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Effects of hormonal priming with gibberellic acid on emergence, growth and yield of chickpea under drought stress

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Chickpea production is low and unstable due to rainfed conditions. Using priming and other pre sowing treatments in many crops have improved seed performance. In order to evaluate the effect of hormonal priming on emergence, growth, physiological traits and yield of chickpea under drought stress, two separate experiments were conducted. In the first experiment, the effect of gibberellic acid on the emergence and vigor of chickpea (cultivar ILC 6266) under drought stress was studied in glasshouse. Different gibberellic acid treatments including zero, 50, 100 and 150 ppm were used in the study. While different drought treatment levels used in the study were 70, 50 and 30 percent of field capacity. The second experiment was conducted under field conditions with drought stress levels as in the first experiment conducted in glasshouse. It was observed that hormonal priming with gibberellic acid increased seed emergence and plant vigor in glasshouse conditions. Moreover, application of gibberellic acid also resulted in improved growth and physiological traits under field condition but could not affect the seed yield. In this experiment drought stress caused a significant reduction in all tested characteristics. Gibberellic acid priming prominently reduced the adverse effects of drought. Of course, this positive effect was more pronounced at higher levels of stress than the control condition. In general, 150 ppm gibberellic acid treatment was the best priming level while with increasing concentration of priming agent, emergence and plant growth indicators also improved. We concluded that priming with appropriate concentration of gibberellin plays an important role in the induction of tolerance to drought and overcome limitations created by the environmental stress such as osmotic effects, ion toxicity and nutritional imbalance.

Keywords: Seed, emergence, field capacity, plant vigor, yield, dry weight.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third most important grain legume crop in the world and first in the Mediterranean basin and South Asia (Turner et al. 2001). In developing countries, chickpea is a rich complement to the cereal diet since it has a high nutritive value. Mainly grown for its edible seeds rich in proteins, this crop can be used for both seed and forage production (Yadav et al. 2011).

Abiotic stresses such as drought and salinity stress are widespread problems around the world (Soltani et al. 2006). Drought, or more generally, limited water availability is the main factor limiting crop production. Drought stress is characterized by reduction of water content, diminished leaf water potential and turgor loss, closure of stomata, and decrease in cell enlargement and growth. The effect of water deficit varies with the variety, degree and duration of stress and the growth stage of the plant (Adejare and Umebese, 2007).

The use of priming technique is one of the solutions to remove these problems in unfavorable conditions. Priming will cause more uniformity in seed germination and better seedling establishment in the field. Primed seeds pass phases I and II of germination and are ready for germination in the base of physiological and biochemical conditions (Eisvand, 2008). Positive priming effects such as improvement in germination and emergence have been shown in most plants especially in vegetables (Kaur et al. 2002). Typical responses to priming are faster and closer spread of times to emergence over all seedbed environments and wider temperature range of emergence, leading to better crop stands, and hence improved yield and harvest quality, especially under suboptimal and stress condition growing conditions in field (Halmer, 2004). Haigh et al. (1986) investigated the primed seeds of carrot, onion and tomato showing that priming these seeds with gibberellin increased the germination percentage and rate. It has been reported that seed priming with gibberellin can decrease the base temperature. Reduced base temperature causes the seed to initiate its germination earlier and as a result it will be more successful than the weeds and it will adapt to the environment conditions more appropriately (MaurMickel and Kavallaro, 1996). Gibberellic Acid (GA3) is the most important growth regulator, which breaks seed dormancy, promotes germination, intermodal length, hypocotyls growth and cell division in cambial zone and increases the size of leaves. GA stimulates hydrolytic enzymes that are needed for the degradation of the cells surrounding the radicle and thus speeds germination by promoting seedling elongation growth of cereal seeds (Rood et al. 1990).

Ashraf and Foolad (2005) reported that seed priming is one of the methods that can be taken to counteract the adverse effects of abiotic stress. Seed priming techniques have been used to increase germination, improve germination uniformity, improve seedling establishment and stimulate vegetative growth in more field crops (Ansari et al. 2012; Patade et al. 2011; Foti et al. 2008) under stressed conditions. Eisvand et al. (2010) reported that use of hormonal priming with 100 ppm gibberellin could improve the vigor index of deteriorated *Agropyron elongatum* in the host seeds with and without the drought stressed conditions.

Comparatively meagre work is on record regarding role of gibberellin on drought tolerance in chickpea. Therefore, the present experiment aims to study the hormonal priming effects with gibberellin on the seedling, growth and yield of chickpea under drought stress.

MATERIALS AND METHODS

The study was conducted in two separate experiments in Ferdowsi University of Mashhad-Iran in 2015.

Glasshouse experiment

In the first experiment the effect of hormonal priming on the emergence and vigor of chickpea (cultivar ILC 6266) were studied. The experiment was conducted as factorial based on completely randomized design with three replications. Each replication includes 15 seed per pot. Gibberellic acid treatments, including concentration of zero, 50, 100 and 150 ppm.

Field experiment

The second experiment was conducted as split-plot based on randomized complete block design with three replications. The inter row spacing was 50 cm and plant-to-plant spacing was kept 8 cm. The cultivar were like to the first experiment. Gibberellic acid treatments, including concentration of zero and 150 ppm (best level of gibberellic acid in test 1).

Priming techniques

Seeds was fully immersed in Gibberellic acid priming media at a temperature of 25°C for durations of 18 hours under dark conditions. Seed were removed from priming media at the same time, then rinsed thoroughly with distilled water and lightly dried using blotting paper and then allowed to dry on paper towels at room temperature.

Drought stress

Three drought stresses were applied. Levels of drought treatment were 70, 50 and 30 percent of field capacity.

Measurement of traits

For a period of 30 days, each day emergence seeds were counted. In this experiment, emergence percentage, emergence rate and plant vigor index were calculated based on the following relationships:

Emergence rate (GR) = Σni / Σti
(ni Germinated seeds at time ti, ti Number of days after germination).

Seed vigor index$_1$ (SVI$_1$) = Shoot length (cm) * Emergence (%)
Seed vigor index$_2$ (SVI$_2$) = Shoot dry weight (mg) * Emergence (%)
Fully expanded leaves from 40 days old plants were submitted to the leaf area meter to determine the sum of leaf area of all leaves (cm^2). And the LAI was calculated as follows:
LAI = Sum of leaf area (cm^2) / Ground area where the leaves collected (cm^2)

Relative water content (RWC):
A composite sample leaf discs, 40 days old, were taken and the fresh weight was determined, followed by floatation in water up to 4 h. The turgid weight was recorded, and the leaves were oven dried to a constant weight at about 85^{oC}. Relative water content (RWC) was, then, calculated as follows:
RWC = [(fresh weight-dry weight) / (turgid weight-dry weight)] * 100
Five grams of leaf in 40 days old from each treatment were soaked in 25 ml distilled water in a beaker and kept at 25 ± 10C temperature. After 24 hours the electrical conductivity of the leachate was measured by digital conductivity meter.
The seed yield obtained from five randomly selected and tagged plants were added to the seed yield of the net plot area for calculation of seed yield and were recorded as seed yield per hectare.

Statistical analysis:
Statistical analyses were carried out using SigmaPlot 11.0. All data were analyzed by analysis of variance (ANOVA) to determine the treatments effects, and LSD test at 5% level was carried out to determine the statistical significance of the differences between treatments means.

RESULTS

Glasshouse experiment

Emergence rate:
At the drought stress level of 70% FC, there was an increase in emergence rate up to level of GA 50 ppm, and no significant change occurred in this trait at concentrations over 50 ppm. At the stress level of 50% FC, a 2.5-time increase was observed in the emergence rate due to the increase in hormo-prime level from 0 to 150. Furthermore, no significant change was observed in the rate of emergence at the stress level of 30% when GA concentration increased from 0 to 100 ppm (Fig 1).

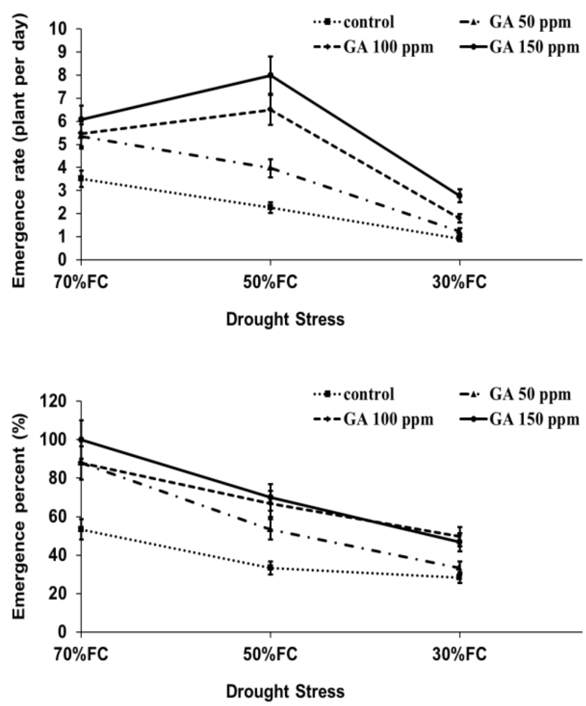

Figure 1: Emergence rate and emergence percent of chickpea under drought stress and gibberellic acid hormonal priming in glasshouse condition.

Emergence percent:
At the stress level of 70% FC, application of 150 ppm GA caused 87% increase in emergence rate compared to the control treatment, so that emergence rate increased from about 53% to 100%. Emergence rate at drought stress levels of 50% and 30% FC increased 110 and 65%, respectively, when concentrations of GA increased from 0 to 150 ppm (Fig 1).

Plant vigor index (SVI1 and SVI2):
Plant vigor 1 and 2 increased at all levels of drought stress along with the increase in GA concentrations. This increase in plant vigor 1 and 2 at drought levels of 50, 70 and 30% FC was 120, 60 and 118%, and 240, 68 and 29%, respectively (Fig 2).

Field experiment

Leaf area index (LAI):

Drought stress reduced leaf area index of pea, although no significant difference was found between 50 and 30% FC levels of drought stress. Drought stress under priming conditions had less impact on leaf area index, so that the index decreased 71% under no priming conditions at drought stress level of 30% FC compared to the 70% FC, but it showed a 40% reduction at GA priming conditions (Fig 3).

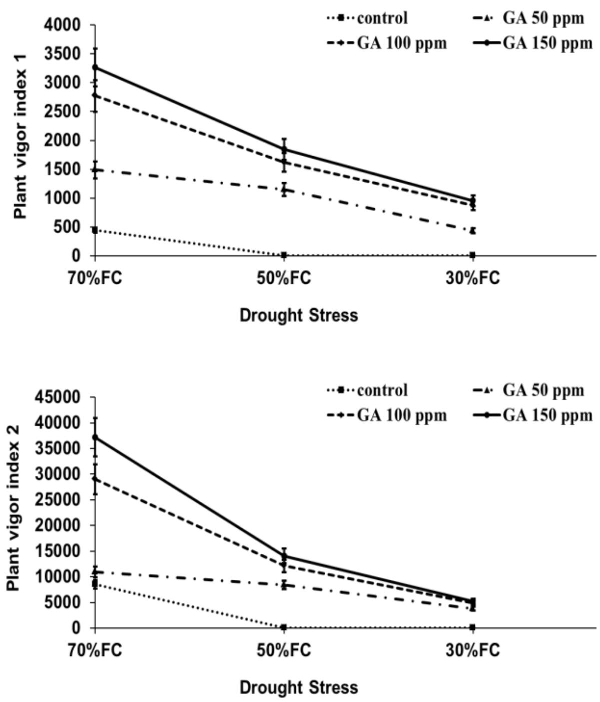

Figure 2: Plant vigor index 1 and plant vigor index 2 of chickpea under drought stress and gibberellic acid hormonal priming in glasshouse condition.

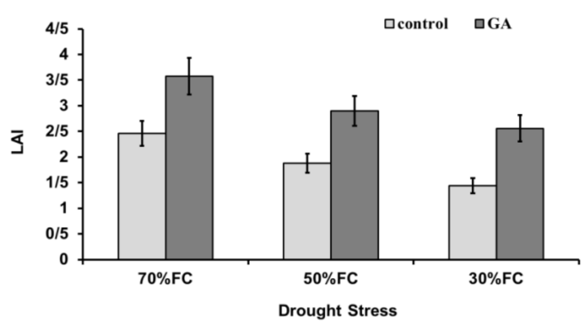

Figure 3: Leaf area index (LAI) of chickpea under drought stress and gibberellic acid hormonal priming in field.

Relative water content (RWC):

Relative water content reduced significantly under drought stress, so that drought stress level of 30% FC caused 170 and 97% reduction in RWC under control and priming conditions, respectively, in comparison with drought stress level of 70% FC. GA priming improved leaf relative water content, so that the trait increased 13, 27 and 55% under drought stress levels of 70, 50 and 30% FC, respectively, by applying of priming (Fig 4).

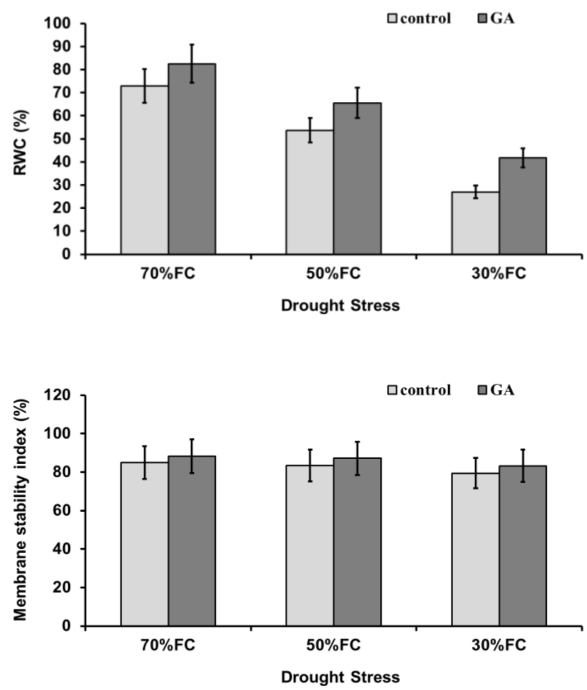

Figure 4: Relative water content (RWC) and Membrane stability index of chickpea under drought stress and gibberellic acid hormonal priming in field.

Membrane stability index:

Under water stress, electrolyte leakage increased but application of GA reduced the damage. Priming increased membrane stability index under drought stress levels of 70, 50 and 30% FC by 4% compared to the control (in all levels of stress) (Fig 4).

Plant length:

Drought stress at both control and priming conditions resulted in a significant reduction in the length of chickpea plant. GA priming could increase the plant length, so that the length increased 33, 36 and 36% compared to control by application of priming at stress levels of 70, 50 and 30% FC, respectively (Fig 5).

Plant dry weight:

Chickpea plant dry weight was not significantly affected by GA priming, but it showed a significant reduction under drought stress. In control (without priming) and GA priming, drought stress of 30% FC reduced dry weight by 66 and 47% when compared with stress level of 70% FC (Fig 5).

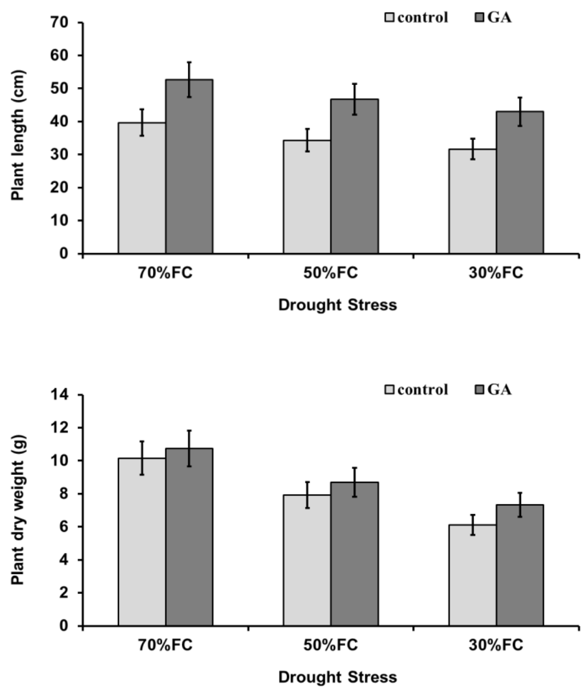

Figure 5: Plant length and plant dry weight of chickpea under drought stress and gibberellic acid hormonal priming in field.

Seed yield:

Although drought stress decreased yield and priming, by contrast, increased yield in this experiment, the effects of drought stress and priming on seed yield were not significant (Fig 6).

Seed protein:

Drought stress decreased protein content of seeds, but application of priming could reduce somehow the damage of drought stress. GA priming at stress levels of 70, 50 and 30% FC increased protein content by 8, 12 and 16% compared to the control conditions (Fig 6).

DISCUSSION

Seed germination is negatively affected by drought stress (Rad and Rad, 2013). In this study, drought stress decreased emergence percentage and emergence rate. Seeds for emergence process must absorb enough water. Due to osmotic pressure loss under drought stress, water absorption process is disrupted and the alpha-amylase enzyme activity is inhibited (Afzal, 2005). Reducing in water absorption by seeds reduces secretion of the stress hormone and enzyme activity and it has negatively effect on seedling growth (Kafi et al. 2005). Also with increasing drought stress, seed vigor decreased.

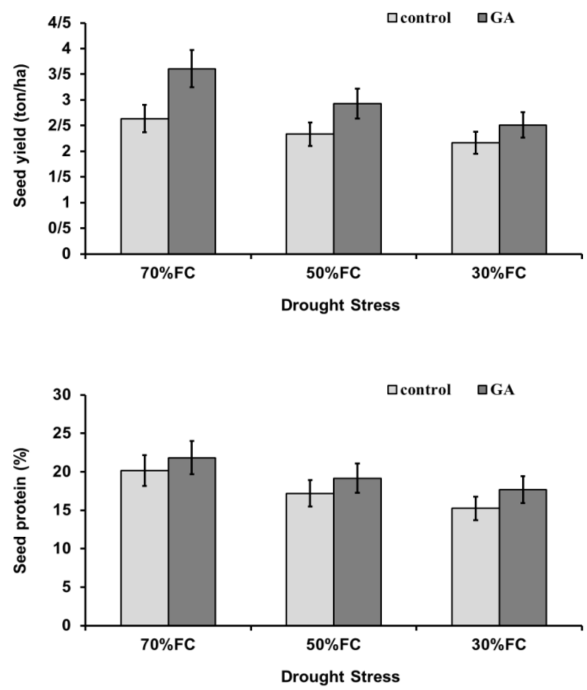

Figure 6: Seed yield and seed protein of chickpea under drought stress and gibberellic acid hormo-priming in field.

Seed priming is one of the methods that can be taken to counteract the adverse effects of abiotic stress (Patade et al. 2011; Ashraf and Foolad., 2005). Also, earlier reports (Patade et al. 2011; Ansari and Sharif-Zadeh 2012; Rouhi et al. 2011) have shown positive effect of priming in relation to germination characteristics. Therefore, our results showed that in across drought stress levels priming increased emergence, growth and yield as compared to the unprimed seeds. One of effective and positive reason for chemical stimuli such as gibberellic acid on the seed emergence is probably due to hormonal balance and decrease proportion of growth inhibiting substances such as abscisic acid (ABA). Gibberellins are necessary for germination of seeds, in which they stimulate the growth of the embryo (Hedden, 2017). Gibberellin improves the synthesis and secretion of hydrolytic enzymes from aleurone cells. These enzymes then mobilize the endosperm storage reserves that fuel germination and growth (Cirac et al. 2004). In stress conditions, ascorbic acid acts as an effective antioxidant. Ascorbic acid can remove the free radicals in stress conditions especially oxygen radicals and it has important role in stimulating and cell expansion and nutrient absorption in the plant cells under environmental stresses which leads to avoid from the oxidation risk (Smirnoff and Wheeler, 2000, Smirnoff**,** 1996).

Overall priming improves seed germination under stress and non-stress conditions (Kolsarıcı, 2006, Murungu et al. 2003). Seed priming reduce time of emergence and cause to accelerate seed emergence. So, it is the reason of superiority of primed seeds in compared with non-primed seeds (Netondo et al. 2004). Seed priming increases antioxidant enzymes such as glutathione in primed seeds and this enzyme may reduce lipid peroxidation activity during germination and this can lead to increase germination (Hus and Sung, 1997, Baalbaki et al. 1995). Our results showed that the use of gibberellic acid significantly increased emergence, growth and yield in chickpea under drought stress.

In most experiments high concentrations of gibberellin were used while the interspaces between consequent concentrations are large (Angrish et al. 2001; Afzal et al. 2005). We observed that priming with low concentrations of gibberellin had no effect on emergence and growth. Priming with appropriate concentration of gibberellin plays an important role in the induction of tolerance to drought and overcome limitations created by the environmental stress such as osmotic effects, ion toxicity and nutritional imbalance (Jamil and Rha, 2007). On the other hand priming with higher concentrations of gibberellin had good effect on emergence and growth (Naeem and Muhammad, 2006). This study helps to find concentrations of gibberellin which has the highest effect on the components of growth which are also economic enough to be suggested to the farmers. However, as the mechanism through which GA regulates development revealed in more and more detail, e.g., the dual role of GA activity in which it first promotes then inhibits flowering, they can be exploited to increase grain production (zhang et al. 2016).

CONCLUSION

We observed that priming with low concentrations of gibberellin had no effect on emergence and growth. Priming with appropriate concentration of gibberellin plays an important role in the induction of tolerance to drought and overcome limitations created by the environmental stress such as osmotic effects, ion toxicity and nutritional imbalance. This study helps to find concentrations of gibberellin which has the highest effect on components of growth which are also economic enough to be suggested to the farmers.

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