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Research article

Genotype-dependent responses of chickpea to high temperature and moderately increased light

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ABSTRACT

Our aim was to understand how moderately increased light intensities influenced the response of chickpea to high temperature. Three chickpea genotypes (Acc#3, Acc#7 and Acc#8) were treated at control (26 °C and 300 μmol m^{-2} s^{-1} photosynthetic photon flux density/PPFD), high temperature (38 °C and 300 μmol m^{-2} s^{-1} PPFD), increased light intensity (26 °C and 600 μmol m^{-2} s^{-1} PPFD) and combination of increased light and temperature (38 °C and 600 μmol m^{-2} s^{-1} PPFD). The net photosynthetic rate (P_N) of Acc#3 and Acc#8 significantly decreased at high temperature regardless of light intensity. The P_N of all three genotypes at increased light intensity was significantly higher than that at high temperature. The intracellular CO_2 concentration (C_i), stomatal conductance (g_s) and transpiration rate (E) of Acc#3 and Acc#8 at increased light intensity with or without high temperature significantly decreased in comparison with control and individually high temperature treatment. The relative water content of Acc#3 at high temperature and the combination treatment decreased as compared with control. The relative water content of Acc#7 at control was higher than the other three treatments. The F_v/F_m (Maximum quantum efficiency of photosystem II) of leaves from the three genotypes at 38 °C were lower than at 26 °C regardless of light intensity. The high temperature decreased chlorophyll content in the lower bottom leaf of Acc#7 and Acc#8 than control. In conclusion, chickpeas showed a higher net photosynthetic rate at increased light intensity to withstand heat stress, which was genotype-dependent. Physiological responses of different chickpea genotypes to increased temperature and light intensity indicated that distinct responsive mechanism of photosynthesis. This study provides information on how chickpea respond to high temperature and increased light intensity, which will help us to improve chickpea to deal with future climate changes.

1. Introduction

Chickpea (*Cicer arietinum* L.) is an important cool-season food legume that is globally grown due to its high nutrition quality and ability to improve soil fertility (Awasthi et al., 2014). Chickpea is one of most significant pulse crop with 14.56 million ha growth area across more than 50 countries (FAOSTAT, 2017). The range in change rate and average value of global-mean temperature is higher by the year of 2020 than the historical period (Smith et al., 2015). The steadily increased temperature, especially in tropical and subtropical regions, lead to growth inhibition and massive yield loss of various crops (Kaushal et al., 2013; Gaur et al., 2014; Balfagón et al., 2019). Optimal temperatures for the growth and development of chickpea is in a broad range from 10 °C to 30 °C (Gaur et al., 2019). Chickpea is quite sensitive to heat stress especially at its reproductive growth stage, resulting in significant yield loss of chickpea at high temperature (Kaushal et al., 2013; Gaur et al., 2019). Chickpea experiences significantly yield losses when exposed to high temperature especially at the reproductive stage (Gaur et al., 2014). The photosynthetic apparatus is recognized as one of the most sensitive components of chickpea to heat stress (Kaushal et al., 2013). Besides, high temperature (≥32 °C) decreased stomatal conductance (g_s), water content, chlorophyll content and photochemical efficiency with a larger effect on heat-sensitive chickpea than heat-tolerant genotypes (Kaushal et al., 2013). Moreover, it is worth noting that large genetic variation in heat susceptibility were reported in chickpea according to Awasthi et al. (2014) and Krishnamurthy et al. (2011),

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leading to heat-sensitive and tolerant chickpea identification. Measurements of chlorophyll fluorescence parameter such as F_v/F_m or maximum quantum efficiency of PSII (photosystem II) is an efficient way to determine the damage of stress on PSII (Baker and Rosenqvist, 2004). Makonya et al. (2019) found that heat-tolerant chickpeas showed not only higher F_v/F_m, but also higher photosynthetic rates and grain yield than sensitive chickpeas at a warmer site in South Africa. These previous studies focused on the physiological effects of the individual heat stress on chickpea genotypes with different heat susceptibilities.

More importantly, plants growing under natural field conditions are affected by the interaction of different environmental factors (Mittler, 2006; Carvalho et al., 2016). Temperature and light are examples of environment factors that could differ between environments and affect plant growth and development (Szymańska et al., 2017). Crop production is based on plant photosynthesis that is affected by temperature and light (Gao et al., 2019). Moreover, high temperatures often occur together with other abiotic stresses such as high light intensity under field condition (Balfagón et al., 2019). The joint effect of heat stress and high light on various plants is well documented (Sandhu and Hodges, 1971; Zhao et al., 2011; Gao et al., 2019). For instance, high temperature (40 °C) and very high light (1500 μmol m^{-2} s^{-1}) for 3 h had negative effects on photosynthetic capacity of bayberry (Gao et al., 2019). Similarly, high temperature (36 °C) and very high light (1800 μmol m^{-2} s^{-1}) stress for 2 h caused damages on D1 protein and photosystem II (PSII) with low photosynthetic capacity of wheat leaves (Zhao et al., 2011). Chickpea plants at 28,063 lux light intensity provided by fluorescent and incandescent lamps for 16 h and 22.5 °C generated more flowers and seeds than the other treatment combination (16,136 lux light intensity, 8 h or 12 h photoperiods and 15 °C or 30 °C) (Sandhu and Hodges, 1971). Tomato plants acclimate better to the combination of high light (800 μmol m^{-2} s^{-1}) and elevated temperature (38/29 °C) for 6 days than individual treatment (Gerganova et al., 2016). However, the physiological response of the chickpea to different light intensity is lacking and the information on the interactive effects of increased light and heat stress on chickpea physiology remained unclear.

With the global climate change, understanding the adaptation responses and strategies to complex environmental conditions is urgent for improving global food security (Bowne et al., 2018; Dhankher and Foyer, 2018). Knowledge on how different chickpea genotypes deal with the co-occurrence of increased temperature and light intensity play a crucial role in crop management and genetic improvement for climate tolerance. Since anthesis stage is a key reproductive growth stage that was more sensitive to high temperature than seedling stage, three chickpea genotypes at anthesis stage with known differences in heat stress were treated at control, high temperature, increased light and their combination in this study. Our aim was to understand the effect of moderately increased light intensity on the physiological response of different chickpea genotypes to heat stress. We hypothesized that a) the physiological responses of chickpeas to the combination of high temperature and increased light depended on genotype and b) moderately increased light intensity might alleviate the damage of high temperature on chickpea's photosynthesis. The knowledge will enhance our ability to understand how chickpea deal with climate changes and be beneficial for crop improvement.

2. Materials and methods

The chickpea seeds of genotypes named Acc#3, Acc#7 and Acc#8 were provided by University of Venda, South Africa. The three genotypes were originally from India, which is the top producer country of chickpea (FAOSTAT, 2017). The three genotypes were chosen since 1) they showed different heat susceptibilities; 2) they reached anthesis stage at similar age in our preliminary trial. The seeds were sown in plastic pots (11 cm/9 cm, diameter/height) with commercial substrate (Pindstrup 2; Pindstrup Mosebrug A/S, Ryomgaard, Denmark) on 7th, Dec 2018 in a greenhouse. The environmental parameters of the greenhouse were set to 23/16 °C (day/night), ambient CO_2 concentration (405 ppm) and 50 ± 10% RH (relative humidity). Supplementary light was provided with LED lamps (Senmatic Fionia Lighting, FL300, Senmatic Søndersø Denmark) and the actual light intensity was 150–220 μmol m^{-2} s^{-1} during the day period in the greenhouse. The plants were irrigated twice a day with nutrient solution (pH = 6, EC = 2.18, NH_4 = 10.9%, N = 191 ppm, P = 35 ppm, K = 275 ppm, Mg = 40 ppm, Ca = 140 ppm).

After 38 days the plants reached anthesis stage and were moved to climate chamber on 15th, Jan 2019 for treatments. The plants were treated at (1) 26 °C and 300 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) (control); (2) 38 °C and 300 μmol m^{-2} s^{-1} PPFD (high temperature treatment); (3) 26 °C and 600 μmol m^{-2} s^{-1} PPFD (increased light treatment) and (4) 38 °C and 600 μmol m^{-2} s^{-1} PPFD (combined treatment). The four treatments started from 16:00 p.m. on day 0 and lasted 18 h with six uniform sized plants per genotype per treatment, during which the plants were irrigated three times to avoid water deficit. The light source in the chambers were Sunlight FL300 (Fionia, Søndersø, Denmark). The light level was determined at the level of the seedlings' height when they were moved in the chambers using an LI-250A quantum sensor (LI-COR, Lincoln, NE, USA). The five locations were randomly chosen in the chambers for the light level measurements.

2.1. Gas exchange

The second full-expanded top leaf was chosen for gas exchange measurements with three replications starting from 16 h of the treatments (8:00 a.m. on day 1) and lasting for 2 h. The 1.7 cm^2 cuvette was fully covered by the whole leaf with no visible gap between each small leaflet. This was feasible by removing the light source before putting the leaf into the cuvette. Then, we covered the cuvette by the whole leaf when we can check though the top of the cuvette. After carefully checking that the cuvette was fully covered by the leaf, the light source was put on. Four parameters including P_N (net photosynthetic rate), C_i (intracellular CO_2 concentration), g_s and E (transpiration rate) were measured using a portable photosynthesis system (CIRAS-2, PP Systems, Amesbury, USA). The cuvette settings were 26 °C and 38 °C with 300 μmol m^{-2} s^{-1} PPFD for control and high temperature, respectively. By comparison, the cuvette settings were 26 °C and 38 °C with 600 μmol m^{-2} s^{-1} PPFD for increased light and combination, respectively. The leaves were placed in 1.7 cm^2 cuvette with 400 μmol m^{-2} s^{-1} CO_2 concentration and 40–70% RH with 0.7–2.4 kPa VPD. The results of the measurements were taken records every 10 s when the four parameters were steady. The last six records were averaged as the result.

2.2. Chlorophyll fluorescence, chlorophyll content and leaf temperature

The second full-expanded top and bottom leaf were used for chlorophyll fluorescence measurements by both Handy PEA (Hansatech Instrument, King's Lynn, England) and Mini PAM (Walz, Effeltrich, Germany). The measurements were performed after 18 h of the treatments when the gas exchange measurements were finished. Then, the leaves were dark-adapted for 30 min with a leaf clip before the measurements of F_v/F_m with four replications.

Chlorophyll content of the top and bottom leaf were determined by Dualex 4 (ForceA, Orsay, France) with four replications from four different plants. For each plant, three leaflets were measured after 18 h of the treatments and the results were averaged per plant.

Leaf temperature with four replications was measured using a Raynger 3i infrared gun (Raytek, Santa Cruz, CA, USA) after about 18 h of the treatments on the top leaf.

2.3. Destructive measurements

The top leaf was sampled after 16 h of the treatments to measure leaf relative water content (three replications) and water loss rate (four

replications). Fresh weight (FW) was immediately determined after cutting. Turgid weight (TW) was measured after immersing the leaf in the dd-H_2O for 4 h at room temperature. Dry weight (DW) was determined after dying the leaf in an oven for 24 h at 80 °C. The relative water content (%) was calculated as [(FW – DW)/(TW - DW)]. Water loss rate (%) was calculated as [(FW_{0min} - FW_{xmin})/FW_{0min}] × 100. The FW of leaf was determined at 0 min, 10 min, 15 min, 20 min, 30 min, 45 min, 1 h, 2 h, 3 h, 4 h and 5 h after taking the samples.

2.4. Data analysis

Analysis of variance (ANOVA) between the physiological parameters of chickpea at the control, high temperature, increased light and combination were performed using SPSS 16.0 (SPSS Inc. Chicago, IL, USA). The ANOVA was conducted among the treatments within each genotype. Pearson correlation between leaf temperature and gas exchange parameters as well as between environmental settings and gas exchange parameters were conducted using SPSS 16.0.

3. Results

The P_N of Acc#3 and Acc#8 at high temperature significantly decreased as compared with 26 °C at both 300 μmol m^{-2} s^{-1} PPFD (44.61%, 43.01%) and 600 μmol m^{-2} s^{-1} PPFD (50.23%, 46.54%) (Fig. 1A). The P_N of Acc#7 at 600 μmol m^{-2} s^{-1} PPFD was significantly higher (61.70%) than that at high temperature (Fig. 1A). As compared with control, the C_i and E of Acc#3 at high temperature significantly increased (12.83%, 53.82%), while that of Acc#3 at 600 μmol m^{-2} s^{-1} PPFD both with 26 °C (40.61%, 49.97%) and 38 °C (20.55%, 43.05%) significantly decreased (Fig. 1B and D). The C_i and E of Acc#8 at 600 μmol m^{-2} s^{-1} PPFD were significantly lower than that at 300 μmol m^{-2} s^{-1} PPFD with both 26 °C (32.15%, 64.49%) and 38 °C (12.86%, 28.17%) (Fig. 1B and D). The g_s of Acc#3 and Acc#8 at 600 μmol m^{-2} s^{-1} PPFD significantly decreased as compared with that at 300 μmol m^{-2} s^{-1} PPFD at both 26 °C (68.86%, 72.39%) and 38 °C (70.05%, 62.20%) (Fig. 1C). The leaf temperature of the three genotypes at 38 °C were significantly higher than that at 26 °C both with 300 μmol m^{-2} s^{-1} PPFD (55.47%, 50.36%, 57.35%) and 600 μmol m^{-2} s^{-1} PPFD (55.13%, 50.59%, 58.09%) (Fig. S1).

The relative water content of Acc#3 at 38 °C at both light levels (300 and 600 μmol m^{-2} s^{-1} PPFD) was significantly lower than control (5.85%, 6.41%) (Fig. 2). The relative water content of Acc#7 at control was significantly higher than that at high temperature (9.11%), increased light (6.01%) and combination (5.97%) (Fig. 2). The water loss rate of Acc#3 and Acc#8 at 38 °C both with 300 and 600 μmol m^{-2} s^{-1} PPFD and at 26 °C with 600 μmol m^{-2} s^{-1} PPFD at the time points

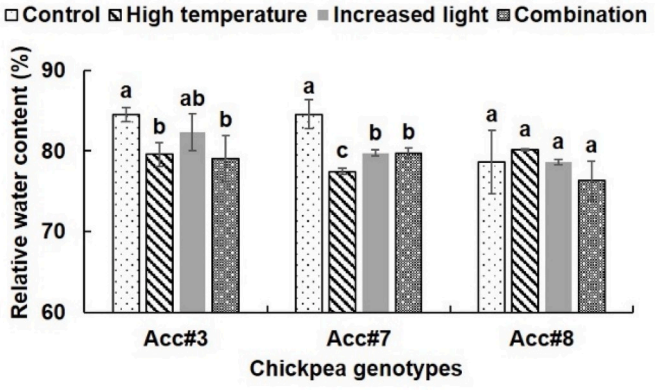

Fig. 2. Relative water content of the second fully expanded top leaves from three chickpea genotypes at the treatments for 18 h 'Control', 26 °C + 300 μmol m^{-2} s^{-1}; 'High temperature', 38 °C + 300 μmol m^{-2} s^{-1}; 'Increased light', 26 °C + 600 μmol m^{-2} s^{-1}; 'Combination', 38 °C + 600 μmol m^{-2} s^{-1}. The data represent average values ± SD (n = 3).

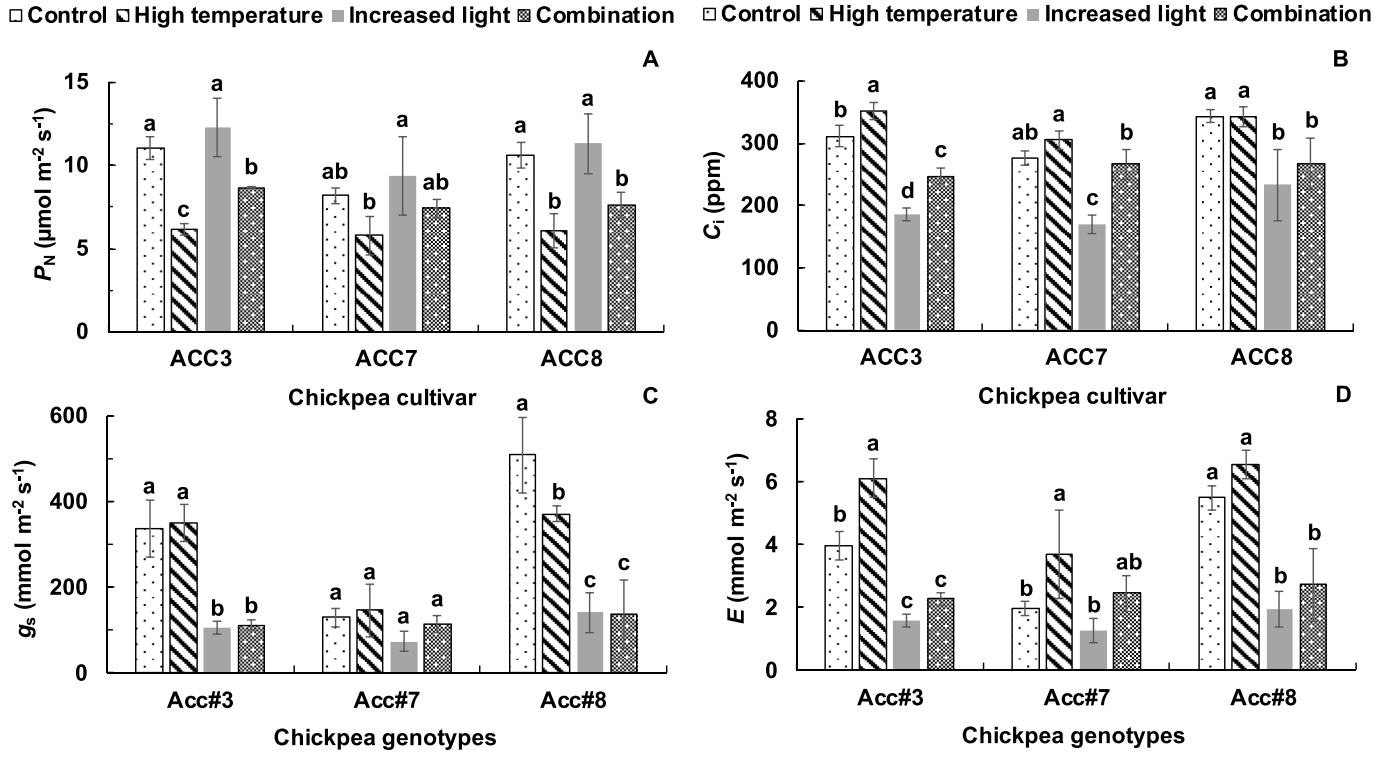

Fig. 1. (**A**) Net photosynthetic rate (P_N), (**B**) intracellular CO_2 concentration (C_i), (**C**) stomatal conductance (g_s) and (**D**) transpiration rate (E) in the second fully expanded top leaves from three chickpea genotypes at the treatments for 18 h 'Control', 26 °C + 300 μmol m^{-2} s^{-1}; 'High temperature', 38 °C + 300 μmol m^{-2} s^{-1}; 'Increased light', 26 °C + 600 μmol m^{-2} s^{-1}; 'Combination', 38 °C + 600 μmol m^{-2} s^{-1}. The data represent average values ± SD (n = 3).

from 10 min to 4 h after taking samples were significantly lower than control (Fig. 3).

The F_v/F_m of the top leaf from Acc#3 at 38 °C irrespective of light level were significantly lower than the control using Handy PEA (Fig. 4A). The F_v/F_m of the top leaf from Acc#7 at control condition were significantly higher than high temperature (3.02%), increased light (1.23%) and combination (2.77%) (Fig. 4A). By comparison, the F_v/F_m of the top leaf from Acc#8 at 38 °C with 600 μmol m^{-2} s^{-1} PPFD was significantly lower than control (3.58%), high temperature (2.09%) and increased light (3.78%) (Fig. 4A). The F_v/F_m of the bottom leaf from all genotypes at 38 °C both with 300 and 600 μmol m^{-2} s^{-1} PPFD were significantly lower than control except that of Acc#3 at 38 °C with 300 μmol m^{-2} s^{-1} PPFD (Fig. 4C). The F_v/F_m of the top and the bottom leaf from the three genotypes at 38 °C both with 300 and 600 μmol m^{-2} s^{-1} PPFD by Mini PAM were significantly lower than control except that of Acc#8 at 38 °C with 300 μmol m^{-2} s^{-1} PPFD (Fig. 4B and D).

The chlorophyll content of both top and bottom leaf from Acc#3 at 600 μmol m^{-2} s^{-1} PPFD was significantly higher than the other three treatments (Fig. 5A and B). By comparison, the chlorophyll content of the top leaf of Acc#7 at 600 μmol m^{-2} s^{-1} PPFD with both 26 °C and 38 °C significantly increased as compared with that at 300 μmol m^{-2} s^{-1} PPFD with 38 °C (30.61% and 19.02%, respectively) (Fig. 5A). The chlorophyll content of the top leaf of Acc#8 at 600 μmol m^{-2} s^{-1} PPFD with 26 °C were significantly higher than that at 300 μmol m^{-2} s^{-1} PPFD with 38 °C (24.98%) (Fig. 5A). The chlorophyll content of bottom leaf from Acc#7 and Acc#8 at 38 °C significantly decreased as compared with that at 26 °C with both 300 μmol m^{-2} s^{-1} (22.37% and 22.63%, respectively) and 600 μmol m^{-2} s^{-1} (29.59% and 23.78%, respectively) PPFD (Fig. 5B).

The leaf temperature by infrared gun and CIRAS was significantly positively correlated (R = 0.988**) (Fig. 6A). The P_N was significantly negatively correlated with the leaf temperature by infrared gun (R = -0.813**) and CIRAS (R = 0.-853**) (Fig. 6B and C). The E was significantly positively correlated with g_s and C_i (R = 0.887** and 0.907**) (Fig. 6D and E). The g_s was significantly positively correlated with the C_i (R = 0.814**) (Fig. 6F). Moreover, temperature was significantly negatively correlated with P_N (R = - 0.825**), while the light intensity was significantly negatively correlated with the C_i, g_s and E (R = - 0.818**, - 0.714** and - 0.741**) (Fig. 7). Thereby, heat stress resulted in lower P_N while increased light caused decreased the C_i, g_s and E.

The three main factors (genotype, temperature and light intensity) had significant effects on the P_N, C_i and E (Table S1). The g_s was significantly affected by genotype and light intensity, while the leaf temperature by infrared gun was significantly affected by temperature and light intensity (Table S1). The interaction between genotype and light intensity had significant effects on g_s and E (Table S1). By comparison, the interaction between genotype and temperature, temperature and light intensity significantly affected leaf temperature by infrared gun and C_i (Table S1).

4. Discussion

Abiotic stresses such as high temperature are expected to aggravate due to climate change, which are primary restrictions to the production of chickpea-a cool season crop (Gaur et al., 2019). In addition to temperature, light intensity is of particular interest among environmental factors, which affect photosynthesis (Szymańska et al., 2017). High light levels usually induce photoinhibition, leading to decreased photosynthetic capacity in plants (Zhao et al., 2011; Gao et al., 2019). Avoidance of photoinhibition is key for plant growth and production (Takahashi et al., 2002). Photoinhibition did not occur in this study as the light intensity were not very high, and no significant decline in P_N and F_v/F_m of all genotypes at higher light was seen. This explained why the increased light did not aggravate the damage of heat stress on chickpea. Accordingly, moderate light levels (1000 μmol m^{-2} s^{-1}) had a protective effect on PSII when barley was exposed to heat stress (42 °C) for 5 h as indicated by fluorescence, thermoluminescence and O_2 evolution (Georgieva et al., 2003). More interesting, tomato plants acclimate better to the combination of high light (800 μmol m^{-2} s^{-1}) and elevated temperature (38/29 °C) for 6 days than to individual treatment from the perspective of both photosystems activity (Faik et al., 2016; Gerganova et al., 2016). The doubling light intensity enhanced the P_N of Acc#3 at high temperature as indicated by higher P_N of Acc#3 at combined treatment than that at high temperature. However, this trend was not observed in Acc#7 and Acc#8, suggesting the effect of higher light intensity on the photosynthesis of chickpea at heat stress was genotype-dependent.

Due to the high sensitivity of the photosynthesis to heat stress, lower net photosynthesis rate was observed in heat-sensitive tomato at heat stress (Zhou et al., 2015). Similarly, heat-sensitive chickpea genotypes had lower photosynthetic function at high temperature (Kaushal et al., 2013; Makonya et al., 2019). In accordance, unfavorably higher leaf temperature due to heat stress resulted in lower P_N since both leaf temperature and temperature setting exhibited significantly negatively correlation with P_N. Here, the negative effects of high temperature were less pronounced in Acc#7 than in Acc#3 and Acc#8 as indicated by P_N under moderate light levels. This implied that Acc#7 was more tolerant to heat stress than the other two genotypes, which was also reported by Makonya et al. (2019). The low P_N could be due to stomatal limitation with decreased C_i and non-stomatal limitation with unaffected C_i (Von Caemmerer and Farquhar, 1981) Reduced P_N together with unaffected C_i in chickpea at high temperature was caused by non-stomatal limitation, which agreed with our findings in tomato (Zhou et al., 2015). However, the low P_N in chickpea at high temperature and higher light

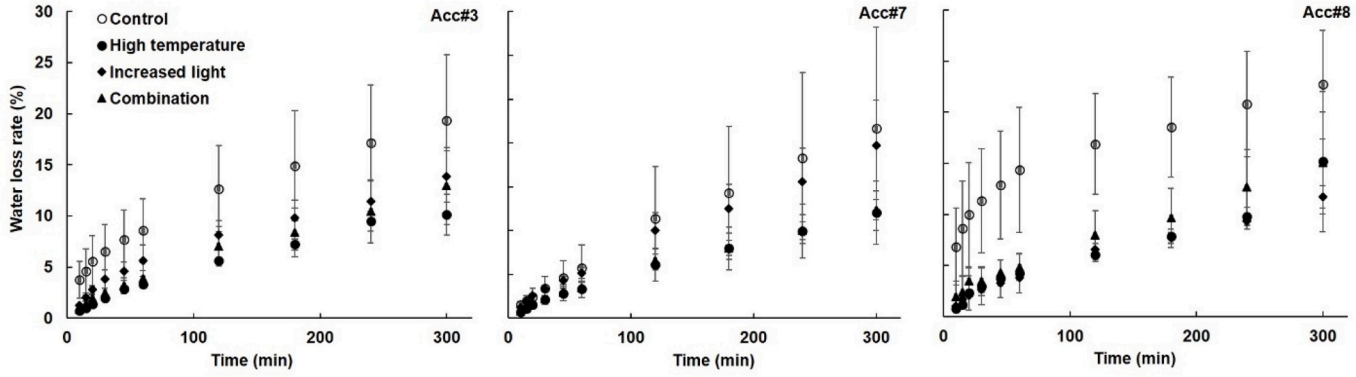

Fig. 3. Water loss rate of the second fully expanded top leaves from three chickpea genotypes at the treatments for 18 h 'Control', 26 °C + 300 μmol m^{-2} s^{-1}; 'High temperature', 38 °C + 300 μmol m^{-2} s^{-1}; 'Increased light', 26 °C + 600 μmol m^{-2} s^{-1}; 'Combination', 38 °C + 600 μmol m^{-2} s^{-1}. The data represent average values ± SD (n = 4).

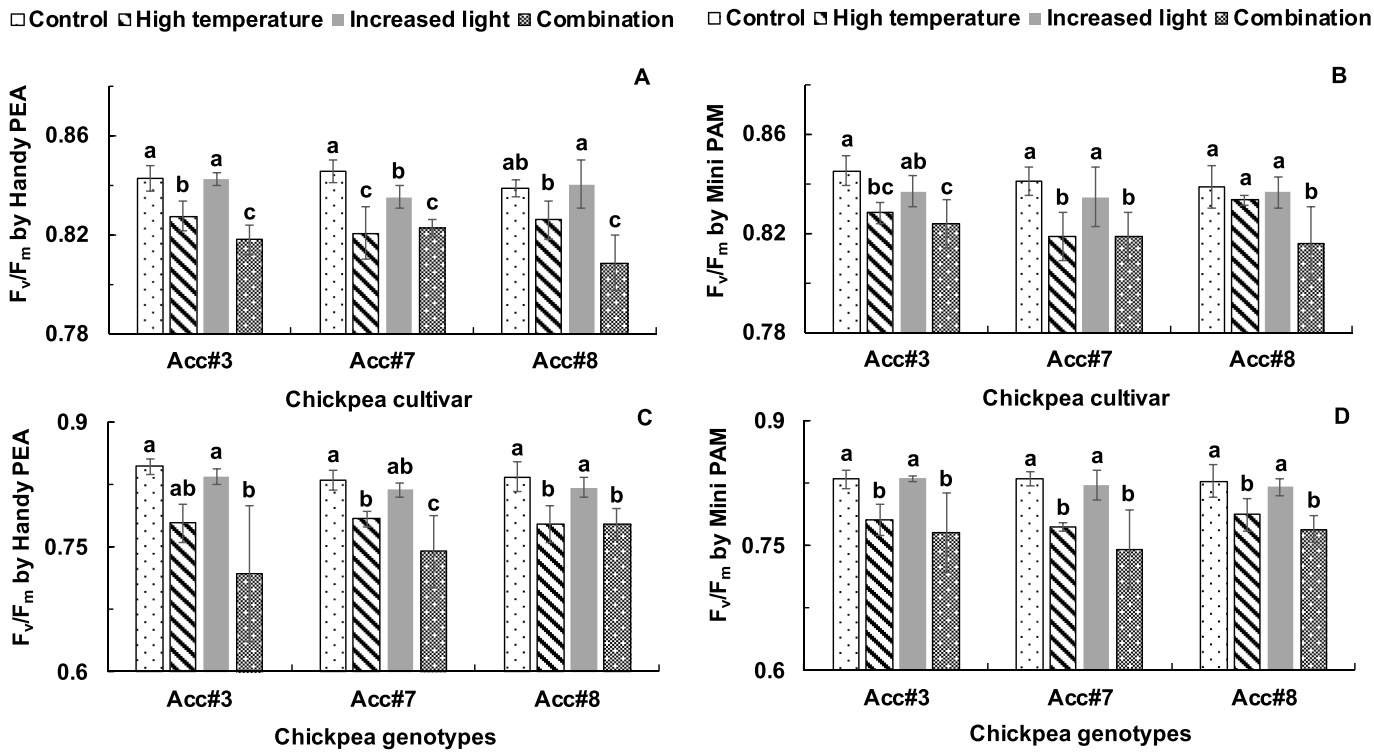

Fig. 4. F_v/F_m by Handy PEA and Mini PAM in the second fully expanded top (**A, B**) and bottom (**C, D**) leaves of three chickpea genotypes at the treatments for 18 h 'Control', 26 °C + 300 μmol m^{-2} s^{-1}; 'High temperature', 38 °C + 300 μmol m^{-2} s^{-1}; 'Increased light', 26 °C + 600 μmol m^{-2} s^{-1}; 'Combination', 38 °C + 600 μmol m^{-2} s^{-1}. The data represent average values ± SD (n = 4).

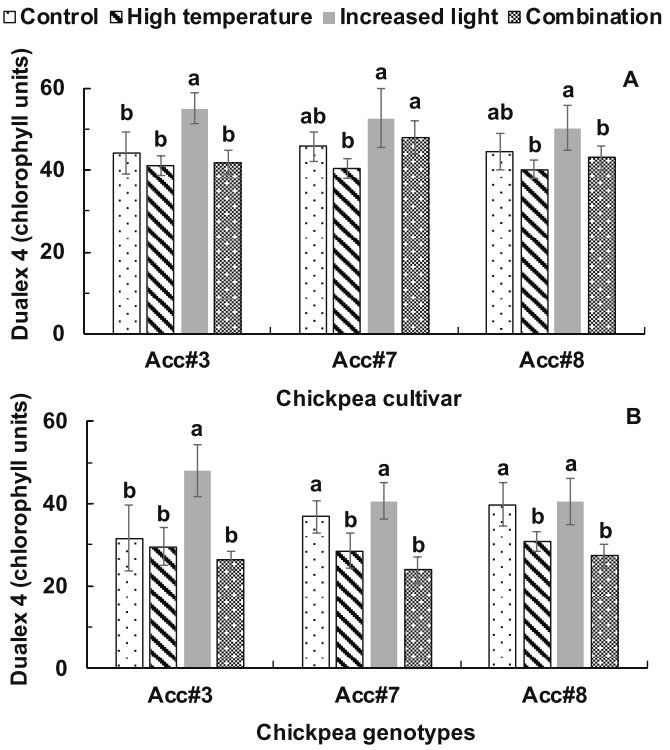

Fig. 5. Non-destructively measured chlorophyll content by Dualex 4 in the second fully expanded top (**A**) and bottom (**B**) leaves from three chickpea genotypes at the treatments for 18 h 'Control', 26 °C + 300 μmol m^{-2} s^{-1}; 'High temperature', 38 °C + 300 μmol m^{-2} s^{-1}; 'Increased light', 26 °C + 600 μmol m^{-2} s^{-1}; 'Combination', 38 °C + 600 μmol m^{-2} s^{-1}. The data represent average values ± SD (n = 4).

intensity was due to stomatal limitation where low g_s was accompanied by decreased C_i. Here, the C_i, g_s and E was significantly correlated with light intensity setting despite the significant correlation between P_N and temperature setting. Thereby, there were different responsive and regulatory mechanism in the chickpea genotypes to high temperature and increased light intensity.

The relative water content, a quantitative indicator of leaf water status, was lower in the four chickpea genotypes with contrasting heat sensitivities at high temperature than control during reproductive stage (Kaushal et al., 2013). This could be due to a reduction in water uptake and increased water loss. The relative water content decreased in three tomato genotypes after five days of drought (no irrigation) but not heat (32/26 °C) (Zhou et al., 2017). We found that relative water content of Acc#3 and Acc#7 at high temperatures irrespective of light intensity was lower than control, while that of Acc#8 showed no significant difference. This suggested that there was genotype difference in leaf water status induced by heat stress. The drop in the relative water content of Acc#7 at high temperature, increased light and their combination corresponded to no difference in g_s. Nevertheless, the difference in relative water content of Acc#8 did not correspond to decreased g_s at the three treatments, indicating that regulation of g_s was closely related to leaf water status (Kaushal et al., 2013).

PSII reaction center in leaf chloroplasts is a primary apparatus that is sensitive to stress conditions such as heat and high light (Su et al., 2014). Chlorophyll fluorescence is a non-invasive and effective index to detect the damage of stress on PSII (Baker and Rosenqvist, 2004). F_v/F_m was reported to decrease in heat-sensitive chickpea at high temperature conditions (Makonya et al., 2019). Here, in most cases, the F_v/F_m of the top and bottom leaves in the three chickpea genotypes decreased at 38 °C regardless of light intensity as compared with control and increased light condition. However, the chlorophyll fluorescence parameters including F_v/F_m was unchanged for heat-tolerant chickpeas such as Acc#7 (Makonya et al., 2019). The inconsistent result could be due to the treatment difference between well-controlled climate chamber and

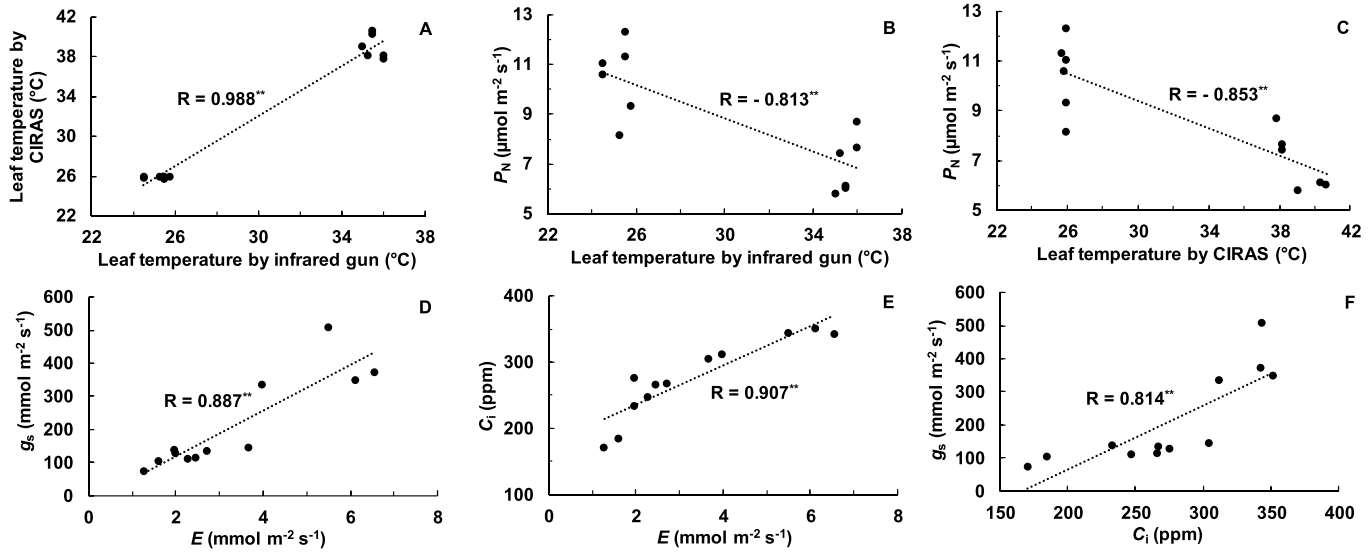

Fig. 6. Correlation between leaf temperature and gas exchange parameters of three chickpea genotypes. ** indicated that the correlation is significant at 0.01 level.

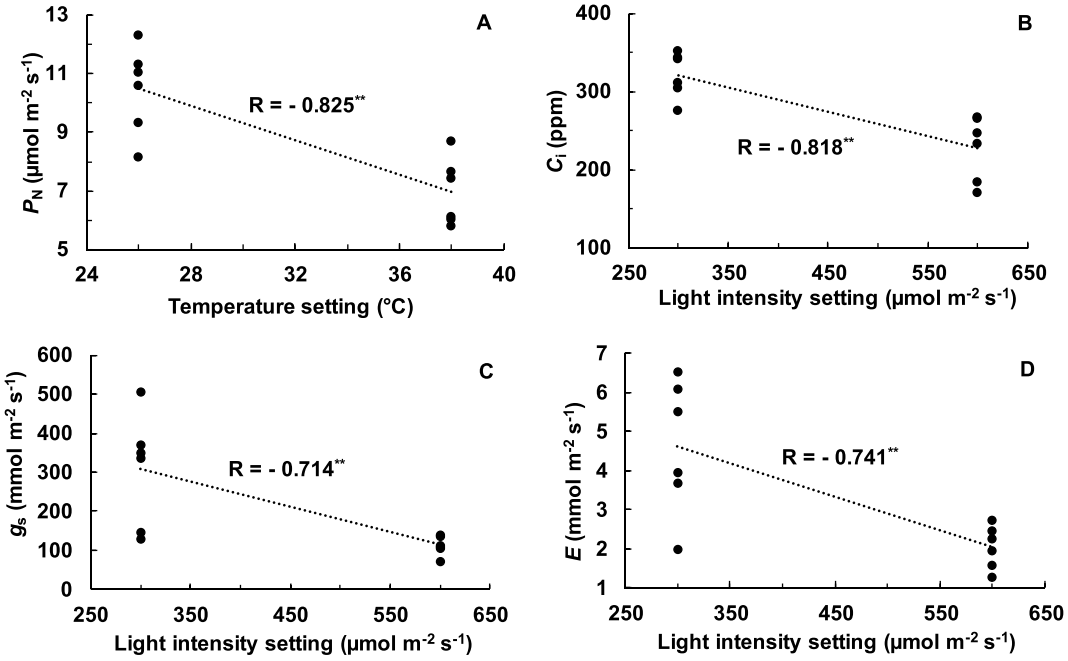

Fig. 7. Correlation between environmental settings and gas exchange parameters of three chickpea genotypes. ** indicated that the correlation is significant at 0.01 level.

field conditions. The direct damage of high temperature on chloroplast membranes in plants might be chlorophyll loss (Kotak et al., 2007). We found that the chlorophyll content of the bottom leaves in Acc#**7** and Acc#8 at high temperature and the combination of treatments were lower than that at control and increased light intensity, indicating that heat stress accelerated the leaf senescence. Our results on chlorophyll loss corresponded to previous studies on chickpea subjected to heat stress (Kumar et al., 2012). The low chlorophyll content, in turn, reduced chlorophyll fluorescence in chickpea at high temperature. Chlorophyll loss also results in a loss of photosynthesis, which occurred to a larger extent in Acc#8 and Acc#3 than Acc#7.

Based on the responses of the three chickpea genotypes, moderately increased light intensity decreased C_i, g_s and E regardless of temperature, while high temperature increased leaf temperature and decreased P_N, F_v/F_m and chlorophyll content regardless of light. Even though the physiological responses of chickpea in the lab could be different from those under open fields, the results are beneficial for understanding plant response and adaption as well as managing crop. Chickpea leaf of Acc#3 showed a higher P_N at the combination of high temperature and moderately increased light than that at high temperature, while it did not occur for Acc#7 and Acc#8. Thereby, each genotype showed specific physiological responses to high temperature and moderately increased light, indicating that chickpea adapt to the different temperature and light intensity. This study help to understand the response of chickpea to high temperature and increased light intensity, which will contribute to chickpea improvement to deal with future climate changes.

Declaration of competing interest

The authors declare no conflict of interest.

CRediT authorship contribution statement

Rong Zhou: Writing - original draft, Writing - review & editing, Formal analysis. **Xiaqing Yu:** Writing - original draft, Writing - review & editing. **Sijie Huang:** Writing - original draft, Writing - review & editing. **Xiaoming Song:** Writing - original draft, Writing - review & editing. **Eva Rosenqvist:** Writing - original draft, Writing - review & editing. **Carl-Otto Ottosen:** Writing - original draft, Writing - review & editing.

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Appendix A. Supplementary data

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