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Full Length Research Paper

Effect of drought stress and N fertilizer on yield, yield components and grain storage proteins in chickpea (*Cicer arietinum* L.) cultivars

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Among the unfavorable environmental conditions, water deficit is the most significant factor that adversely affects plant growth, development and productivity. To determine the effects of drought stress and N fertilizer on yield, yield components and grain protein profiling pattern of chickpea (*CICER ARIETINUM* L.) a field experiment was conducted on a clay soil, in Razi University of Kermanshah, 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 N levels (0 and 25 kg/ha). The results showed that the effects of drought stress on yield and yield component, effect of cultivars on grain yield and protein yield were significant. With increase 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 them. Application of small starter N fertilizer (25 kg/ha) had better effect on grain yield, yield components and grain protein yield compared to the control (0 kg /ha N). Therefore, we can increase yield and grain protein yield of chickpea by irrigation and application of small starter N fertilizer. Also the results revealed that drought stress and N fertilizer no effects on grain protein banding patterns in chickpea cultivars.

Key words: Chickpea, drought stress, N fertilizer, protein, Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), yield.

INTRODUCTION

The rapid increase in the world population demands parallel increases in food production, particularly of chickpea. The global water crisis seriously influences crop productivity particularly in most of the Asian countries where irrigated agriculture accounts for 90% of total diverted fresh water (Huaqi et al., 2002). 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). Chickpea is an important self-pollinated grain legume crop, grown mainly in West, Asia, North Africa and the Indian subcontinent, where it is a basic component of the human diet (Talebi et al., 2008).

Drought stress is the second important constraint of yield in chickpea after disease (Singh et al., 1994). Low soil moisture during the early stages of the chickpea growth decreases nodule formation (Gan et al., 2005), and low moisture during late vegetative to early flowering period decreases efficiency of N_2 fixation (Beck et al., 1991) and decreased yield and yield components of it. The study of genetic parameters of chickpea under irrigated and rain-fed management conditions revealed significantly positive effect of irrigation on all the parameters including yield (Anwar et al., 2003). Several studies have also shown that optimum yield can be obtained with irrigation at branching, flowering and pod formation stages (Prihar and Sandhu, 1968). The yield in chickpea depends on two factors: number of grains/m^2 at harvest time and the grain weight (Wry, 1986).

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Abbreviations: HI, Harvest index, Sodium Dodecyl Sulfate poly acrilamid gel electrophoresis (SDS-PAGE), **S,** drought stress, **N,** nitrogen fertilizer, **V,** cultivar, **m,** marker; **DAP,** day after podding.

Number of seed per pod has the most stability than other yield components of pulse crop (Kochaki, 1997). Number of seed per pod, number of seed per plant and nuber of pod per plant decreased with increasing of drought stress level (Khurgami et al., 2009).

In recent years, grain legumes have played a primary role in the search for vegetable sources of proteins owing to the high protein content of the seed, ranging from 20% in pea to 40% in lupin (Cereletti, 1979). Chickpea seeds contain essential amino acids like isoleucine, leucine, lysine, phenylalanine and valine (Karim and Fattah, 2006). The protein in chickpea is highly digestible (70 to 90%) (Williams and Singh, 1980). 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 and nitrogen availability (Altenbach et al., 2002; Luo et al., 2000; Ottman et al., 2000; Rharrabti et al., 2001; Tea et al., 2004). In recent years, the applications of proteomic tools have become popular, and the tools are powerful methodologies for detecting and examining changes in protein composition accurately. Accumulation of specific proteins and other compounds for nutrient storage to high levels is one of the characteristic events during seed development (Suoiy et al., 2009). Improvement of storage protein in seed is being given more and more attention all over the world (Kim et al., 1990). 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). It has been widely suggested that such banding patterns could be important supplemental method for cultivars identification, particularly when there are legal disputes over the identity of a cultivar or when cultivars are to be patented (Tanksley and Jones, 1981). Seed storage protein is useful tool for studying genetic diversity of wild and cultivated rice (Thanh and Hirata, 2002). Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is most economical simple and extensively used biochemical technique for analysis of genetic structure of germplasm (Amjad et al., 2009). 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 N fertilizer on yield, yield component, seed storage proteins, protein yield and protein banding pattern of chickpea cultivars.

MATERIALS AND METHODS

Experimental design

A field experiment was conducted at Razi University of Kermanshah, Iran, on a clay soil. The experiment was laid out in a split-factorial design with drought stress in main plots and cultivar with nitrogen fertilizer in subplots with three replications. The experimental treatments consisted of three levels of drought stress [sever drought stress (S_2), moderate drought stress (S_1) and no drought stress (S_0)] in the main plots and four cultivars of chickpea, Azad, Bivanij, Hashem and ILC482 and 2 N levels in the sub plots. Plants were either not given any fertilizer N (0 N), or supplied with fertilizer N at the rate of 25 kg/ha (25 N). The fertilizer N was applied in the form of ammonium nitrate in solution at the time of sowing. The plots were fertilized with, P_2O_5 at the rate of 40 kg/ha as basal application. The seeds were sown in rows on April 8, 2009. Each cultivar was planted in a 5 m long, 6-row plot. Row to row and plant - plant distance was maintained at 25 and, respectively. Seeds were placed at 3 to 5 cm depth in each row. The crop field was weeded twice to control weeds.

Yield and yield components

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.

100 grain weight (g)

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

Biomass yield

The biomass production was measured on 10 plants treatment at 40 day after podding (DAP).

Harvest Index

The harvest index was accounted for with the following:
HI = (economical yield / biological yield)

Grain protein and electrophoresis

A single seed was grounded with a mortar and pestle and 10 mg (0.01 g) out of this seed flour was taken into a 1.5 ml micro-tube. 400 µl 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 1 g 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

Table 1. Analysis of variance (mean squares) for yield, yield components and protein yield in chickpea cultivars under drought stress and N fertilizer.

Source of variation	df	Means of square								
		Number of pod per plant	Number of grain per pod	Number of grain per plant	100 grain weight	Grain yield	Biomass yield	Harvest Index	Grain proteins	Protein yield
Repetition	2	424	0.005	87.6	7.87	938519.3	418114.1	93.7	0.109	30956
Drought stress	2	5569**	0.021**	1698.8**	24.01^{ns}	25713402.7**	38766584**	1696**	0.205**	827050**
Error (Ea)	4	19	0.005	6.8	8.1	970269.4	62798.3	6.8	0.023	18996
N fertilizer	1	2^{ns}	0.003^{ns}	14.9^{ns}	7.79^{ns}	816011.1^{ns}	369871.3^{ns}	14.9^{ns}	0.022^{ns}	8024^{ns}
Cultivar	3	195**	0.69**	44.7^{ns}	183.2**	4155662.9**	1504421^{ns}	44.7^{ns}	0.026^{ns}	84199**
N fertilizer* stress	2	24^{ns}	0.0008^{ns}	21.3^{ns}	2.17^{ns}	279752.7^{ns}	1203572^{ns}	21.3^{ns}	0.005^{ns}	14386^{ns}
N fertilizer* cultivar	3	50^{ns}	0.01^{ns}	22^{ns}	3.9^{ns}	200018.5^{ns}	460928^{ns}	22^{ns}	0.109^{ns}	9889^{ns}
cultivar* stress	6	95^{ns}	0.001^{ns}	28.2^{ns}	8.54^{ns}	936594.4^{ns}	605401.1^{ns}	28.2^{ns}	0.03^{ns}	0.03^{ns}
Stress* cultivar* N fertilizer	6	69^{ns}	0.005^{ns}	10^{ns}	3.31^{ns}	2826761.1**	450704.7^{ns}	10^{ns}	0.095^{ns}	3695^{ns}
Error (Eb)	42	380.02	0.043	465.94	11.55	181122.41	23061040	34.36	0.431	14584
CV		28.7	6.9	25.2	12.51	25.8	25.8	14.8	12.55	32.58

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

fixing gel was washed with distilled water for 15 min, with changing the water after every 5 min.

Protein yield

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).

Statistical analysis

The statistical analyses to determine the individual and interactive effects of drought stress, N fertilization and cultivar were conducted using JMP 5.0.1.2 (Statistical analyses system Institute incorporated ,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

Number of pod per plant

Number of pod per plant is one of the most important yield components. 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 (40) number of pod per plant and the S_2 treatment has the lowest number of pod per plant (11.9) and the difference is significant. Among the cultivars treatments, the highest number of pod per plant (26.9) was belonged to the ILC482 cultivar and the lowest number of pod per plant (19.1) was belonged to the Bivanij cultivar (Table 3). Similar results were reported by Mansur et al. (2010), Khurgami et al. (2009) and Arya and Khushwa (2000) in chickpea.

Number of grain per pod

The effects of drought stress and cultivar treatments on number of grain per pod were 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 grain per pod (Table 3) shows that S_1 treatment has the highest (1.13) number of grain per pod and the S_0 treatment has the lowest number of grain per pod (1.07) and the difference is significant. Among the cultivars treatments, the highest number of grain per pod (1.19) was belonged to the Hashem cultivar and the lowest number of grain per pod (1.04) was belonged to the Bivanij cultivar (Table 3). Similar results were reported by Khurgami et al. (2009) in chickpea.

Number of grain per plant

The effect of drought stress treatment on number of grain 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 grain per plant (Table 3) shows that S_0 treatment has the highest (22.5) number of grain per plant and the S_2 treatment has the lowest number of grain per plant (7.1) and the difference is significant. Among the cultivars treatments, the highest number of grain per plant (14.8) belonged to the ILC482 cultivar and the lowest number of grain per plant (11) belonged to the Bivanij cultivar (Table 3). Similar results were reported by Khurgami et al. (2009) in chickpea.

100 grain weight (g)

Grain weight, an important yield determining factor, reflects the extent of grain development. 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 (31.6 g) 100-grain weight and ILC482 cultivar has the lowest (24.3 g) 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.

Grain yield

The analysis of variance in Table 1 shows the effects of drought stress, cultivar and interaction of drought stress × variety × N fertilizer treatments on grain yield are significant at 1% level and the effects of N fertilizer non-significant on it. Comparison of average grain yield in different irrigation treatments indicated that the S_0 treatment has the highest grain yield (2229.6 kg /ha) and the S_2 treatment has the lowest grain yield (815 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 N fertilizer treatments, the highest grain yield (1438 kg/ha) was belonged to the N_1 treatment and the lowest grain yield (1388 kg/ha) was belonged to the N_0 treatment (Table 3). Among the cultivars treatments, the highest grain yield (1675.5 kg/ha) was belonged to the Bivanij cultivar under non stress conditions and the lowest grain yield (914.4 kg/ha) was belonged to the Hashem cultivar under stress conditions (Table 3). Interaction effect of drought stress × variety × N fertilizer (S × V × N) shows that $S_0N_1V_2$ has the highest grain yield (2730 kg/ha) and $S_2N_0V_3$ has the lowest grain yield (150 kg/ha) (Table 2). The significance of this interaction clearly shows the differential response of plants under different water regimes to N fertilizer. Similar results were reported by Mansur et al. (2010) Singh and Dixit (1992) and Arya and Khushwa (2000) in chickpea.

Biomass yield

The effect of drought stress treatment on biomass yield was significant at 1% 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 (4277 kg/ha) biomass yield and the S_2 treatment has the lowest biomass yield (1771 kg/ha) and the difference is significant. There is no significant difference in biomass yield between N_0 and N_1 treatments. Among the cultivars treatments, the highest biomass yield (3279.2 kg/ha) was belonged to the Hashem cultivar and the lowest biomass yield (2600.6 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.

Harvest index

Chickpea cultivars differed significantly for harvest index both under stress and non stress conditions. 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 (41.9%) harvest index and the S_2 treatment has the lowest harvest index (36.6%) and the difference is significant. Among the cultivars treatments, the highest harvest index (47.2%) was belonged to the ILC482 cultivar and the lowest harvest index (23.3%) was belonged to the Hashem cultivar (Table 3). Similar results were reported by Mansur et al. (2010) and Arya and Khushwa (2000) in chickpea.

Grain proteins

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 (1.55 mg/ml) grain protein and the S_0 treatment has the lowest grain protein (1.34 mg/ml) and the difference is significant.

Among the cultivars treatments Hashem cultivar has the highest (1.47 mg/ml) grain protein and the Bivanij cultivar has the lowest grain protein (1.4 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).

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

Cultivar	No stress (S_0)		Moderate stress (S_1)		Severstress (S_2)	
	No fertilizer (N_0)	Application of fertilizer (N)	No fertilizer (N_0)	Application of fertilizer (N_1)	No fertilizer (N_0)	Application fertilizer (N_1)
Azad	2583^{a}	2000^{abcd}	1130^{def}	1356_{bcdef}	923^{efg}	1116^{def}
Bivanij	2106^{abc}	2730^{a}	1343_{bcdef}	1696^{bcde}	1143^{def}	1033^{ef}
Hashem	1580_{bcdef}	2196^{ab}	670^{fg}	733^{fg}	150^{g}	156^{g}
ILC482	2076^{abc}	2563^{a}	1280^{cdef}	$1360b^{cdef}$	1066^{ef}	930^{efg}

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

Table 3. Mean comparisons for yield, yield components and protein yield in chickpea cultivars under drought stress and N fertilizer.

Characteristics	Number of pod per plant	Number of grain per pod	Number of grain per plant	100 grain weight (g)	Grain yield (kg/ha)	Biomass yield (kg/ha)	Harvest index (%)	Grain proteins (mg/ml)	Protein yield (kg/ha)
No stress	40^{a}	1.07^{b}	22.5^{a}	27.9	2229.6^{a}	4277^{a}	41.9^{a}	1.34^{b}	457.5^{a}
Moderate stress	15.8^{b}	1.13^{a}	9.1^{b}	27.5	1196.3^{b}	2654.8^{b}	39.6^{a}	1.43^{ab}	263.6^{b}
Sever stress	11.9^{c}	1.12^{a}	7.1^{b}	26	815^{b}	1771.2^{c}	36.6^{b}	1.55^{a}	192^{c}
LSD	3.5	0.05	2	2.28	558.2	200.8	2.5	0.122	110.47
N fertilizer									
No fertilizer	22.4	1.1	12.5	26.7	1388.8^{a}	2972.7	38.5	1.37	308.62
Application of fertilizer	22.8	1.11	13.4	27.4	1438.3^{b}	28.29	40.2	1.44	329.73
LSD	3	0.03	2.1	1.61	120.6	390.5	2.7	0.087	49.47
Cultivars									
Azad	21.2^{b}	1.12^{b}	12.5^{ab}	26.8^{b}	1518.3^{a}	2777^{ab}	45^{ab}	1.47	348.6^{a}
Bivanij	19.1^{b}	1.04^{c}	11^{b}	31.6^{a}	1675.5^{a}	2947^{ab}	41.8^{b}	1.4	362.8^{a}
Hashem	$23a^{b}$	1.19^{a}	13.4^{ab}	25.7^{bc}	914.4^{b}	3279.2^{a}	23.3^{c}	1.49	217.1^{b}
ILC482	26.9^{a}	1.08^{bc}	14.8^{a}	24.3^{c}	1546.1^{a}	2600.6^{b}	47.2^{a}	1.46	348.1^{a}
LSD	4.37	0.05	3	2.28	170.5	552.3	3.9	0.12	69.97

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

Protein yield

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 (457.5 kg/ha) protein yield and the S_2 treatment has the lowest protein yield (192 kg/ha) and the difference is significant. Among the cultivars treatments, the highest protein yield (362.8 kg/ha) was belonged to the Bivanij cultivar and the lowest protein yield (217.1 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) and Ottman et al. (2000).

Simple correlation study

The correlation matrix (Table 4), indicated strong

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

Correlation characteristics	(GY)	(BY)	(HI)	(NPP)	(NGPod)	(NGPlant)	(100GW)	(GP)	(PY)
Grain yield (GY)	1.00 ^{ns}	0.39 ^{ns}	0.6 ^{ns}	0.8**	-0.72*	0.8**	0.04 ^{ns}	-0.8*	0.99*
Biomass yield (BY)		1.00	-0.07 ^{ns}	0.48 ^{ns}	-0.11 ^{ns}	0.47 ^{ns}	0.1 ^{ns}	-0.43 ^{ns}	0.33 ^{ns}
Harvest Index (HI)			1.00	0.19 ^{ns}	-0.75*	0.17 ^{ns}	0.14 ^{ns}	-0.3 ^{ns}	0.64 ^{ns}
Number of pod per plant (NPP)				1.00	-0.3 ^{ns}	0.99**	-0.03 ^{ns}	-0.65 ^{ns}	0.79*
Number of grain per pod (NGPod)					1.00	-0.27 ^{ns}	-0.55 ^{ns}	0.55 ^{ns}	-0.71*
Number of grain per plant (NGPlant)						1.00	-0.02 ^{ns}	-0.64 ^{ns}	0.78*
100 grain weight (100GW)							1.00	-0.48 ^{ns}	0.37 ^{ns}
Grain proteins (GP)								1.00	-0.77*
Protein yield (PY)									1.00

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

and significant ($p<0.01$) correlation of grain yield with number of pod per plant and number of grain per plant (r=0.8 and 0.8) respectively. These results were agreement with the previously reported ones (ICARDA, 1993). 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 had negative but significant correlation coefficient (r= -0.72 and -0.8) of 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=-0.75). The number of pod per plant was positively and significantly ($p<0.01$) correlated with number of grain per plant (r=0.99). However the Protein yield was negatively and significantly ($p<0.05$) correlated with Grain protein (r=-0.77) and number of grain per pod (r=-0.71) respectively.

SDS-PAGE protein analysis

The grain storage proteins patterns for 4 cultivars of chickpea under drought stress and used of starter nitrogen fertilizer and no nitrogen fertilizer after SDS-PAGE are shown in Figures 1 and 2 respectively. In total 29 to 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 nitrogen 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.

1, 2, 3, 4 = No drought stress treatment(S_0); 5, 6, 7, 8= Moderate drought stress treatment (S_1) 9, 10, 11, 12 = severe drought stress treatment (S_2); m= Marker; 1, 5, 9= Azad cultivar; 2, 6, 10= Bivanij cultivar; 3, 7, 11= Hashem cultivar; 4, 8, 12= ILC482 cultivar.

1, 2, 3, 4 = No drought stress treatment (S_0); 5, 6, 7, 8= moderate drought stress treatment (S_1) 9, 10, 11, 12= sever drought stress treatment (S_2); 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 shown 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 71% 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 27% increase over the Bivanij 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.

The number of grain per plant in the non-stress condition (S_0) giving a 69% increase over the severe drought stress condition (S_2) (Table 3). The number of grain per plant in ILC482 cultivar giving a 26% 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 (Araus et al., 2002; Seghatoleslami et al., 2008). Also, the results showed that

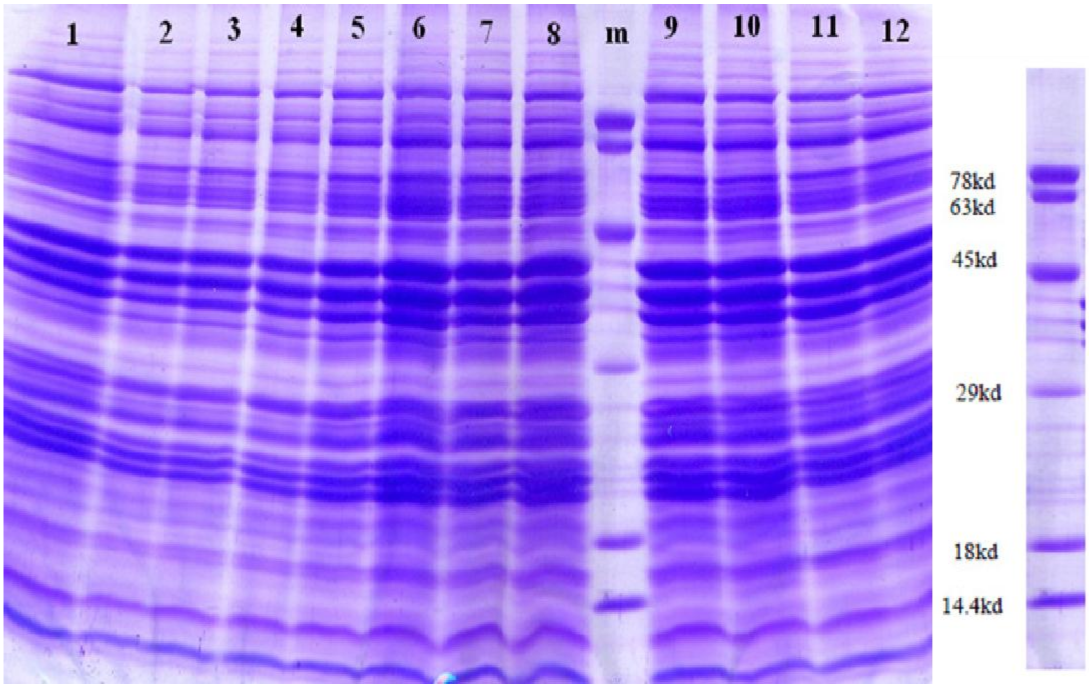

Figure 1. Grain protein banding patterns in chickpea cultivars under drought stress with used of N fertilizer.

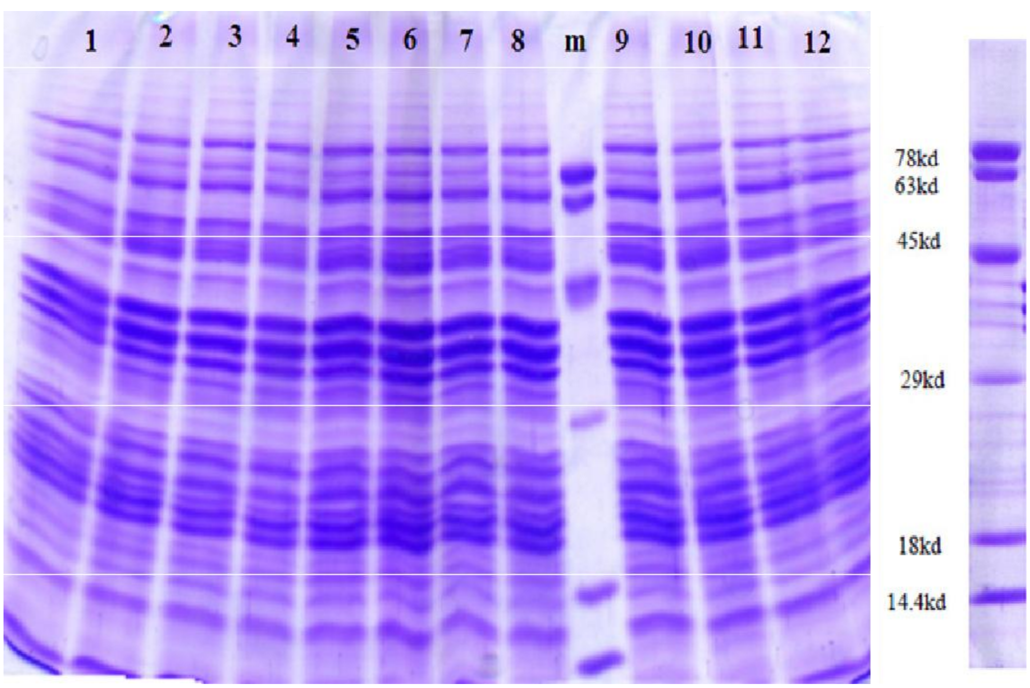

Figure 2. Grain protein banding patterns in chickpea cultivars under drought stress with no N fertilizer.

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) giving a 64% increase over the severe drought stress condition (S_2) (Table 3). Cultivars differ in their response to drought stress at different growth stages. However, Bivanij cultivar gave the highest grain yield and Hashem cultivar the lowest. The grain yield in the Bivanij cultivar giving a 46% increase over the Hashem cultivar (Table 3). N fertilizer had a positive effect on the grain 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 thise 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 59% increase over the sever drought stress condition (S_2) (Table 3). The biomass yield in the Hashem cultivar giving a 21% increase over the ILC482 cultivar. Latiri-Soki et al. (1998) reported that, irrigation and N 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) attributed the effect of drought on HI to the reduction in assimilate supply. 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 14% increase over the non-stress condition (S_0), but the protein yield in the non-stress condition (S_0) giving a 58% increase over the sever drought stress condition (S_2) (Table 3). The grain protein yield in the Bivanij cultivar giving a 41% increase 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 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 N fertilizer, while sever drought stress (S_2) produced minimum production. Therefore, Bivanij cultivar had highest production of chickpea (grain yield and grain protein yield) and Hashem cultivar had a lowest them. Also, results of these experiment showed that application of small starter N fertilizer (25 kg/ha) had better effect on grain yield, yield components and grain protein yield compared to the control (application of 0 kg/ha starter N fertilizer). Therefore, we can increase yield and grain protein yield of chickpea by irrigation and application of small starter N fertilizer. Also the results revealed that drought stress and N fertilizer no effect on grain protein banding patterns in chickpea cultivars.

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