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ORIGINAL ARTICLE

Seed priming with gibberellic acid rescues chickpea (*Cicer arietinum* L.) from chilling stress

Tariq Aziz[1] · **Erkut Pekşen**[1]

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Abstract

Chickpea is sensitive to low temperature stress, especially during germination and stand establishment. Present study was planned to rescue the chickpea seedlings from chilling stress through seed priming with gibberellic acid. A study comprising of two chickpea cultivars (cv.) viz. Çağatay (a chilling tolerant cultivar: CTC) and Akçin (a chilling sensitive cultivar: CSC) in combination with 0, hydropriming, 5 µM, 10 µM, 15 µM, 20 µM gibberellic acid (GA_3) seed priming treatments was tested in completely randomized design under chilling temperature. Primed and unprimed seeds were sown at 9 ± 0.5 °C day temperature for 14 h and 7 ± 0.5 °C night temperatures for 10 h. Final emergence percentage (FEP) in both cultivars was noted higher in 10 µM GA_3 seed priming. Coefficient of uniformity of emergence (CUE) was increased and time taken to 50% emergence (E_{50}) was shorten with the application of 5 or 15 µM GA_3. Mean emergence time (MET) was reduced in both cultivars in 20 µM GA_3 seed treatment. Emergence energy (EE) and emergence index (EI) of CTC were increased in 15 µM GA_3. In CSC 5 µM GA_3, seed treatment was most productive treatment under low temperature. Higher doses of GA_3 seed treatments in CSC were proved very effective in maintaining high relative water contents and low electrolyte leakage. Plant height, root length and number of flowers were also increased in GA_3 primed treatments. In conclusion, seed priming with GA_3 can be used in chickpea for good stand establishment, crop growth, reducing electrolyte leakage and maintaining high relative water contents.

Keywords Gibberellic acid · Chilling stress · Chickpea · Seed priming · Cultivars

Introduction

Chickpea ranks third among food grain legume crops in the world. Although, it is a member of cool season legume crops, but gives poor response to low temperatures (Singh et al. 1993). Normally, chickpea requires 21–29 °C temperatures during day and 20 °C during night time for optimum growth and development (Kulkarni and Chimmad 2014). Low temperature adversely affects its growth and development (Croser et al. 2003). The seed germination phase is relatively more sensitive to chilling stress. The performance of lateral growth stages is associated with competence of seed germination (Farooq et al. 2017). Chilling can slowdown or inhibit hydrolytic enzymes activities in germinating seeds. Hydrolytic enzymes convert complex food reserve to the simple useable form for embryo growth during seed germination (Szopińska and Politycka 2016). Chilling can disrupt the function of cellular membrane and disturb the physiological and biochemical processes. Cell membranes are sites considered to be the primary targets of hormonal actions in plants to perform different processes (Trewavas and Gilroy 1991). These sites are very vulnerable to environmental stresses (Kuiper 1985; Lyons 2012). Stability of membrane constituents, particularly lipids are very important in maintaining membrane integrity and cell functionality (Mazliak 1983; Leshem et al. 1990). Huge changes in membrane's lipid composition occurred in higher plants to maintain chlorophyll activity during chilling stress (Pham et al. 1982, Quartacci et al. 1995). Such changes might be the result of an increase of unsaturated fatty acids ratio in the galactolipids. It decreases the temperature of transition phase of the total thylakoid lipid (Moon et al. 1995), resulting in higher membrane stability when temperature touches lower critical

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✉ Tariq Aziz
tariqbwp@gmail.com

[1] Department of Field Crops, Faculty of Agriculture, Ondokuz Mayıs University, 55139 Samsun, Turkey

levels. It has been reported that the impact of chilling is not restricted to germination it may also change nutrients dynamics, water uptake, interrupt exchange of gases and photosynthesis (Yadav 2010), thereby, reducing plant growth and yield. Low temperature directly effects the photosynthetic machinery by disturbing thylakoid membranes that is the main site of light reactions during photosynthesis (Zhou et al. 2007). In chickpea, poor initial crop stand increases the chances of chilling vulnerability; therefore, can cause considerable damage and yield losses (Croser et al. 2003).

Gibberellic acid is a growth hormone involved in seed germination. Earlier research findings demonstrated that low-temperature stress harmed seedling emergence in rice but seed treatment with GA_3 significantly increased seedling emergence (Chen et al. 2005). In many other studies, it has been reported that GA_3 improves abiotic stress tolerance in chickpea (Kaur et al. 1998). Seed priming with growth hormones or salts is one of the pragmatic methods used to improve seed germination and seedling establishment under chilling stress (Farooq et al. 2017). Up to now, many studies have been reported about the progressive role of seed priming in developing tolerance in plants (Farooq et al. 2008) but the role of GA_3 via seed priming under chilling stress still not investigated. So, using seed priming to overcome chilling stress could be one of possible options to grow up crops under abnormal temperatures. In the current research, we studied the role of seed priming with gibberellic acid to improve chilling tolerance in chickpea during seed germination and early growth stages under controlled conditions.

Materials and methods

Plant material

Seeds of chickpea cultivars were obtained from Black Sea Agricultural Research Institute, Samsun, Turkey, and were used as an experimental material. The selection of the cultivars was made on the bases of our own earlier screening experiments. Out of 24 Turkish released chickpea cultivars, two cultivars (Çağatay and Akçin) were selected for further study. Çağatay cultivar was known to be chilling tolerant cultivar (CTC) and Akçin cultivar to be chilling sensitive cultivar (CSC).

Method

The experiment was conducted in plastic pots. The dimensions of the pots were comprising of 22 cm width × 28 cm in height having capacity of carrying 9 kg soil. The soil used in the pots was passed through 5 mm sieve to get off all coarse materials out. Sieved soil was mixed with peat soil and farm yard manure by keeping 1:1:1 ratio of each material. Pots were filled with that well homogenized mixture. Three pots were excessively irrigated and left overnight allow to draining out extra water to determine the field capacity by calculating the actual amount of water hold by the soil mixture. Primed and unprimed seeds were sown in pots. After sowing, pots were irrigated with calculated amount of water to bring them at field capacity. After irrigation, pots were shifted to growth room by setting growth room temperatures at 9 ± 0.5 °C day: 7 ± 0.5 °C night. Photoperiod was set to 14 h and 10 h for day and night, respectively. Light intensity was set at 350 mE m^2 s^1 photon flux density.

Seed priming

Seed priming was carried out by dipping the seeds in well aerated solution of gibberellic acid consisting of 0 (hydropriming or HP), 5, 10, 15 and 20 µM for 4.5 h at 23 ± 2 °C. Seed weight to solution volume ratio was kept 1:5 (w/v) (Farooq et al. 2006). After each seed priming treatment, seeds were surface washed with running tap water and placed in the shade under forced air at 23 ± 2 °C, until initial moisture level was achieved. Finally, seeds were packed in plastic bags and kept in a cool place at 4 °C till sowing (Lee and Kim 2000). Seeds without any treatment were considered as a control treatment.

Stand establishment and seedling vigour evaluation

The number of germinated seeds was calculated every day according to the protocol described in seedling evaluation Handbook of Association of Official Seed Analysis (1990). Time taken to 50% emergence (E_{50}) was measured following the equation described by Coolbear et al. (1984), and revised by Farooq et al. (2005). Mean emergence time (MET) was determined using the formulae of Ellis and Roberts (1981). The energy of emergence (EE) was calculated according to Farooq et al. (2008). The coefficient of uniformity of emergence (CUE) was measured by applying the equation of Bewley and Black (1994). The emergence index (EI) was measured as described in the association of official seed analysis (AOSA 1983). Seedling dry weight was determined after drying the plant samples at 70 °C for 72 h days.

Measurement of electrolyte leakage

Membrane stability was estimated on the bases of electrolyte leakage following the protocol of Blum and Ebercon (1981). Leaf segments, weigh 0.5 g were rinsed with distilled water and immersed in test tubes having 10 ml distilled water. Heads of all test tubes were covered with aluminum foil and placed in a water bath (NÜVE BS 302) at 40 °C for 30 min. All the

test tubes were taken out and kept at room temperature to bring their temperature down to 25 °C, followed by measuring EC_1 of the test tubes with a conductivity meter (Delta OHM HD 8706). After measuring EC_1 of the all test tubes containing same leaf samples were again placed in water bath for 10 min at 100 °C. Samples were taken out and cool down to 25 °C. The electrical conductivity of killed tissues (EC_2) was measured. Electrolyte leakage was calculated as the ratio between EC_1 and EC_2.

Relative water content (RWC)

Fresh leaves were used to measure the relative water contents. Leaf samples (0.5 g; W_f) were floated on distilled water in Petri dishes for the period of 2 h to absorb water. After due time, saturated leaves were taken out and were surface dried with soft tissue paper. The saturated weight (W_s) were taken and then dried the leaves samples for 24 h at 80 °C in oven for determining the dry weight (W_d) (Jukanti et al. 2012).

Relative water content (RWC) was calculated by the following equation (Barr and Weatherley 1962):

$$RWC\ (\%) = [(W_f - W_d)/(W_S - W_d)] \times 100$$

Phenological parameters

Phenological parameters like time taken from sowing to first flowering (days), time taken from sowing to 50% flowering (days), time taken from sowing to 100% flowering (days) and time taken from sowing to physiological maturity (days) were considered when almost 50% of plants reached to their particular stage.

Statistical analysis

Experiments were conducted in a completely randomized design (CRD) with factorial arrangement using four replications. For the comparison of treatment means, standard errors were figured out using Microsoft Excel program. Data from the study were analysed using 'Statistix 10′ window program at 0.05% probability level. Means showing statistically significance were separated by LSD test at $p < 0.05$ and mean separation were shown on the columns on the graphs for each parameter. Graphical presentation of means with error bars based on the standard error of the mean were made using Microsoft Excel program.

Results

Stand establishment

Low temperature is a major constraint to the seed germination, stand establishment and early seedling growth of chickpea. The state of crop establishment and the level of its productivity is related to the vigorous seed germination. Chilling stress has many adverse effects on seed germination and stand establishment. In our study, chilling stress reduced final emergence percentage (FEP) but seed priming with 10 µM GA_3 improved FEP in both cultivars followed by 5 µM GA_3 seed treatment in CTC (Fig. 1a). CSC showed lower FEP than that of CTC. Emergence energy (EE) was recorded higher in CTC in combination with 15 µM GA_3 seed priming while EE of CSC was recorded higher in 5 µM GA_3 seed treatment (Fig. 1b). Although, chilling stress delayed time taken to 50% emergence (E_{50}) and mean emergence time (MET) in both cultivars but seed priming with GA_3 reduced E_{50} and MET. E_{50} and MET were distinctly higher in untreated control treatment as compared to the GA_3-treated seeds. Minimum E_{50} and MET were recorded in seed priming with 10 and 15 µM GA_3, respectively (Fig. 2a, b). The data related to the comparison of coefficient of uniformity of emergence (CUE) showed that both cultivars differ significantly with each other. GA_3 seed treatments at the rate of 5 and 15 µM increased CUE in CSC and CTC, respectively. Likewise, emergence index (EI) was recorded higher in CSC in 15 µM GA_3 seed treatment. In CTC, HP improved EI as compared to other seed priming treatments (Fig. 3a, b).

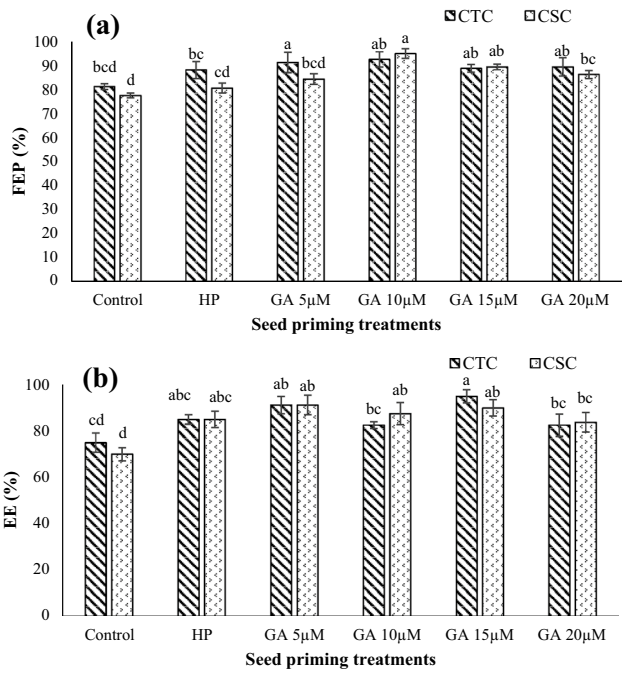

Fig. 1 Influence of pre-sowing gibberellic acid (GA_3) seed treatments on **a** final emergence percentage (FEP) and **b** energy of emergence (EE) under chilling stress using four replications. Means with the same letter are not significantly different ($p < 0.05$) and bars on the columns show standard error (±S.E) of the means. Whereas *CTC* chilling tolerant cultivar, *CSC* chilling sensitive cultivar

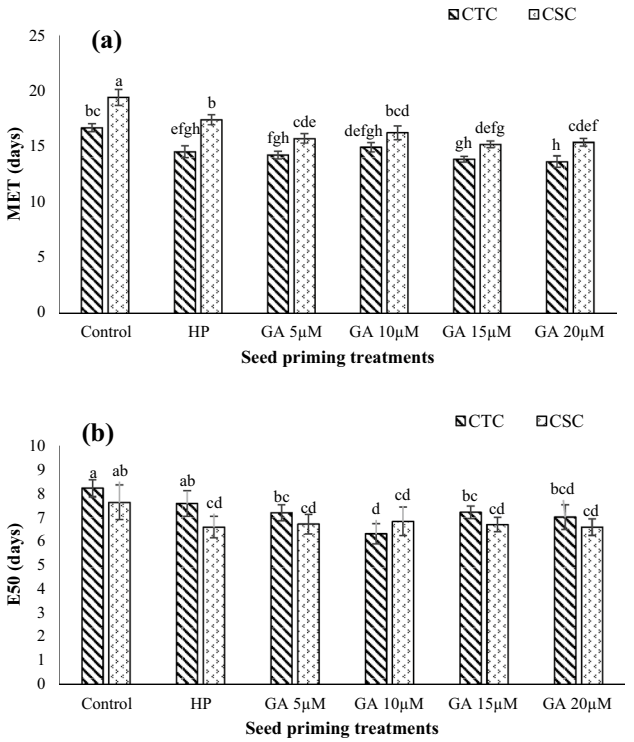

Fig. 2 Influence of pre-sowing GA_3 seed treatments on **a** mean emergence time (MET) and **b** time taken to 50% emergence (E_{50}) of chickpea cultivars under chilling stress using four replications. Means with the same letter are not significantly different ($p < 0.05$) and bars on the columns indicate standard error (±S.E) of the means. *CTC* chilling tolerant cultivar, *CSC* chilling sensitive cultivar

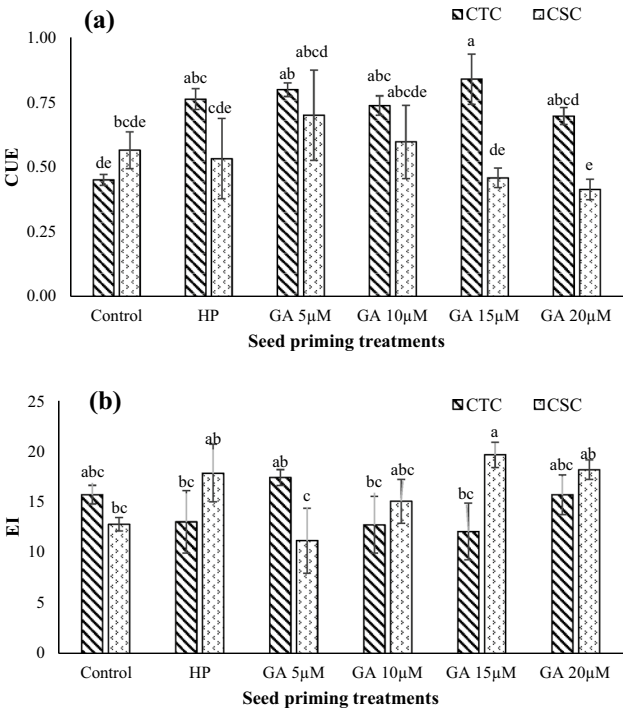

Fig. 3 Influence of pre-sowing GA_3 seed treatments on **a** coefficient of uniformity of emergence (CUE) and **b** emergence index (EI) of chickpea cultivars under chilling stress using four replications. Means with the same letter are not significantly different ($p < 0.05$) and bars on the columns indicate standard error (±S.E) of the means. *CTC* chilling tolerant cultivar, *CSC* chilling sensitive cultivar

Periodic shoot length (cm)

Shoot length (SL) was recorded five times each after 5 days interval to observe the shoot growth pattern under chilling stress. Influence of GA_3 on shoot length (SL) is presented in Fig. 4. Under low temperature stress, SL was suppressed but seed priming treatment has increased or maintained SL of both cultivars (Fig. 4). There was distinct difference between shoot elongation rate during chilling period and after shifting the pots to the ambient growth conditions in the greenhouse. Maximum SL was noted in CTC with 10, 15 or 20 µM GA_3 seed priming as compared with control treatment. Although, increasing trend of SL was observed in both but the growth rate of CSC was bit slower than that of CTC under chilling temperature. In CSC, during initial growth days, the rate of increasing SL was relatively high in 10 and 20 µM GA_3 pre-sowing treatments but after 07 March, it was preceded by the 15 µM GA_3 seed treatment. SL of the CSC increased substantially with higher doses (10 or 20 µM GA_3) of seed treatments as compared with control and other seed priming treatments. CTC grow vigorously and performed better than that of CSC under chilling stress. Chilling tolerance was further increased by pre-sowing seed priming with GA_3 treatments.

Relative water content (RWC) and electrolyte leakage (EL%)

Chilling stress resulted decrease in relative water contents (RWC) in both cultivars irrespective to seed priming. The results showed that RWC of chilling tolerant cultivar were less disturbed under chilling stress as compared to the chilling sensitive cultivar (Fig. 5a). Seed priming with GA_3 effectively improved RWC than that of control treatment. Seed priming with 5 or 10 µM GA_3 maintained higher RWC in CTC while in CSC seed priming with 15 or 20 µM GA_3 treatments were very effective in maintaining higher RWC (Fig. 5a). Chilling stress induced the EL (the solutes come out from the cells), and was recorded higher in control treatment. The extent of EL is an indicator of cell membrane damage. Cell membrane is known for one of the swift targets of many plant stressors. Application of GA_3 proved very effective in reducing electrolyte leakage by stabilizing the cell membrane. Low EL was recorded in 20 µM GA_3 followed by 10 µM GA_3 seed treatments

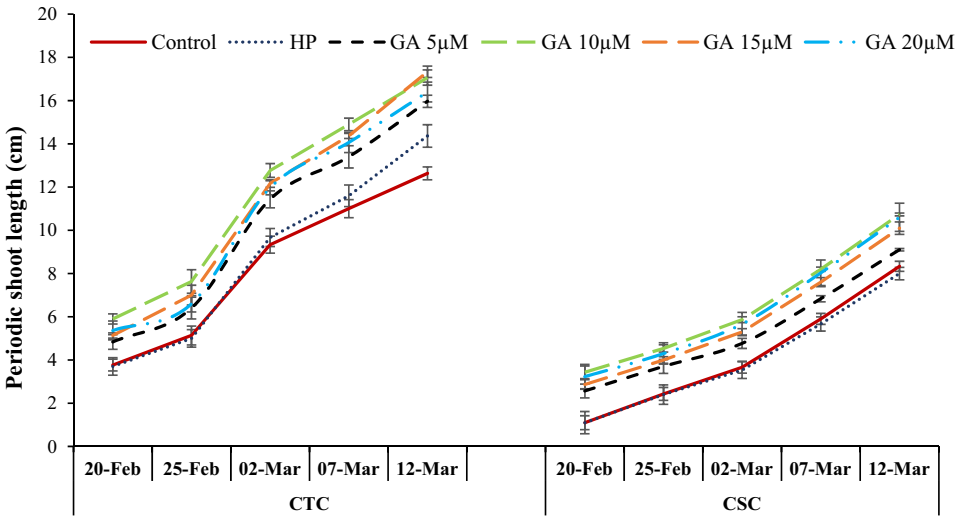

Fig. 4 Role of seed priming with GA_3 on periodic shoot length (cm) of two chickpea cultivars sown under chilling stress using four replications. Bars on the lines indicate standard error (± S.E) of the means

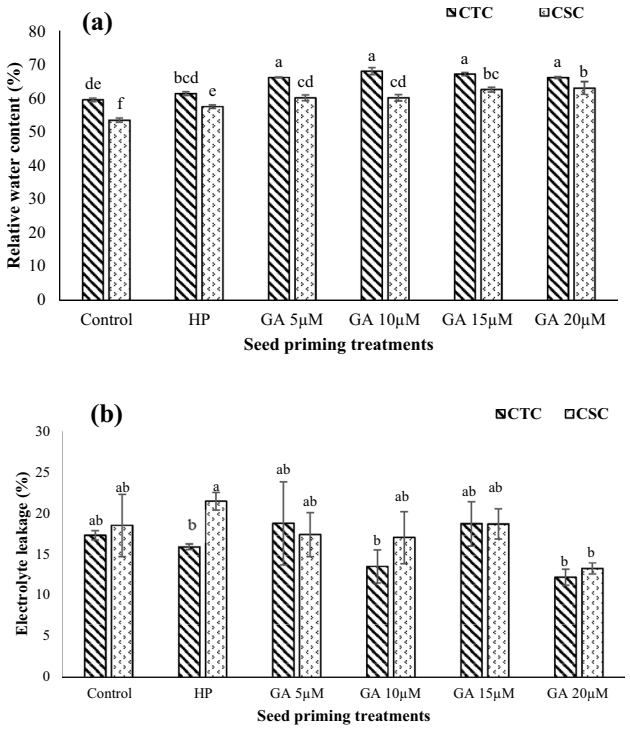

Fig. 5 Role of seed priming with GA_3 on **a** relative water content (RWC) and **b** electrolyte leakage (EL) of two chickpea cultivars sown under chilling stress using four replications. Means with the same letter are not significantly different ($p < 0.05$) and bars on the columns indicate standard error (± S.E) of the means. *CTC* chilling tolerant cultivar, *CSC* chilling sensitive cultivar

indicates that seed priming can be effectively use for ameliorating cell membrane damage under low temperature stress. Similarly, our results were promising in reducing the effect of chilling stress in both cultivars either tolerance or sensitive (Fig. 5b).

Chlorophyll contents (µmol m^{-2})

Chilling stress disordered photosynthesis by lowering and disrupting chlorophyll pigments. However, the pretreated seeds with GA_3 well-maintained chlorophyll contents as compared with the control treatment. Leaf chlorophyll contents of CTC and CSC cultivars were recorded higher in 15 µM and 5 µM GA_3 seed treatments, respectively (Fig. 6a). Chlorophyll contents of the plants after 1 week of their shifting to the greenhouse (ambient environment) revealed higher chlorophyll recovery in CSC as compared with CTC. In CTC, chlorophyll contents were already higher than CSC so less change was observed. Seed priming with 20 µM GA_3 was the best treatment for CSC. In CTC, there was no significant difference in chlorophyll contents between seed priming and control treatment (Fig. 6b).

Plant growth parameters

Pre-sowing GA_3 seed priming increased shoot dry weight significantly in CSC, while there was no significant difference in CTC was found as compared with control treatment. Seed priming with 5 µM GA_3 was the most effective seed treatment in increasing plant dry weight (Fig. 7a). Seed priming has influenced root dry weight in both chickpea cultivars (Fig. 7b). Maximum root dry weight was recorded in the CTC sown after 20 µM GA_3 seed treatment. Similarly, seed priming with 10 µM GA_3 increased root dry weight of CSC cultivar (Fig. 7b), however, seed priming with 20 µM GA_3 resulted lowest root dry weight in CSC (Fig. 7b).

Plant height and root length (cm)

Seed priming was helpful in improving plant height and root length (RL) of both chickpea cultivar. Maximum plant height was measured in CSC in seed priming with 10 µM GA_3

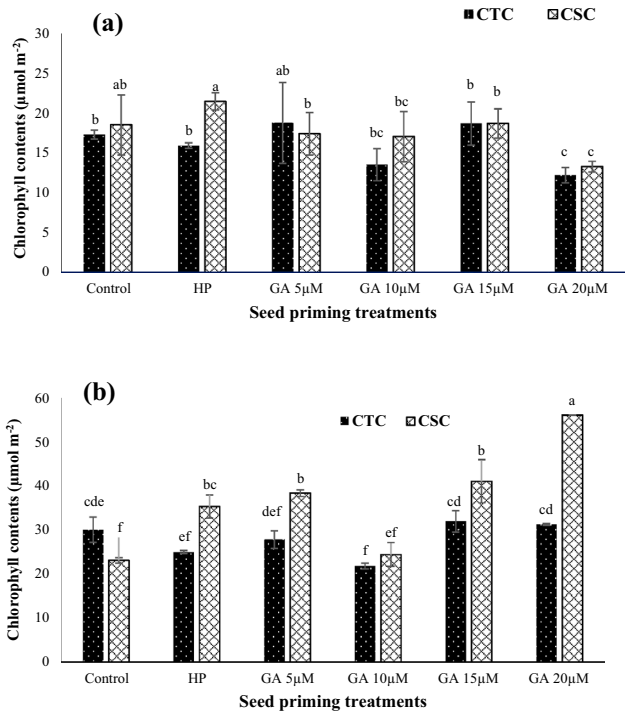

Fig. 6 Role of seed priming with GA_3 on **a** chlorophyll contents (µmol m^{-2}) during chilling stress **b** chlorophyll contents (µmol m^{-2}) after chilling stress of two chickpea cultivars sown under chilling stress using four replications. Means with the same letter are not significantly different ($p < 0.05$) and bars on the columns indicate standard error (±S.E) of the means. *CTC* chilling tolerant cultivar, *CSC* chilling sensitive cultivar

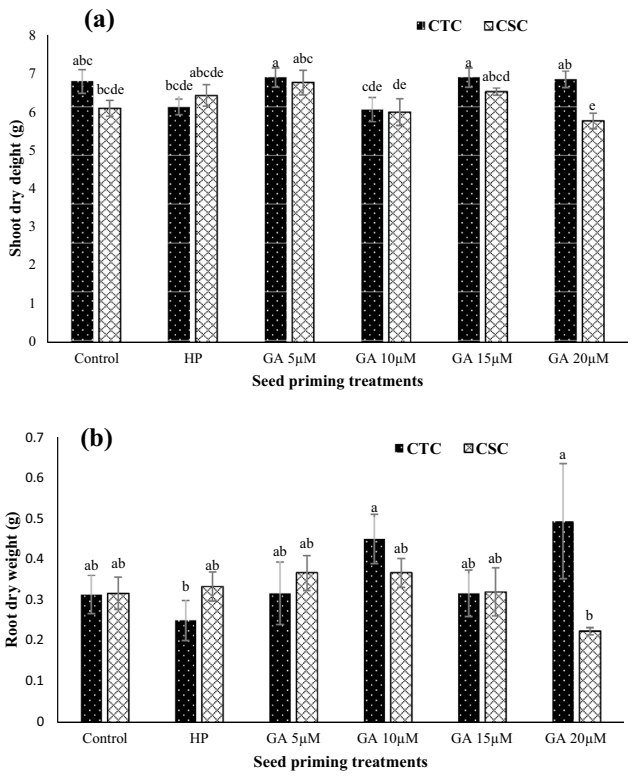

Fig. 7 Role of seed priming with GA_3 on **a** shoot dry weight **b** root dry weight of two chickpea cultivars sown under chilling stress using four replications. Means with the same letter are not significantly different ($p < 0.05$) and bars on the columns indicate standard error (±S.E) of the means. *CTC* chilling tolerant cultivar, *CSC* chilling sensitive cultivar

and minimum plant height was in control treatment. Plant height of CTC varied between 53.66 and 60 cm (Fig. 8a). Root lengths of CSC and CTC were ranged between 6.33 and 16.33 cm and 11.33–17.33 cm, respectively (Fig. 8b). Maximum root length was recorded in CTC in 10 µM GA_3 seed treatment (Fig. 8b) and the minimum RL was in control treatment. Overall CTC produced long roots as compared with CSC of all priming treatments except seed priming with 20 µM GA_3 where CSC performed better than CTC (Fig. 8b).

Number of flowers and branches

Number of flowers were differed in primed and non-primed seeds. Seed priming with higher GA_3 concentration improved number of flowers in both cultivars (Fig. 9a). Seed priming with 10 or 15 µM GA_3 doses produced higher number of flowers in both cultivars. Maximum number of flowers were counted in CTC with 10 µM GA_3 seed treatment. Number of branches were improved in CSC when seeds were sown after seed priming with GA_3 as compared with untreated seeds. Overall number of branches were observed higher in CSC in priming treatments while under control treatments number of branches were higher in CTC. It means CSC was more responsive to GA_3 seed priming in term of production of branches (Fig. 9a).

Discussion

Chilling temperature stress affected seed germination, stand establishment and early growth of both tested cultivars (Figs. 1, 2, 3). The present findings are in line with the Yusefi-Tanha et al. (2015). They reported poor seedling emergence under-chilling temperature stress in peas, but seed priming improved final emergence percentage. The reasons behind low germination were might be the adverse effect of reactive oxygen species and poor enzymatic activities. Chilling stress can ramp up the production of reactive oxygen species (ROS) which disturbed electron flow during metabolism. ROS damage the cell membranes and caused electrolyte leakage and proscribe seed germination (Baalbaki et al. 1999; Yusefi-Tanha et al. 2019). In seeds, GA_3 involves in physiological and metabolic processes to initiate seed germination (Pipinis et al. 2012), and also help up to

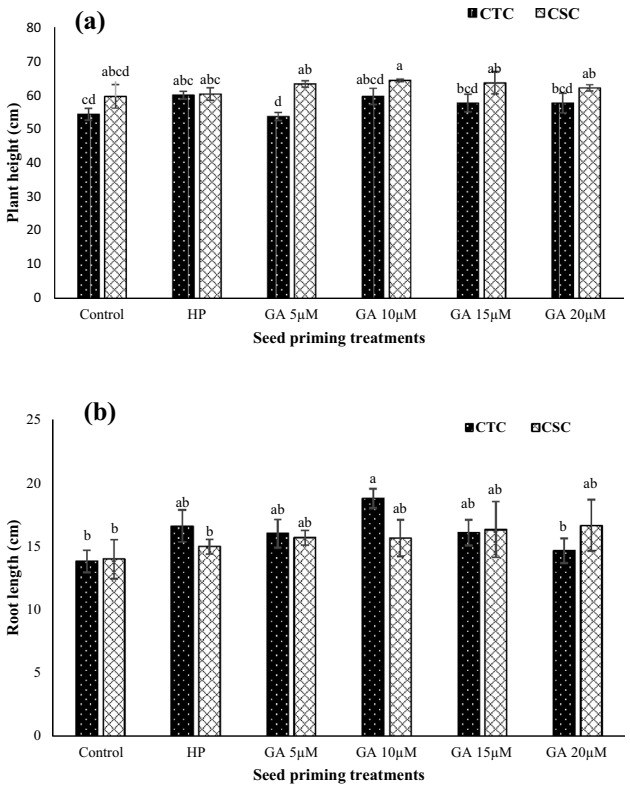

Fig. 8 Role of seed priming with GA_3 on **a** plant height and **b** root length of two chickpea cultivars sown under chilling stress using four replications. Means with the same letter are not significantly different ($p < 0.05$) and bars on the columns indicate standard error (±S.E) of the means. *CTC* chilling tolerant cultivar, *CSC* chilling sensitive cultivar

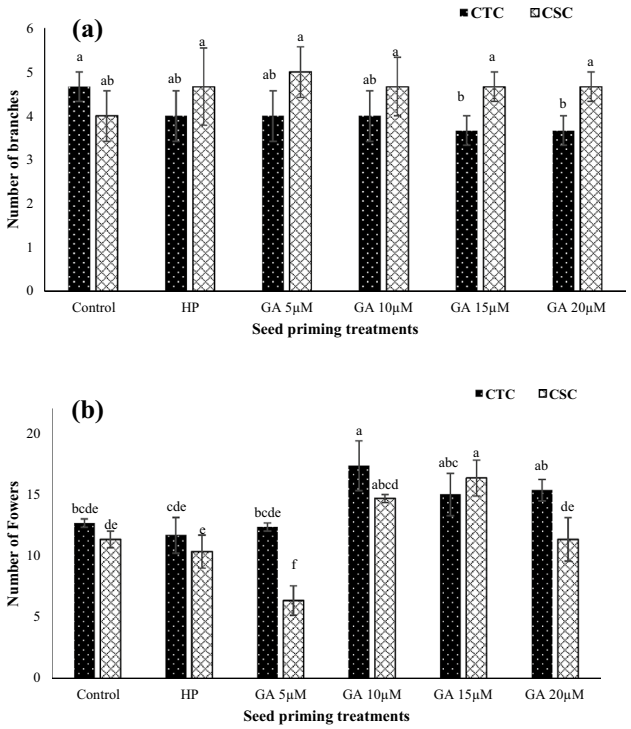

Fig. 9 Role of seed priming with GA_3 on **a** shoot dry weight and **b** root dry weight of two chickpea cultivars sown under chilling stress using four replications. Means with the same letter are not significantly different ($p < 0.05$) and bars on the columns indicate standard error (±S.E) of the means. *CTC* chilling tolerant cultivar, *CSC* chilling sensitive cultivar

alleviate the adverse effect of stress as found in our study (Fig. 1a, b). Kirmizi et al. (2010) reported that application of 150 ppm GA_3 under low temperature increases germination percentage in *Pedicularis olympica* (Scrophulariaceae). In the current research, seed priming with GA_3 improved seedling emergence percentage and reduced E_{50} and MET (Fig. 2a, b). Gibberellic acid takes part in inducing hydrolytic enzyme activities such as hydrogenase and α-amylase (Gupta et al. 2013) to initiate germination process in the seed and speed up germination process. Seed priming with GA_3 can hastens seed germination rate by stimulating and activating the food-digestive and food mobilizing enzymes (Hartmann et al. 1997). Taiz and Zeiger (2002) reported that embryo naturally releases GA_3 that activate particular genes for mRNA transcription by α-amylase during seed germination. The activation of α-amylase and couple of other enzymes degrades the food reserves and makes available for embryo consumption. The other possible reason behind good stand establishment was that primed seeds had already completed the first two phases of seed germination; in other words, seeds were at the brink of germination. On the other hand, enzymes must have to digest food reserves first to convert complex food to simple available form in unprimed seeds for the embryo utilization. Therefore, embryo takes much longer time to grow in untreated seeds due to low and slow availability of soluble food.

Earlier research findings showed that chilling stress reduces plant growth but seed treatment with GA_3 stimulates plant growth (Rehman and Park 2000). Thakare et al. (2011) also reported that seed priming with GA_3 enhances chickpea height under low temperature stress. Higher plant height in GA_3-treated seedlings, might be the result of higher cell division, cell elongation (Naylor 1984), in addition to high cell wall acid invertase activity (Kaur et al. 1998; Kaya et al. 2006). Cell wall acid invertase enzymes catalyze the irreversible breakdown of sucrose to free glucose and fructose and are the fundamental enzymes take part in controlling cell differentiation and plant development (Koch 1996). GA_3 also trigger the metabolic consumption of soluble sugars to form new cell constituents which involves in growth process (Jasmine and Merina 2012). In short statured pea (*Pisum sativum*) plants, GA_3 treatment triggered shoot length by activating cell-wall invertase enzyme (Wu et al. 1993). In beans (Phaseolus vulgaris), GA_3-treated plants showed high invertase activity in elongating internodes (Morris and

Arthur 1985), and resulting stem elongation. GA_3 has natural character that boost up impaired cell division and cell elongation under unfavorable conditions (Kaya et al. 2006). This might be the result of less water requirements or high water up take in the presence of plant growth hormones during seedling growth (Kaur et al. 1998). Chilling stress induces production of ROS, causes reduction in cell division and cell elongation (Kaya et al. 2006) and modify biochemical changes in cell wall during cell growth and restrict cell wall extension (VanVolkenburgh and Boyer 1985).

Relative water contents give the information about internal plant water status and all types of metabolic activities are directly linked with the presence of water in the plant tissues (Flower and Ludlow 1986). RWC is associated with leaf water potential and correlated with plant yield (Lafitte 2002). Seed priming with 5 or 10 µM GA_3 maintained higher RWC in CTC while in CSC seed priming with 15 or 20 µM GA_3 treatments were very effective in maintaining high RWC (Fig. 5a). Under low temperature stress, low RWC were possibly due to the decrease of cell metabolites and solutes available to keep the water within the cells. The main effect of low temperature stress on developing seedlings were related to turgor loss, as a consequence of turgor loss, plant showed wilting as well as drought symptoms (Croser et al. 2003). So, in this regard, high RWC of plant leaf under low temperature could be used as an indicator of chilling tolerance (Singh et al. 2012; Ghosh et al. 2016). In 20 µM GA_3-treated seeds low electrolyte leakage occurred followed by 10 µM GA_3 seed treatments indicates that seed priming can be effectively used for ameliorating cell membrane damage under low temperature stress (Fig. 5b). Chilling stress triggers the production of reactive oxygen species that disturbed the electron flow across the plasma membrane. Under chilling stress, membrane lipids usually transform from fluid to gel or semisolid or solid states, which limits membrane permeability (Leshem 2013). Under such conditions, ROS may react with membrane's polyunsaturated fatty acids and induce lipid peroxidation and damage cell membrane. It might be thought that GA_3 increases Ca^{2+} ion in the leaf cells which help to keep water balance. Likewise, Ali et al. (2012) reported that the role of GA_3 is obvious in maintaining high RWC of leaf. Kaya et al. (2006) also reported similar results that GA_3 increase relative water contents by increasing root length and water uptake. In nutshell, seed priming with GA_3 proved effective in decreasing EL and increasing RWC under low temperature stress (Fig. 5a, b).

Chlorophyll contents of CTC and CSC cultivars were improved with 15 and 5 µM GA_3 seed treatments, respectively (Fig. 6a). Tatar et al. (2013) noted that chilling stress inhibit chlorophyll contents, damage its structure and function in non-treated seeds. They also reported that Sarı-98 and VDI-5 cultivars differ in chlorophyll content under chilling conditions. Georgia et al. (2010) reported that externally applied GA_3 increased quantum yield and the ratio of Fv/Fm in chilies. Another study report stated that GA_3 increased chlorophyll content in grape (Lim et al. 2004). Seed priming with 200 ppm GA_3 increased chlorophyll content in papaya seedlings by accelerating nitrogen uptake (Ramteke et al. 2016). Under low temperature stress, reduction in chlorophyll contents might be the result of proteolytic enzymes synthesis such as chlorophyllase that involved in chlorophyll degradation (Sabater and Rodriquez 1978), and harm the photosynthetic apparatus (Yasseen 1983).

Seed priming with 5 µM GA_3 was the most effective seed treatment in increasing plant dry weight (Fig. 7a). Maximum plant height and root length was measured in CSC in combination with seed priming with 10 µM GA_3 and minimum plant height was recorded in control treatment (Fig. 8a, b). Many researchers reported that seed priming with GA_3 accelerates root and shoot length, fresh and dry weight and increases leaf water content (Little and MacDonald 2003; Ghodrat and Rousta 2012; Shehzad et al. 2014). In another study, no significant change in dry matter accumulation was reported in indian grass under chilling stress when the seeds were sown after soaking in 1000 ppm GA_3 solution (Watkinson and Pill 1998). The contradictory results in dry matter accumulation in Indian grass might be the seeds required higher concentration of GA_3 to stimulate dry matter accumulation in seedlings. In sum up, application of seed priming with gibberellic acid in chickpea has positive effect on plant shoot and root dry weight.

In our study, number of the flowers and branches were differed in plants grown from primed and non-primed seeds with GA_3 treatment (Fig. 9a, b). Some previous research investigation showed that seed priming can increased the number of branches from 6.63 to 10.43 per plant (Chavan et al. 2014). But in another study, contradictory results were reported by Khan et al. (2005). GA_3 involves in flower induction by increasing invertase enzyme activity in sugar cane (Sacher et al. 1963). In phalaenopsis plants, application of GA_3 up to 1000 ppm increased flower numbers almost double without giving any harm to plants (Cardoso et al. 2012). Some other studies revealed that GA_3 plays a subsidiary role in flower induction (Dong et al. 2017). Our findings are agreed with Zhang et al. (2016), they were reported that GA_3 regulates flowering in wheat.

On the bases of above study, it is concluded that seed priming could help in maintaining stand establishment, increasing shoot length, root length, leaf relative water content, seedling dry weight, number of flowers and decreasing electrolyte leakage in chickpea cultivars under chilling stress. Seed priming could be successfully used for growing chickpea in low temperature areas to improve the plant performance.

Author contribution statement EP and TA discussed the research concept and designed the experiment. TA conducted the experiment, took the data, did the plant and statistical analysis which were finally verified by EP. TA prepared the initial manuscript draft which was improved through a series of changes made by EP.

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