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# Response of chickpea (Cicer arietinum L.) cultivars to integrated application of Zinc nutrient with water stress

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**ABSTRACT:** To determine the effects of drought stress and Zinc fertilizer on yield, yield components and grain protein profiling pattern of chickpea a field experiment was conducted on a clay soil, in Bu-Ali Sina University of Hamedan, Iran. The experiment was a split- factorial design with three replications. The main treatment was drought stress (sever drought stress, moderate drought stress and no drought stress). The sub treatment was four cultivars of chickpea, Azad, Bivanij, Hashem and ILC482 and 2 Zn levels (using hand sprayer 1 L and control). The results showen that the effects of drought stress on yield and yield components, effect of cultivars on grain yield and protein yield were significant. With increasing level of drought stress yield, yield components and protein yield decreased. Therefore, Bivanij cultivar had highest production of chickpea (grain yield and grain protein yield) and Hashem cultivar had a lowest among of them. Application of Zinc fertilizer had better effect on grain yield, yield components and grain protein yield compared to the control and grain yield and biomass yield increased with application of it significantly. Therefore, we can increase yield and grain protein yield of chickpea by irrigation and application of Zinc fertilizer. Also the results revealed that drought stress and Zn fertilizer had not effects on grain protein banding patterns in chickpea cultivars.

***Key words:*** Chickpea, drought stress, Zn fertilizer, protein, yield

## INTRODUCTION

Chickpea is an important pulse crop of rainfed areas in semiarid/arid climate. Average chickpea yield in different countries in the world looked such as China (4135 kg ha-1), Canada (1427 kg ha-1), USA (1391 kg ha-1) and Pakistan (785 kg ha-1). Environmental stress is a primary cause of crop loss worldwide, resulting in average yield losses of more than 50% for major crops every year (Brya, 2004; Chaves and oliveira, 2004). Drought stress causes deceleration of cell enlargement and thus reduces stem length by inhibiting inter nodal elongation and also checks the tillering capacity of plants (Ashraf and O'Leary, 1996). Drought Several studies have also shown that optimum yield can be obtained with irrigation at branching, flowering and pod formation stages (Prihar and Sandhu, 1968). Chickpea yield is depend of nutrient elements. Zinc is one of these important elements, in addition it has high pH that affects the absorption ability of phosphorus as macro-element. Zinc plays an important role as a metal component of enzymes (alcohol dehydrogenase, superoxide dismutase, carbonic anhydrase and RNA polymerase) or as a functional, structural, or regulator cofactor of a large number of enzymes (Marschner, 1986). Mahady (1990) found that foliar application of Zn SO for faba bean plants increased number of pods/plant and seed yield/fed. Guenis et al. (2003) and Soleimani (2006) reported marked increase in number of grains spike-1 of wheat for foliar application of boron and zinc, respectively. Soleimani (2006) reported increase in biological yield for foliar application of zinc. Torun et al. (2001) and Grewal et al. (1997) reported increased wheat production with application of zinc and boron over control. Grain protein content and baking quality highly depend on genetic background and environmental factors, especially influence of drought and heat stress, during the grain filling period (Luo et al., 2000; Ottman et al., 2000). Storage protein is a method to investigate genetic variation and to classify plant varieties (Isemura et al., 2001). Seed storage proteins are not sensitive to environmental fluctuations; its banding pattern is very stable which advocated for cultivars identification purpose in crop (Javid et al., 2004; Iqbal et al., 2005). Despite the fact that the response of protein composition to environmental factors in mature wheat grain results from changes in protein deposition during plant development, very few studies has examined the effects of water stress and nitrogen fertilizer on protein profiling of grains(Sumera and Asghari, 2009). Therefore this study was planned to examine effect of drought stress and Zinc fertilizer on yield, yield components, seed storage proteins, protein yield and protein banding pattern of chickpea cultivars.

## MATERIALS AND METHODS

This study was conducted in the Faculty of Agriculture, Bu-Ali Sina University and Biology department of medicine college, Hamedan, Iran during the growing seasons 2009. Soil of the experiment was clay with pH 7.4, organic matter content 1.63 , total P 0.15% and and 0.9 mg/kg of zinc. The experiment was laid out in a split-factorial design with drought stress in main plots and cultivar with Zinc microelement in subplots with three replications. The experimental treatments consisted of three levels of drought stress [severe drought stress (S2), moderate drought stress (S1) and no drought stress (S0)] in the main plots and four cultivars of chickpea, Azad, Bivanij, Hashem and ILC482 and 2 Zinc levels in the sub plots [Zn0 (application of Zinc fertilizer) and Zn1 (non application of Zinc fertilizer)]. Tow spraying with Zinc (Tap water, 0.02 and 0.06% Zn EDTA 14% Zn). Zinc chelate (organic material) was used in the form of Ethylene Diamine Tetra Acetic Acid. The seeds were sown in rows on March 31, 2009. Chickpea plants were sprayed once with the aqueous solution of this chelated Zn at 45th days after planting, while control plants were sprayed with tap water. The volume of the sprayed aqueous solution of this zinc was 400 L/fed (using hand sprayer 1 L.).

To determine yield, we removed and cleaned all the seeds produced within a per square meter area in the field. The seeds were air-dried and weighed, and seed yield recorded on a dry weight basis. Yield was defined in terms of grams per square meter and quintals per hectare. The number of pod per plant, the number of grain per pod and the number of grain per plant were determined.

Replicated samples of clean seed (broken grain and foreign material removed) were sampled randomly and 100-grain were counted and weighed.

The biomass production was measured on 10 plants treatment at 40 day after podding (DAP).
The harvest index was accounted with follow:

HI = (Economical yield / Biological yield)

A single seed was grounded with a mortar and pestle and 10mg (0.01 g) out of this seed flour was taken into a 1.5ml micro-tube. 400µl of the protein 10% glycerol, 5% β-mercaptoethanol, 5 M urea and 0.0001% bromo-phenol blue) was added and mixed well by vortexing. The crude homogenates were then centrifuged in micro-centrifuge machine at room temperature with 13000 rpm for 20 min. The supernatant was separated and used for protein profiling. Protein concentration of extracts was measured by dye binding assay as described by Bradford (1976). Supernatant was mixed (4:1) with cracking solution (10 ml containing 1g SDS, 0.01 g bromo-phenol blue, 2 ml β-mercaptoethanol, 1.5 ml 0.5 *M* tris, pH 6.8, 5 g sucrose and 6.5 ml water) on vortex mixer and heated in a boiling water bath for five minutes to denature the proteins. Proteins profiling of samples was performed using SDS- polyacryl amide gels as described by Laemmli (1970). Equal quantities of proteins (150 micro grams) from each sample along with protein molecular weight marker were loaded into 10% gels. Electrophoresis was performed at constant voltage (100 volts). At end of electrophoresis, gels were dye in coomassie blue G-250 for 45 min. Then gel fixed in solution containing 10% Acetic acid and 40% Ethanol overnight, with constant agitation on a shaker. After fixing gel was washed with distilled water for 15 min, with changing the water after every 5 min.

Finally, amount of grain protein yield was accounted with follow (Sinkai et al., 1993; Khan et al., 2002):

Grain Protein yield (kg/ha) = Grain protein percentage (%) × Grain yield (kg/ha)

The statistical analyses to determine the individual and interactive effects of drought stress, Zinc fertilization and cultivar were conducted using JMP 5.0.1.2 (SAS Institute Inc., 2002). Statistical significance was declared at $P \leq 0.05$ and $P \leq 0.01$. Treatment effects from the two runs of experiments followed a similar trend, and thus the data from the two independent runs were combined in the analysis.

## RESULTS

The effect of drought stress and cultivar treatments on number of pod per plant was significant at 1% level (Table 1), but the other treatments were not significant on it. The comparison of the mean values of the number of pod per plant (Table 3) shows that $S_0$ treatment has the highest (45) number of pod per plant and the $S_2$ treatment has the lowest number of pod per plant (9) and the differences were significant. Among the cultivars treatments, the highest number of pod per plant (31.2) was belonged to the ILC482 cultivar and the lowest number of pod per plant (21.2) was belonged to the Azad cultivar (Table 3). Similar results were reported by khurgami et al (2009) and Arya and Khushwa (2000) in chickpea and Mirakhori et al (2009) in soybean Max.

The effects of drought stress and cultivar treatments on number of grain per pod were significant at 5% level (Table 1) but the other treatments were not significant on it. The comparison of the mean values of the number of grain per pod (Table 3) shows that $S_1$ treatment has the highest (1.9) number of grain per pod and the $S_0$ treatment has the lowest number of grain per pod (1.1) and the differences were significant. Among the cultivars treatments, the highest number of grain per pod (2) was belonged to the Hashem cultivar and the lowest number of grain per pod (1.01) was belonged to the Bivanij cultivar (Table 3).

The effect of drought stress treatment on number of grain per plant was significant at 5% level (Table 1) but the other treatments were not significant on it. The comparison of the mean values of the number of grain per plant (Table 3) shows that $S_0$ treatment has the highest (31.55) number of grain per plant and the $S_2$ treatment has the lowest number of grain per plant (9.3) and the differences were significant. Among the cultivars treatments, the highest number of grain per plant (21.7) was belonged to the ILC482 cultivar and the lowest number of grain per plant (11.2) was belonged to the Bivanij cultivar (Table 3). Similar results were reported by khurgami et al (2009) in chickpea.

Table 1 shows that effect of cultivar treatment on 100-grain weight is significant at 1% level but the other treatments were not significant on it. The comparison of the mean values of the 100-grain weight (Table 3) shows that Bivanij cultivar has the highest (32.1 g) 100-grain weight and Hashem cultivar has the lowest (24g) 100-grain weight. Drought stress imposed from flowering to maturity resulted in 100 grain weight as compared to non stress chickpea plants. Decrease in 100 grain weight under stress conditions might be due to lower photosynthetic translocation in the developing grain. Similar results were reported by Mansur et al (2010) and Arya and Khushwa (2000) in chickpea. and seghatoleslami et al (2008) in millet and Nabipour et al (2007) in safflower and Mirakhori et al (2009) in soybean Max.

The analysis of variance in Table 1 shows the effects of drought stress, cultivar and interaction of drought stress × variety × Zn fertilizer treatments on grain yield are significant at 1% level and effects of Zn fertilizer was significant on it at 5%. Comparison of average grain yield in different irrigation treatments indicated that the $S_0$ treatment has the highest grain yield (2645.2 kg /ha) and the $S_2$ treatment has the lowest grain yield (917 kg/ha) and the difference is significant (Table 3).
Mahalakshmi and Bidinger (1985) reported that drought stress at grain filling stage reduced grain yield up to 50%. Among the Zn fertilizer treatments, the highest grain yield (1526 kg/ha) was belonged to the $Zn_1$ treatment and the lowest grain yield (1298 kg/ha) was belonged to the $Zn_0$ treatment (Table 3). Among the cultivars treatments, the highest grain yield (2126 kg/ha) was belonged to the Bivanij cultivar under non stress conditions and the lowest grain yield (1125 kg/ha) was belonged to the Hashem cultivar under stress conditions (Table 3). Interaction effect of drought stress × variety × Zn fertilizer (S × V × Zn) shows that $S_0Zn_1V_2$ has the highest grain yield (2987 kg/ha) and $S_2Zn_0V_3$ has the lowest grain yield (397 kg/ha) (Table 2). The significance of this interaction clearly shows the differential response of plants under different water regimes to Zn fertilizer. Similar results were reported by Mansur et al (2010) Singh and Dixit (1992) and Arya and Khushwa (2000) in chickpea and seghatoleslami et al (2008) in millet and Nabipour et al (2007) in safflower and Mirakhori et al (2009) in soybean Max.

The effect of drought stress treatment and Zn fertilizer on Biomass yield were significant at 5% level (Table 1) but the other treatments were not significant on it. The comparison of the mean values of the Biomass yield (Table 3) shows that $S_0$ treatment has the highest (5326 kg/ha) Biomass yield and the $S_2$ treatment has the lowest Biomass yield (1922 kg/ha) and the differences were significant. Among the Zn fertilizer treatments, the highest grain yield (3526 kg/ha) was belonged to the $Zn_1$ treatment and the lowest grain yield (3125kg/ha) was belonged to the $Zn_0$ treatment (Table 3). Among the cultivars treatments, the highest Biomass yield (3856kg/ha) was belonged to the Hashem cultivar and the lowest Biomass yield (2866 kg/ha) was belonged to the ILC482 cultivar (Table 3).

Similar results were reported by Mansur et al (2010) and Singh and Dixit (1992) in chickpea and Kenan and Cafer (2004) in sugar beet and Penuelas et al (1993) in pepper and beans.

The effect of drought stress treatment on harvest index was significant at 1% level (Table 1) but the other treatments were not significant on it. The comparison of the mean values of the harvest index (Table 3) shows that $S_0$ treatment has the highest (44%) harvest index and the $S_2$ treatment has the lowest harvest index (37.6%) and the differences were significant. Among the cultivars treatments, the highest harvest index (49.1%) was belonged to the ILC482 cultivar and the lowest harvest index (37%) was belonged to the Hashem cultivar (Table 3). Similar results were reported by Mansur et al (2010) and Arya and Khushwa (2000) in chickpea and Mirakhori et al (2009) in soybean Max.

Table1. Analysis of variance (mean squares) for yield, yield components and protein yield in chickpea cultivars under drought stress and Zn fertilizer

| | | MS | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Source of variation | df | num of pod per plant | num of grain per pod | num of grain per plant | 100grain weight | grain yield | Biomass yield | Harvest Index | grain Proteins |
| repetation | 2 | 511 | 0.004 | 92.5 | 8 | 865721 | 582103.3 | 101.2 | 0.09 |
| Drought stress | 2 | 6132* | 0.03** | 1855* | 30ns | 29325063** | 40125936* | 1724.9** | .021** |
| Error (Ea) | 4 | 21 | 0.0051 | 8.5 | 7.6 | 854720 | 712053 | 6.6 | 0.0196 |
| Zn fertilizer | 1 | 4ns | 0.003 ns | 19.3 ns | 9 ns | 913025.8 * | 412036.5 * | 15.6 ns | 0.022 ns |
| cultivar | 3 | 209* | 0.76** | 55.3 ns | 2000.6** | 40123695** | 1856230.2 ns | 55 ns | 0.019 ns |
| Zn fertilizer* stress | 2 | 21 ns | .000085 ns | 33.7 ns | 3.3 ns | 310256 ns | 1803265 ns | 33 ns | .00048 ns |
| Zn fertilizer* cultiva | 3 | 61 ns | 0.011 ns | 30 ns | 4.3 ns | 210250 ns | 512458.5 ns | 29.3 ns | 0.111 ns |
| cultivar* stress | 6 | 102 ns | .0013 ns | 31.8 ns | 9 ns | 865410 ns | 685421.5 ns | 33.2 ns | 0.055 ns |
| stress* cultivar* Zn fertilizer | 6 | 74 ns | 0.006 ns | 9 ns | 4.6 ns | 1965280.1** | 463206.3 ns | 19 ns | 0.15 ns |
| Error (Eb) | 42 | 411 | 0.55 | 33325 | 12.3 | 203204.3 | 32503201 | 33.25 | 0.522 |
| CV | | 11.2 | 8.9 | 15.3 | 9 | 19.3 | 18.1 | 8.3 | 11.9 |

ns: Non-significant, * and **: Significant at 5% and 1% probability levels, respectively

Table 2. Interaction effect of drought stress × variety × Zn fertilizer on grain yield

| | No stress(S0) | | Moderate stress(S1) | | Sever stress(S2) | |
|---|---|---|---|---|---|---|
| cultivars | No Zinc($Zn_0$) | Application of Zinc($Zn_1$) | No Zinc($Zn_0$) | Application of Zinc($Zn_1$) | No Zinc($Zn_0$) | Application of Zinc($Zn_1$) |
| Azad | 2141bcd | 2151abc | 1111def | 1292cdef | 653gh | 892fg |
| Bivanij | 2543abc | 2987a | 1356cdef | 21456bcd | 911fg | 1156ef |
| Hashem | 1972cde | 2456ab | 960fg | 1322fg | 397h | 462h |
| ILC482 | 2451abc | 2700a | 1156def | 1943cde | 928fg | 722gh |

ns: Non-significant, * and **: Significant at 5% and 1% probability levels, respectively

The effect of drought stress treatment on grain protein was significant at 1% level (Table 1) but the other treatments were not significant on it. The comparison of the mean values of grain protein (Table 3) showed that $S_2$ treatment has the highest (1.66 mg/ml) grain protein and the $S_0$ treatment has the lowest grain protein (1.41 mg/ml) and the differences were significant. Among the cultivars treatments Hashem cultivar has the highest (1.51 mg/ml) grain protein and the Bivanij cultivar has the lowest grain protein (1.46 mg/ml) and the difference is not significant (Table 3). Similar results were reported by Kim et al (1990) and Suoyi Han et al (2009).

The effects of drought stress and cultivar treatments on protein yield were significant at 1% level (Table 1) but the other treatments were not significant on it. The comparison of the mean values of protein yield (Table 3) showed that $S_0$ treatment has the highest (521 kg/ha) protein yield and the $S_2$ treatment has the lowest protein yield (210 kg/ha) and the differences are significant. Among the cultivars treatments, the highest protein yield (374.4 kg/ha) was belonged to the Bivanij cultivar and the lowest protein yield (254.6 kg/ha) was belonged to the Hashem cultivar and the difference is not significant (Table 3). These results were in agreement with the findings of Luo et al (2000) Ottman et al (2000).

The correlation matrix (Table 4), indicated strong and significant ($p<0.01$) correlation of grain yield with number of pod per plant and number of grain per plant ($r=0.91$ and 0.89) respectively. These results were agreement with the previously reported ones. Also results showed had significant ($p<0.05$) correlation between grain yield and protein yield. Such results indicated that selection for these traits would lead to the increase in grain yield of chickpea (El-gizawy and Mehasen, 2004). Also results showed that, had a negative and significant correlation coefficient ($r= -0.52$ and -0.95) between grain yield with number of grain per pod and grain protein respectively. However number of grain per pod was negatively and significantly (p<0.05) correlated with HI (r=-0.79). The number of pod per plant was positively and significantly (p<0.01) correlated with number of grain per plant (r=0.88). However the Protein yield was negatively and significantly (p<0.05) correlated with Grain protein (r=-0.82) and number of grain per pod (r=-0.8) respectively.

The grain storage proteins patterns for 4 cultivars of chickpea under drought stress and used of Zinc fertilizer and no Zinc fertilizer after SDS-PAGE are shown in fig 1 and fig 2 respectively. In total 29- 31 bands (since below 14 kDa until over 78 kDa molecular weight band) per cultivars were detected in electrophoregrams. The SDS-PAGE results revealed no effects treatments (drought stress and Zn fertilizer) on the grain protein banding patterns but the related sever drought stress bands were chromatic, because they have highest protein concentration. These results were in agreement with the findings of Tanksley et al (1981) Javid et al (2004) and Iqbal et al (2005) in wheat.

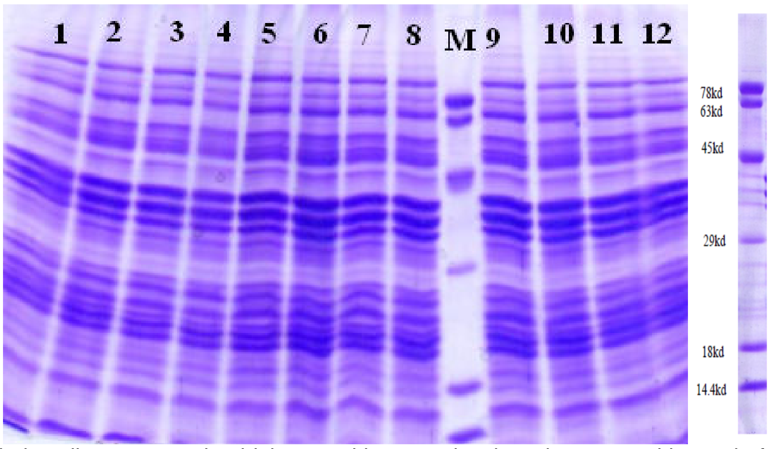


figure 1. Protein banding patterns in chickpea cultivars under drought stress with used of Zn fertilizer
1,2,3,4= No drought stress treatment(S0); 5,6,7,8= Moderate drought stress treatment (s1)
9,10,11,12= sever drought stress treatment (S2); m= Marker; 1,5,9= Azad cultivar; 2,6,10= Bivanij cultivar ; 3,7,11= Hashem cultivar; 4,8,12= ILC482 cultivar

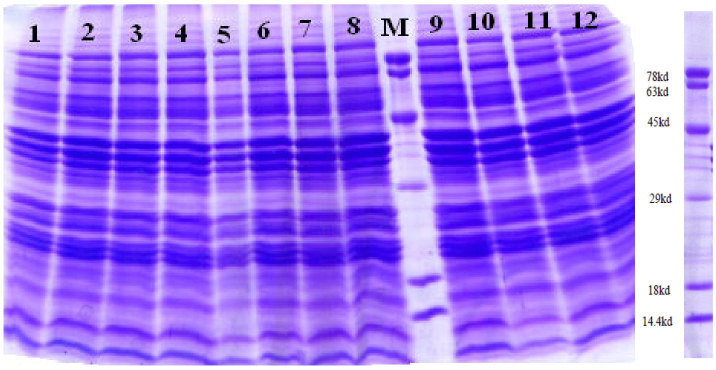


figure 2. Protein banding patterns in chickpea cultivars under drought stress with no Zn fertilizer
1,2,3,4= No drought stress treatment(S0); 5,6,7,8= Moderate drought stress treatment (s1)
9,10,11,12= sever drought stress treatment (S2); m= Marker;
1,5,9= Azad cultivar; 2,6,10= Bivanij cultivar ;
3,7,11= Hashem cultivar; 4,8,12= ILC482 cultivar

## DISCUSSION

Drought is deleterious for plant growth, yield and mineral nutrition (Garg et al., 2004; Samarah et al., 2004). Soil moisture status during the reproductive phase of chickpea plays an important role to determine the impact of yield component in final grain yield (Singh and Bhushan, 1980). This study has showed that chickpea grain yield and yield components decreased significantly with the increase of drought stress. The reduction in number of grain per pod under drought stress treatments may be attributed to the limitation of dry matter partitioning to the reproductive sink or even grain formation factors as has been reported by Turk et al (1980). The number of pod per plant in the non-stress condition ($S_0$) giving a 80% increase over the sever drought stress condition ($S_2$) (Table 3).

The significant reduction in number of harvested pods per plant under drought stress may be attributed to the abscission of the reproductive structures. Ziska and Hall (1983) and Gwathmey and Hall (1992) reported similar results. The number of pod per plant in the ILC482 cultivar giving a 32% increase over the Azad cultivar. The differential behavior of various cultivars to drought stress may be attribute to their variable genetic make up and impaired physiological mechanism of plants carried out in the presence of water.

Table3. Mean comparisons for yield, yield components and protein yield in chickpea cultivars under drought stress and Zinc fertilizer

| | Num of pod per plant | Num of grain per pod | Num of grain per plant | 100grain Weight (g) | Grain yield (kg/ha) | Biomass yield (kg/ha) | Harvest Index (%) | Grain proteins (mg/ml) | Protein yield (kg/ha) |
|---|---|---|---|---|---|---|---|---|---|
| Drought stress | | | | | | | | | |
| No stress | 45a | 1.9a | 31.55a | 29a | 2645.2a | 5326a | 44a | 1.41c | 521a |
| Moderate stress | 21.6b | 1.6a | 18.3b | 28.5a | 1425b | 3623b | 41a | 1.51b | 311.2b |
| Sever stress | 9c | 1.1b | 9.3c | 24b | 917c | 1922c | 37.6 | 1.66a | 210c |
| LSD | 2.2 | 0.052 | 3.2 | 2.6 | 611.3 | 216.3 | 3.1 | 0.213 | 117 |
| Zn fertilizer | | | | | | | | | |
| No Zn fertilizer | 24.2 | 1.33 | 15.5 | 27 | 1295b | 3125b | 41.2 | 1.49 | 321 |
| Application of Zn fertilizer | 26.5 | 1.41 | 16.2 | 28.3 | 1526a | 3526a | 43.3 | 1.52 | 332 |
| LSD | 3.2 | 0.041 | 3.2 | 1.71 | 111.3 | 400.3 | 2.89 | 0.09 | 51.23 |
| Cultivars | | | | | | | | | |
| Azad | 21.2b | 1.4b | 13.1b | 28b | 1946ab | 3255bb | 46b | 1.46 | 312.6b |
| Bivanij | 22b | 1.01c | 11.2b | 32.1a | 2126a | 3125b | 44b | 1.45 | 374.4a |
| Hashem | 25.3b | 2a | 18.1ab | 24c | 1125c | 3856a | 37c | 1.51 | 254.6c |
| ILC482 | 31.2a | 1.39b | 21.7a | 24.3c | 1452b | 2866bc | 49.1a | 1.47 | 304.6b |
| LSD | 5.1 | 0.049 | 3.4 | 2.9 | 182 | 550.6 | 4.2 | 0.18 | 72.23 |

Means by the uncommon letter in each column are significantly different ($p<0.05$)

Table 4. Correlation matrix of mean productivity, effect of drought stress and Zinc fertilizer on yield, yield components and protein yield in chickpea cultivars

| | (GY) | (BY) | (HI) | (NPP) | (NGPod) | (NGPlant) | (100GW) | (GP) | (PY) |
|---|---|---|---|---|---|---|---|---|---|
| grain yield (GY) | 1.00 $^{n}$ | 0.42 $^{n}$ | 0.71 $^{ns}$ | 0.91 $^{**}$ | -0.52 $^{*}$ | 0.89 $^{**}$ | 0.01 $^{ns}$ | -0.95 $^{*}$ | 0.85 $^{*}$ |
| Biomass yield (BY) | | 1.00 | -0.09 $^{n}$ | 0.55 $^{ns}$ | -0.09 $^{ns}$ | 0.52 $^{ns}$ | 0.22 $^{ns}$ | -0.55 $^{ns}$ | 0.22 $^{ns}$ |
| Harvest Index (HI) | | | 1.00 | 0.33 $^{ns}$ | -0.79 $^{*}$ | 0.11 $^{ns}$ | 0.11 $^{ns}$ | -0.41 $^{ns}$ | 0.37 $^{ns}$ |
| number of pod per plant (NPP) | | | | 1.00 | -0.25 $^{ns}$ | 0.88 $^{**}$ | -0.09 $^{ns}$ | -0.55 $^{ns}$ | 0.85 $^{*}$ |
| number of grain per pod (NGPod) | | | | | 1.00 | -0.27 $^{ns}$ | -0.62 $^{ns}$ | 0.11 $^{ns}$ | -0.8 $^{*}$ |
| number of grain per plant (NGPlant) | | | | | | 1.00 | -0.11 $^{ns}$ | -0.33 $^{ns}$ | 0.65 $^{*}$ |
| 100grain weight (100GW) | | | | | | | 1.00 | -0.59 $^{ns}$ | 0.11 $^{ns}$ |
| Grain proteins (GP) | | | | | | | | 1.00 | -0.82 $^{*}$ |
| Protein yield (PY) | | | | | | | | | 1.00 |

ns :Non-significant , * and **: Significant at 5% and 1% probability levels, respecti

The number of grain per plant in the non-stress condition ($S_0$) giving a 42% increase over the sever drought stress condition ($S_2$) (Table 3). The number of grain per plant in Hashem cultivar showed a 49.5% increase over the Bivanij cultivar.

The yield of chickpea in the stress condition was restricted by limited moisture availability. Drought occurrence in relation to anthesis stage causes a drastic reduction in yield and yield components (Seghatoleslami et al., 2008). Also, the results showed that under non drought-stressed conditions chickpea cultivars significantly gave better grain yields than under drought-stressed conditions and the Bivanij cultivar comparatively was the highest grain-yielding cultivar under both conditions. The grain yield of chickpea in the non-stress condition ($S_0$) had a 65% increasing over the sever drought stress condition ($S_2$) (Table 3). Cultivars differed in their response to drought stress at different growth stages. However, Bivanij cultivar gave the highest grain yield (2987 kh/ha) under non stress condation and application of Zn fertilizer but Hashem cultivar had lowest grain yield (397 kh/ha) under drought stress condation and non-application of Zn fertilizer (Table2). Under non stress condation the grain yield in the Bivanij cultivar in application of Zn fertilizer treatment giving a 33% increase over the Hashem cultivar in non-application of Zn fertilizer treatment (Table 3). Zn fertilizer had a positive effect on the grain yield and biomass yield of chickpea. In chickpea, the final grain yield is dependent upon the number of pods per plant, number of grains per pod and the extent to which grains are filled. In the present study, the reduction in grain yield under drought stress was associated with dramatic decrease in all yield components (Table 3). Supporting evidences were reported by many researchers (Ziska and Hall, 1983; Ludlow and Mushow, 1990; Gwathmey et al., 1992). They attributed the reduction in grain yield under drought stress to the reduction in number of pods per plant, number of grain per pod and grain weight. Turk and Hall (1980) attributed the reduction in grain yield under drought stress to the secondary detrimental effects of drought avoidance on $CO_2$ assimilation. This result suggests that chickpea cultivars exhibit reproductive plasticity under drought stress conditions.

Decrease biomass yield under lower soil moisture might be due to reduction of leaf area and photosynthesis rate (Sinaki et al., 2007). In different irrigation treatments indicate with increasing drought stress increased the biomass yield significantly. The biomass yield in the non-stress condition ($S_0$) giving a 64% increase over the sever drought stress condition ($S_2$) (Table 3). The biomass yield in the Hashem cultivar had a 25% increase over the ILC482 cultivar. Latiri-Soki et al (1998) reported that, irrigation and fertilizers increased biomass yield and grain yield. They suggested the increase might be due to increased leaf area index (LAI) and an increase in the period for which the crop remained green which resulted in increased capture efficiency of radiation energy and consequently more dry matter production.

Also, Ziska and Hall (1983) the effect of drought on HI to the reduction in assimilate supply attributed. Harvest index also varied significantly among cultivars, with the introduced cultivar (ILC482) having the highest value compared to the other cultivars (Table 3). This suggests that chickpea cultivars which gave higher grain yield under drought-stressed conditions could play an important role in sustaining crop production in semi arid regions.

With increasing levels of drought stress, chickpea grain protein significantly increased compared to control ($P<0.01$). The grain protein in the sever drought stress ($S_2$) condition giving a 15% increase over the non-stress condition ($S_0$), but the protein yield in the non-stress condition ($S_0$) giving a 60% increase over the sever drought stress condition ($S_2$) (Table 3). The grain protein yield in the Bivanij cultivar had a 32% increasing over the Hashem cultivar. The electrophoregrams of grain protein banding patterns in chickpea cultivars indicated that not obvious any new band and not deleted any bands. These findings were indicated that grain protein banding pattern is very stable and not sensitive to environmental changes (Tanksley and Jones, 1981).

## CONCLUSION

The present study concluded that maximum production of chickpea (grain yield, yield components and grain protein yield) was recorded for non stress treatment ($S_0$) and was followed by application of Zn fertilizer, while sever drought stress ($S_2$) produced minimum production. Therefore, Bivanij cultivar had highest production of chickpea (grain yield, 100 grain weight and grain protein yield) and Hashem cultivar had a lowest them. Also, results of these experiment showed that application of Zn fertilizer had better effect on grain yield, yield components and grain protein yield compared to the control. Therefore, we can increase yield and grain protein yield of chickpea by irrigation and application of Zn fertilizer. Also the results revealed that drought stress and Zn fertilizer no effect on grain protein banding patterns in chickpea cultivars.

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