicts the data to be highly significant with *P* value < 0.001

Effect of heat stress on root branching after 7 days – Under control conditions, the root branches became prominent in all the genotypes after 7 days. A comparative analysis among all the genotypes was done after 7 days that includes 5 days of continuous stress under three temperature followed by a recovery period of next 2 days. In all the chickpea genotypes grown under control condition had average no. of branches in the range of 5.73 ± 0.15–8.30 ± 0.26. Plants grown under heat stress 30 °C showed a number of branches in the range of 5.3 ± 0.43–13.96 ± 0.25. In most of the genotypes grown under heat stress 35 °C had less no. of branches ranging from 2.03 ± 0.05 to 9.46 ± 0.25 (Fig. 4). Heat stress treatment under 40 °C showed almost lethal effect on all the genotypes that lacks growth and root branching after second day of recovery.

A comparative study among all the varieties showed that CSJD-884 and RSG-895 were more tolerant with more number of branches (13.96 ± 0.25 and 8.66 ± 0.49) under heat stress (30 °C). This shows that the recovery after 5 days of stress was fast in these two genotypes. Heat stress (35 °C) was observed to be harmful for root branching, but after recovery, the genotypes CSJD-884 and RSG-895 showed tolerant behaviour towards heat stress in comparison with other genotypes with average number of branches 9.46 ± 0.25 and 7.23 ± 0.15, respectively (Fig. 4). Genotype C-235 was the most sensitive for heat stress under 30 and 35 °C with average no. of 6.96 ± 0.37 and 2.03 ± 0.05 branches (Fig. 4).

Heat stress effects on average shoot length and root length of chickpea genotypes after 10 days of recovery – On the 15th day after recovery, the shoot and root length measurement were taken in control and stressed plants. Genotypes growing under stress 30 °C showed fast recovery in most of the genotypes when transferred to optimum temperature (control condition 25 °C) for growth. As we had found the positive effect of heat stress on seedling growth after 24, 48, and 72 h, shoot length and root length were also increased in most of the genotype after recovery (Fig. 5a, b) with average length 2 ± 0.26–9 ± 0.26 cm as compared to control plants that were contentiously growing under 25 °C for 15 days. Plants under 35 °C also showed recovery, and the shoot length was in the range of 2.6 ± 0.10–3.8 ± 0.10 cm (Fig. 5a, b). This range was almost similar with the control plants of most of the genotypes. Five days of heat stress at 40 °C that showed the lethal effect on various genotypes also showed recovery (Fig. 5a, b), and the shoot length was in the range of 0.8 ± 0.10–4 ± 0.10 cm.

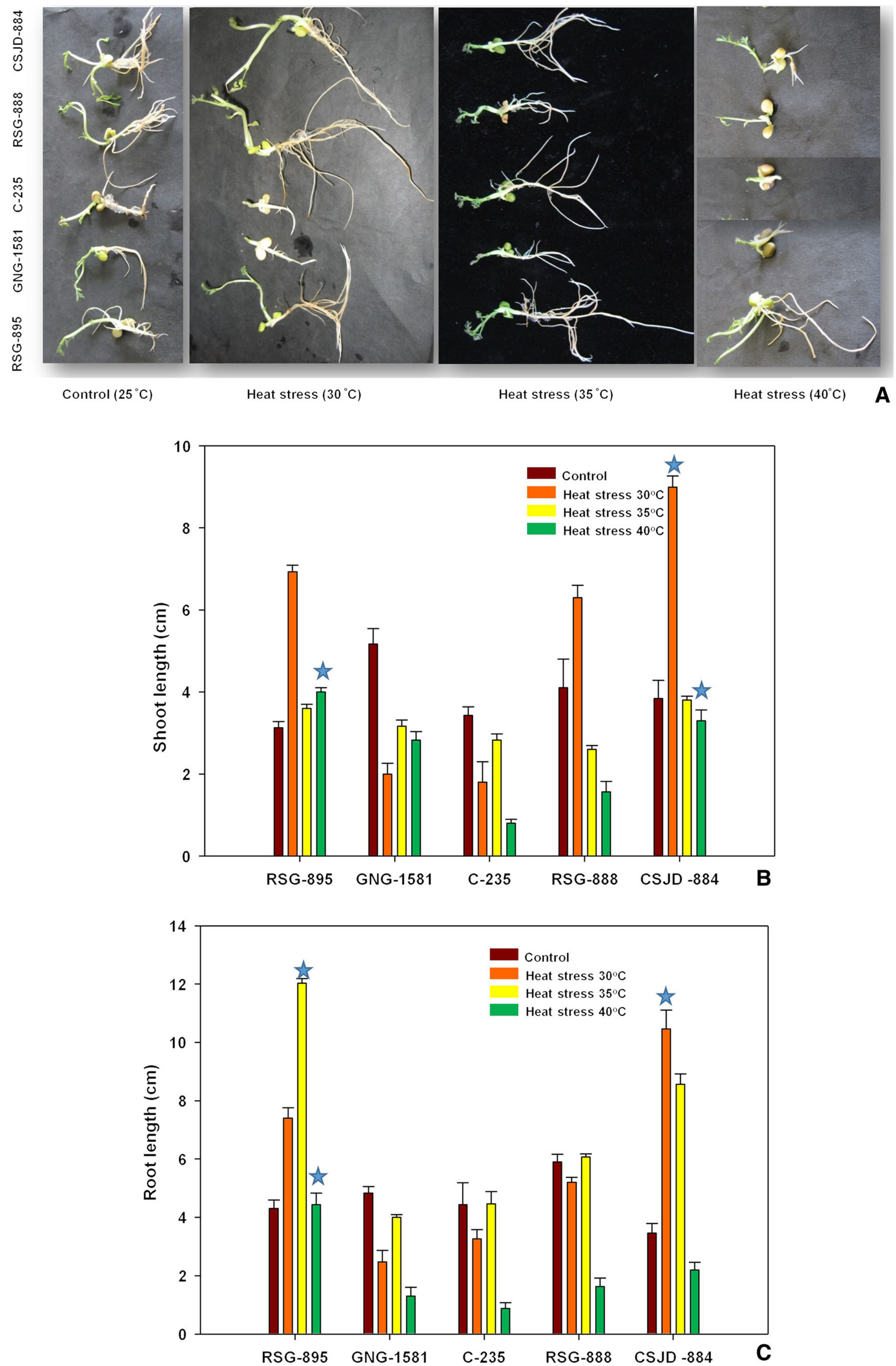
Root length was also compared in control versus stressed plant on 15th day after completion of recovery period.

Fig. 5 **a** Evaluation of root length and shoot length in control and stress-treated plants of chickpea genotypes after 5 days of heat treatment followed by 10 days of recovery. **b** Comparative analysis of average shoot length of five chickpea genotypes after 10 days of recovery, under control and heat stress conditions (30, 35 and 40 °C). Data represented here are the mean ± SE of three replicates. Star depicts the data to be highly significant with P value < 0.001. **c** Comparative analysis of average root length of five chickpea genotypes after 10 days of recovery, under control and heat stress conditions (30, 35 and 40). Data represented here are the mean ± SE of three replicates. Star depicts the data to be highly significant with P value < 0.001

As it was expected, stressed plant at 30 and 35 °C recovered fast and had an increased root length under 30 °C range from 2.4 ± 0.40 to 10.4 ± 0.64 cm (Fig. 5c). The range of the root length was from 4 ± 0.10 to 12 ± 0.15 cm for stressed plants at 35 °C. The control plants showed root length in the range of 3.4 ± 0.32–5.9 ± 0.26 cm. Plants treated under 40 °C also showed recovery with average root length of 0.8 ± 0.18–4.4 ± 0.40 cm (Fig. 5a, c).

Comparative evaluation of root length and shoot length of different chickpea genotypes after recovery – Under all the temperatures, genotypes CSJD-884 and RSG-895 showed fast and good recovery. Genotype CSJD-884 had average root length 10.4 ± 0.64 and 8.5 ± 0.35 cm and average shoot length 9 ± 0.26 and 3.8 ± 0.10 cm under heat stress 30 and 35 °C, respectively. Genotype RSG-895 showed shoot length 6.9 ± 0.15 and 3.6 ± 0.10 cm and root length 7.4 ± 0.36 and 12 ± 0.15 cm under heat stress 30 and 35 °C, respectively. These measurements were comparable with other varieties growing under heat stress and control conditions. At 40 °C also, these two genotypes (CSJD-884 and RSG-895) showed the tolerant behaviour during recovery stage with average root length 2.2 ± 0.26 and 4.4 ± 0.40 cm and shoot length 3.3 ± 0.26 and 4 ± 0.10 cm, respectively. Genotype C-235 was the most sensitive variety with average shoot length of 1.8 ± 0.50, 2.8 ± 0.15 and 0.8 ± 0.10 cm under 30, 35 and 40 °C, while average root length was measured as 3.2 ± 0.30, 4.4 ± 0.41 and 0.8 ± 0.18 cm, respectively (Fig. 5b, c).

Effect of heat stress on % RWC of chickpea genotypes – The analysis of % RWC was performed in control and stress-treated plants after 10 days of recovery. The % RWC was significantly reduced under increasing temperature 30, 35 and 40 °C in all the five genotypes (Fig. 6). Genotypes CSJD-884 and RSG-895 showed high percentage of RWC during comparative analysis with average value of 93.64 ± 1.29 and 91.81 ± 1.59 under heat stress 30 °C, 83.73 ± 1.51 and 82 ± 1.63 under heat stress 35 °C, 66.71 ± 0.63 and 70.57 ± 0.57 under heat stress 40 °C, respectively (Fig. 6). C-235 showed an average value of


CSJD-884
RSG-888
C-235
GNG-1581
RSG-895
Control (25˚C)
Heat stress (30˚C)
Heat stress (35˚C)
Heat stress (40˚C)
A
Shoot length (cm)
0
2
4
6
8
10
Control
Heat stress 30ºC
Heat stress 35ºC
Heat stress 40ºC
RSG-895
GNG-1581
C-235
RSG-888
CSJD -884
B
Root length (cm)
0
2
4
6
8
10
12
14
Control
Heat stress 30ºC
Heat stress 35ºC
Heat stress 40ºC
RSG-895
GNG-1581
C-235
RSG-888
CSJD -884
C

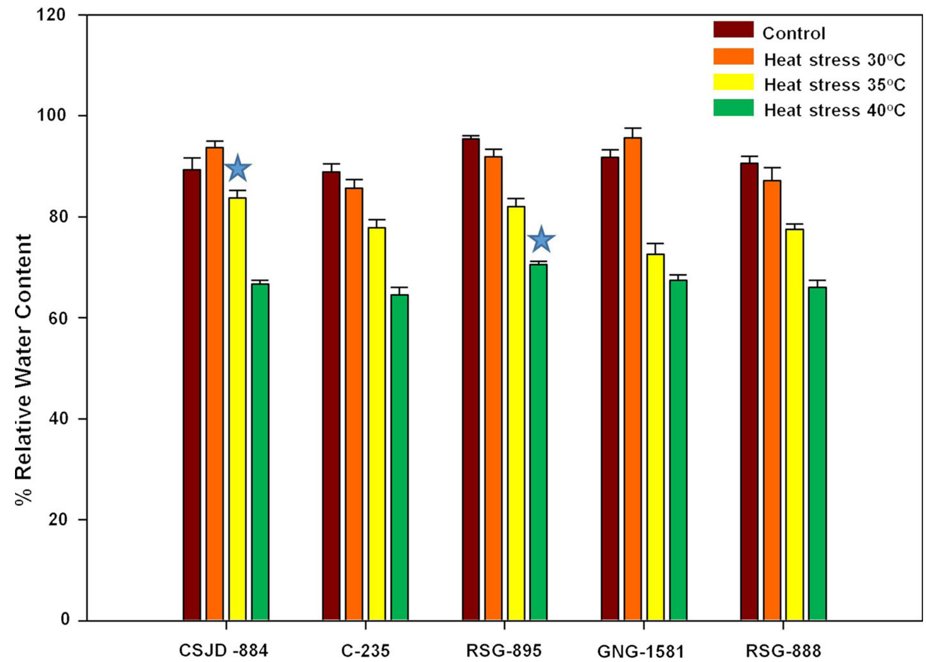


Fig. 6 Comparative analysis of % RWC of five chickpea genotypes after 10 days of recovery, under control and heat stress conditions (30, 35 and 40 °C). Data represented here are the mean ± SE of three replicates. Star depicts the data to be highly significant with *P* value < 0.001

85.62 ± 1.69, 77.83 ± 1.56 and 64.52 ± 1.42 under 30, 35 and 40 °C, respectively (Fig. 6).

Photosynthetic pigments estimation under control and stressed condition in all the genotypes – In all the genotypes, the temperature rise was generally associated with a gradual fall in pigment biosynthesis, chlorophylls i.e. chl *a*, chl *b* and carotenoids. Maximum decrease as compared to control was observed under heat stress at 40 °C (Fig. 7). At 35 °C also, there was significant decrease in chlorophyll pigments as compared to control.

When a comparative analysis was performed among all the genotypes, C-235 was the most sensitive towards all the three temperatures with a significant decrease in pigments (Fig. 7). The range of chl *a*, chl *b* and carotenoid was 1.41 ± 0.05, 0.94 ± 0.03 and 0.42 ± 0.07 under heat stress at 30 °C, respectively. At 35 °C, it was 1.48 ± 0.13, 1.30 ± 0.17, 0.44 ± 0.04, and at 40 °C it was 10.75 ± 0.02, 0.66 ± 0.04 and 0.27 ± 0.01, respectively (Fig. 7). Genotypes CSJD-884 and RSG-895 had less effects on photosynthetic pigments among all the genotypes during increasing stress that again showed a tolerant behaviour (Fig. 7).

4 Discussion

Plants being a sessile organism use a complex network of metabolic and interconnected signalling pathways to cope up with the set of stress factors. An increase in temperature, above the optimum growth temperature for extended period of time, is termed as heat stress that may cause damage and can limit the plant growth (Bita and Gerats 2013). High temperature adversely affects several physio-morphological aspects that include seed germination, photosynthesis, morphology, water transport, membrane stability, seed quality, modulations of hormones and metabolites, etc. (Chen et al. 1982; Wahid et al. 2007). The level of heat stress effect depends on plant exposure to high temperature, as well as genotypes, with intra- and inter-specific variations in their abilities to cope with stress conditions (Bita and Gerats 2013). The ability of plants to acclimatize successfully an instalment of heat stress, referred to as basal thermotolerance, and is commonly measured by plant's ability to survive under heat stress (Larkindale and Vierling 2008; Suzuki et al. 2008). Due to inherent ability of plants to survive in the temperature exposure above the optimum, they exhibit basal thermotolerance and also have remarkable ability to acquire tolerance to lethal heat stress (Larkindale et al. 2005). In conditions such as heat stress, changes in gene expression pattern lead to modification of physiological and biochemical processes that govern heat tolerance in the form of adaptation (Hasanuzzaman et al. 2010; Moreno and Orellana 2011).

The current study describes the effect of heat stress on five different chickpea genotypes under three temperature regimes. Chickpea genotypes have been analysed for changes in morphological and physiological parameters that include seedling growth, root branching, growth of roots and shoots, RWC and photosynthetic pigments at different time points. Simultaneously, a comparative study

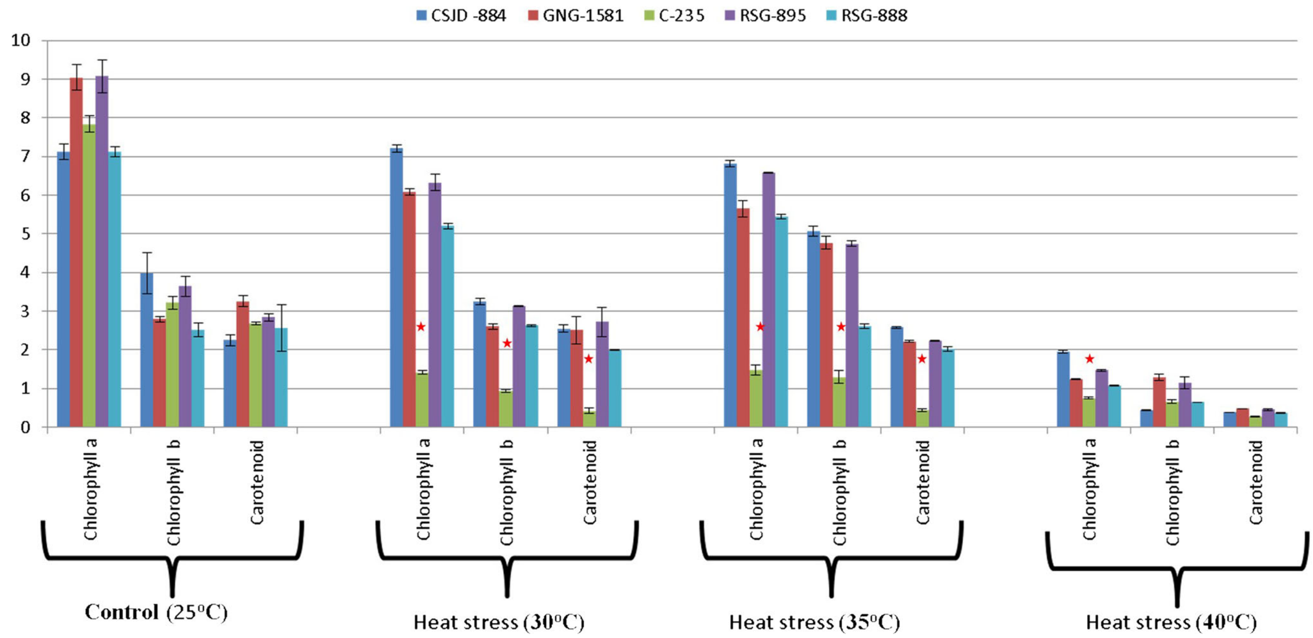


Fig. 7 Photosynthetic pigment estimation under control and stressed condition among all the chickpea genotypes. Data represented here is the mean ± SE of three replicates. Star depicts the data to be highly significant with P value < 0.001

has been performed among five chickpea genotypes for analysing their thermotolerance behaviour. The results showed that heat stress to a certain extent hastens the seedling growth, root and shoot development and root branching as compared to plants grown under control conditions. Earlier studies showed that enhanced crop development and shortened growth period were reported under warmer temperatures (Sadras and Monzon 2006; Tao et al. 2006; Challinor and Wheeler 2008). Kumari et al. (2015) have reported that heat stress under 30, 35 and 40 °C was supporting the germination frequency of chickpea genotypes as compared to control. According to Chakrabarti et al. (2013) heat stress to a certain extent reduced the crop growth period and hastened maturity of wheat and chickpea crop throughout the growth period.

The current study showed that the seedling growth under heat stress at 30 and 35 °C was increased as compared to control, while increased duration for stress at 40 °C showed lethal effect on seedling length. This is in agreement with the findings on mung bean (*Phaseolus aureus* Roxb.) obtained by Basra et al. (1997), Thind et al. (1997) and Kumar et al. (2011). They reported that temperatures exceeding from 35/25 °C was stressful for the growth of mung bean seedlings and the seedling length was compromised. Seedlings of genotypes CSJD-884 and RSG-895 were healthier and showed a higher seedling length than C-235 under heat stress. According to Rodríguez et al. (2005) and Wahid et al. (2007), plants show dynamic behaviour to high temperature that depends upon plant type, environmental factors, duration and certain extent of heat stress.

The results showed that the root and shoot lengths were improved and differential response was observed among the chickpea varieties growing under heat stress after recovery as compared to control. This study was in accordance with Piramila et al. (2012) who reported the effect of heat treatment on dry black gram seeds at 50 °C for 10, 20 and 30 min. An increase in the length of roots and shoots was observed from the day of analysis up to 5th day after heat treatment as compared to the seeds that were not given heat treatment (control) and showed reduction in shoot and root length. In the current study, temperature exceeding from 30/35 °C showed inhibition of shoot length and root length after recovery. Kumar et al. (2011) reported that the root and shoot growth was not inhibited at 35/25 °C but inhibited at 40/30 °C and 45/35 °C in mung bean. According to Kumar et al. (2012), shoot growth was inhibited to a greater extent than the root growth with elevation of temperature to 40/35 °C and 45/40 °C in rice and maize. CSJD-884 and RSG-895 growing under all the temperatures showed quick and good recovery along with highest root and shoot length. Shaheen et al. (2016) reported a comparative analysis of 191 tomato (*Solanum lycopersicum* L.) genotypes under high temperature (40/32 °C day/night temperature) and observed L00090 and L0009 to be heat-tolerant genotypes, while CLN1462A and CLN 1466E were comparatively sensitive.

Heat stress also showed effects on branches of roots. The number of root branches per plant was higher in stressed plant after 96 h, while there was no branching in control plants after 96 h. Genotypes CSJD-884 and RSG-895 among all had more number of root branches as compared to other genotypes under heat stress and in comparison with most of the genotypes growing under control condition after 7 days. Heat stress treatment under 40 °C showed almost lethal effect on all the genotypes which completely lacks growth and root branching after recovery also. Lather et al. (2001) reported poorly developed roots in emerging cotton seedlings in response to heat stress. Temperature and root branching correlation has also been previously reported (Pregitzer et al. 2000). Nagel et al. (2009) reported that temperature gradients mimicking natural soil conditions was related to responses in the root system and subsequently affected the entire root system. High temperatures are related to pre- and post-harvest damages, sunburns on leaves and twigs, branches and stems, abscission and leaf senescence, shoot and root growth inhibition, fruit damage and reduced productivity (Ismail and Hall 1999; Vollenweider and Gunthardt-Goerg 2005).

The measurement of RWC showed a significant decrease under heat stressed plants as compared to control. Among the genotypes studied, CSJD-884 and RSG-895 showed high percentage of % RWC after recovery. Our observations are in accordance with the earlier reports that showed reduction in RWC due to heat stress in wheat (Sairam et al. 2000), turfgrass (Jiang and Huang 2001) and Kentucky bluegrass (Liu et al. 2008).

Hasanuzzaman et al. (2013) studied the seedling stage of wheat (*Triticum aestivum* L.) under 38 °C heat stress and found decreased RWC. Significant decrease in relative leaf water content at 40/30 °C (10% over control) and at 45/35 °C (12% over control) has been reported in mung bean (Kumar et al. 2011). To understand the effect of high temperature on photosynthetic capabilities, the status of photosynthetic pigments, viz. chl *a*, chl *b* and carotenoid, was determined in all the varieties after 10 days of recovery. Most of the varieties showed an initial decline in photosynthetic pigments under heat stress, as compared to control plants after recovery. Similar result was also reported by Truong et al. (2017) that chlorophyll levels fell rapidly by more than 40% after 12 h of heat treatment at 45 °C in stressed seedlings followed by a recovery period of 6 days. Due to increased temperature, the loss of chlorophyll was in accordance with similar reports in tomato (Camejo and Torres 2001), mulberry (Chaitanya et al. 2001), rice (Sohn and Back 2007) and wheat (Almeselmani et al. 2009), and it was ascribed to photo-oxidation of chlorophyll (Guo et al. 2006). The decreased chlorophylls *a*, *b* and total chlorophyll in sorghum have also been reported in response to elevated temperature and in maize and rice (Jagtap et al. 1998; Kumar et al. 2012). In our study, CSJD-884 and RSG-895 showed better maintenance of the photosynthetic pigments, whereas maximum damage was observed in C-235 variety. There is a report that showed a decrease in total chlorophyll biosynthesis under heat stress in faba bean cultivars which may be due to inhibition of photosynthetic electron transport chain (Mohanty et al. 1989) and the enzymes of chl biosynthesis, such as d-aminolevulinic acid (Tewari and Tripathy 1998; Shalygo et al. 1999).

In conclusion, high temperature stress to a certain extent (30 and 35 °C) hastened the early growth of the chickpea genotypes. Seedling growth, branching, shoots and root growth were enhanced under high temperature, and photosynthesis pigment as well as relative water content was getting reduced. Heat stress under 40 °C showed the lethal effect on physio-morphological aspects and growth parameter. Among the chickpea genotypes, CSJD-884 and RSG-895 showed the more thermotolerant behaviour towards physio-morphological changes and C-235 was found to be the most sensitive genotype amongst all. We can assume that the thermotolerance and the sensitivity of these genotypes can be linked with production of antioxidants, compatible solutes or the temperature conditions of different originating centres of these genotypes. The data presented in Table 2 (Rathore et al. 2013) show per year mean temperature (minimum and maximum) trends of Rajasthan and Punjab. These data explain a 95% significant increase in temperature of Rajasthan, while there is a significant decrease in annual, seasonal and monthly temperature trend of Punjab per year. In the current study, we found that C-235 genotype from Punjab region was the most sensitive variety as compared to four other varieties from Rajasthan under heat stress. From the trends displayed in Table 2, we can see that in Punjab the per year decrease in annual mean temperature and winter mean temperature is − 0.01 and − 0.02 °C, respectively, while Rajasthan shows the per year significant increase in annual and winter mean temperature, i.e. + 0.01 °C. As chickpea is a cool-season crop, we can assume the per year significant decrease in the temperature trend of Punjab makes C-235 chickpea genotype more sensitive towards heat stress than other chickpea genotypes of Rajasthan region. If we see the monthly mean (maximum and minimum) temperature trend especially from October to March, Rajasthan is showing a significant rise in the temperature per year, while Punjab shows the decreased temperature pattern per year. So, it can be possible that the higher sensitivity of C-235 is somehow linked with this type of climatic aspect of their geographical centre or a less warm weather conditions of Punjab affecting the C-235 thermotolerance behaviour and making this genotype more sensitive under heat stress. The foregoing discussion is well correlated according to Berry and Björkman (1980) that plants growing under cool temperature zones tend to have a limited potential for adaptation to high temperature stress.

Reliable indicator of heat sensitivity in plants is damage to membranes (Liu and Huang 2000; Gulen and Eris 2004). In some food legumes (Srinivasan et al. 1996) and cowpeas (Ismail and Hall 1999), heat-tolerant genotypes possess the better membrane integrity than heat-sensitive ones. According to Kumar et al. (2012), rice genotypes as compared to maize genotypes showed the greater damage to membranes. Thus, the loss of membrane integrity may be one of the reasons for their greater sensitivity towards heat stress.

Many organisms respond to elevation of temperature through synthesis of several heat shock proteins (HSPs) (Vierling 1991). Thermotolerance acquisition is related to accumulation of HSPs (Vierling 1991; Koskull-Döring et al. 2007) and gets regulated by the heat shock transcription factor (HSF) family (Schöffl et al. 1998; Baniwal et al. 2004). Molecular studies of chloroplast-localized small HSPs (CP-sHSP) are related to protective role towards heat-labile components of photosystem II (Joshi et al. 1997; Downs et al. 1998; Barua et al. 2003). Inter- and intraspecific variation in CP-sHSPs accumulation is related to the degree of heat tolerance in different plants, viz. *Ceanothus* (Knight and Ackerly 2001), *Chenopodium* (Barua et al. 2003), bentgrass (Wang and Luthe 2003).

Under stress, different plant species may accumulate a variety of osmolytes such as proline, sugars and polyols, tertiary and quaternary ammonium compounds, etc. as a key adaptive mechanism for thermotolerance (Sairam and Tyagi 2004). Synthesis of glycine betaine (GB) under stress conditions differs from species to species (Ashraf and Foolad 2007). High level of GB accumulation was reported in maize (Quan et al. 2004) and sugarcane (Wahid and Close 2007) under water deficit or high temperature. Similarly, accumulation of soluble sugars under heat stress has been reported in sugarcane, which may be related to heat tolerance (Wahid and Close 2007).

Plants must be protected from heat-induced oxidative stress so that they can survive under high temperature. Tolerant plants have a protection tendency against damaging effects of ROS with the synthesis of various enzymatic and non-enzymatic ROS scavenging and detoxification systems (Apel and Hirt 2004). Antioxidant enzyme activities are temperature sensitive, and activities of these enzymes increase with increasing temperature. Chakraborty and Pradhan (2011) observed that enzymatic antioxidants like peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD) and glutathione reductase (GR) showed an initial increase under heat stress. Rani et al. (2013) exposed 5-day-old thermotolerant genotype, namely BPR-542-6, and thermo susceptible genotype, namely NPJ-119, of *Brassica juncea* (L) Czern & Coss. to high temperature (45.0 ± 0.5 °C) stress. They observed that the activities of SOD, POX, CAT, APX and GR increased under heat stress, but the increase was significantly higher in tolerant genotype.

We may conclude that study related to plant developmental response to climate change specially temperature will be helpful for crop improvement. In future, the growing conditions will keep the plants exposed to heat stress, thereby having influence on global agriculture, so further research in this important area is critical. Currently, our ability to understand and predict plant developmental responses to climate change is limited by the number of experiments that are conducted in physiologically relevant stress conditions. So, molecular knowledge of stress response and tolerance mechanisms will pave the way for engineering plants in future that can tolerate heat stress.

Acknowledgements The authors are thankful to the Agriculture Research Station (ARS) Durgapura, Jaipur, India, for the supply of different chickpea genotypes. We acknowledge Dr. H.R. Kushwaha, International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi, India, for critically reading the manuscript.

Author's contributions (1) PK, SY and SS were involved in project preparation and experimental design; (2) PK contributed to experimental work; (3) PK and SY prepared the manuscript; (4) PK and SY analysed the data, and all of the authors read, corrected and approved the manuscript in its final form.

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