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Effect of drought stress and Zink fertilizer of protein profile and seeds proteins of chickpea (*Cicer arietinum* L.) cultivars

Ezatollah Hasanpoor[1*], Samira Khasamiri Argane[2]

1- Department of plant roduction and genetic Engineering, Islamic Azad University, Khorramabad Brach, Khorramabad, Iran.
2- Department of plant production and genetic Engineering, Lorestan University, Khorramabad, Iran

Corresponding author: **Ezatollah Hasanpoor**

ABSTRACT: Chickpea is an important crop in the cropping pattern supplying cheap protein diet especially for poor people. Over the years, however, low yields are more prominent declining acceptability of this crop. this study was planned to examine effect of drought stress and Zn ferilizer on protein content and protein banding pattern of chickpea cultivars. The experiment was laid out in a split-factorial design with drought stress in main plots and cultivar with nitrogen Zn ferilizer in subplots with three replications. The experimental treatments consisted of three levels of drought stress [sever drought stress (S2) , moderate drought stress (S1) and no drought stress(S0)] and four cultivars of chickpea (*Cicer arietinum* L.) , Arman, Bivanij, Flipe and GREET and 2 levels of Zn fertilizers. Plants were either not given any Zn ferilizer, or supplied with Zn ferilizer at the rate of 25 kg ha-1. The results showed that the effects of drought stress on seed storage proteins and protein yield, effect of cultivars on protein yield were significant($P<0.01$). With increase drought stress seed storage proteins was increased and protein yield decreased. Also, results showed that No effects treatments (Drought stress and Zn ferilizer) on protein banding patterns. Also, results indicated that not obvious any new band and not deleted any bands.

Key words: Chickpea, drought, electrophoresis, nitrogen and protein

INTRODUCTION

Chickpea (*Cicer arietinum* L.) belongs to the family *Leguminosae*. It is one of the important grain legume cultivated in the world. 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 (Javid et al, 2004). The protein in chickpea is highly digestible (70-90%) (Sumera et al, 2009). The seed storage proteins are non-enzymatic and have the sole purpose of providing proteins (nitrogen and sulphur source) required during germination and establishment of a new plant.

Seed 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; Dupont and Altenbach 2003; 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 (Singh et al, 1993). Storage protein is a method to investigate genetic variation and to classify plant varieties (Iqbal et al, 2005). Seed protein is not sensitive to environmental fluctuations; its banding pattern is very stable which advocated for cultivars identification purpose in crop. 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 (Singh et al, 1994). Seed storage protein is useful tool for studying genetic diversity of wild and cultivated rice (Singh et al, 1994). Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is most economical simple and extensively used biochemical technique for analysis of genetic structure of germplasm. As seed storage proteins are largely independent of environmental fluctuations, their profiling using SDS-PAGE technology is particularly considered as a consistent tool for economic characterization of germplasm (Javid et al., 2004; Iqbal et al., 2005). Accumulation of specific proteins and other compounds for nutrient storage to high levels is one of the characteristic events during seed development (Singh et al, 1993). Improvement of storage protein in seed is being given more and more attention all over the world (Kim et al., 1990). Protein is the performer of life activity, the change of plant morphology corresponds with the change of relative proteins (Singh et al, 1993). 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 Zn fertilizer on protein profiling of grains (Iqbal et al, 2005). For gave to highest seed yield in agriculture addition to both nitrogen and phosphate fertilizer is very important (Shaban, 2013a,b). For gave the highest seed yield and protein yield in rapeseed (Kiani et al, 2013) and maize (Beyranvand et al, 2013) should apply both nitrogen and phosphate bio fertilizers. Drought stress is the second important constraint of yield in chickpea after disease (Singh et al., 1994). Several studies have also shown that optimum yield can be obtained with irrigation at branching, flowering and pod formation stages (Prihar and Sandhu, 1968). Therefore this study was planned to examine effect of drought stress and Zn fertilizer on protein content and protein banding pattern of chickpea cultivars. The results showed that with increase drought stress seed storage proteins was increased and protein yield decreased. Also, results indicated that not obvious any new band and not deleted any bands.

2. Materials and Methods

2.1. 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 Zn fertilizer 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)] and four cultivars of chickpea (*Cicer arietinum* L.) , Arman, Bivanij, Flipe and GREET and 2 N levels. Plants were either not given any Zn fertilizer, or supplied with Zn fertilizer at the rate of 25 kg ha-1. The Zn fertilizer was applied in the form of ammonium nitrate in solution at the time of planting. The plots were fertilized with, P2O5 at the rate of 40 kg/ha as basal application. The seeds were sown in rows on April 8, 2009. Each variety was planted in a 5m long, 6-row plot. Row to

row and plant - plant distance was maintained at 25cm and 10cm, respectively. Seeds were placed at 2-3 cm depth in each row. The crop field was weeded twice to control weeds.

2.2. Seed protein and Electrophoresis

A single seed was grounded with a mortar and pestle and 10mg (0.01g) 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 13000rpm 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,0.01g bromo-phenol blue, 2ml β-mercaptoethanol, 1.5ml 0.5*M* tris, pH 6.8, 5g 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.

2.3. Protein yield

Finally, amount of grain protein yield was accounted with follow (17):

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

2.4. 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 (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.

3. Results and discussion

3.1. Seed storage proteins

The effect of drought stress treatment on seed protein was significant at 1% level (Table 1) but the other treatments were not significant on it. The comparison of the mean values of the seed protein (table2) showed that S2 treatment has the highest (1.55mg/ml) seed protein and the S0 treatment has the lowest seed protein (1.34mg/ml) and the difference is significant. Among the cultivar treatments Flipe cultivar has the highest (1.47mg/ml) seed protein and the Bivanij cultivar has the lowest seed protein (1.4mg/ml) and the difference is not significant (table2). Similar results were reported by Kim et al., 1990; Suoyi Han et al, 2009.

3.2. Protein yield

The effect of drought stress and variety 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 the protein yield (table2) showed that S0 treatment has the highest (457.5 kg ha-1) protein yield and the

S2 treatment has the lowest protein yield (192 kg ha-1) and the difference is significant. Among the cultivar treatments, the highest protein yield (362.8 kg ha-1) was belonged to the Bivanij variety and the lowest protein yield (217.1 kg ha-1) was belonged to the Flipe variety and the difference is not significant (Table 2). These results were in agreement with the findings of Luo et al. 2000; Ottman et al. 2000; Rharrabti et al. 2001.

3.3. SDS-PAGE Protein Analysis

The seed storage proteins patterns of 4 varieties of chickpea under drought stress and starter nitrogen Zn fertilizer after electrophoresis are shown in Figure 1. In total 29- 31 bands (since below 14kDa until over 78kDa molecular weight band) per variety were detected in electrophoregrams. The SDS- PAGE results revealed no effects treatments (Drought stress and nitrogen Zn fertilizer) on the protein banding patterns but the related sever drought stress bands were chromatic, because they have highest protein concentration (figure 1). These results were in agreement with the findings of Tanksley et al, 1981; Javid et al., 2004; Iqbal et al., 2005. However, this results indicated that not obvious any new band and not deleted any bands. This findings were indicated that grain protein banding pattern is very stable and not sensitive to environmental changes (Tanksley and Jones, 1981).

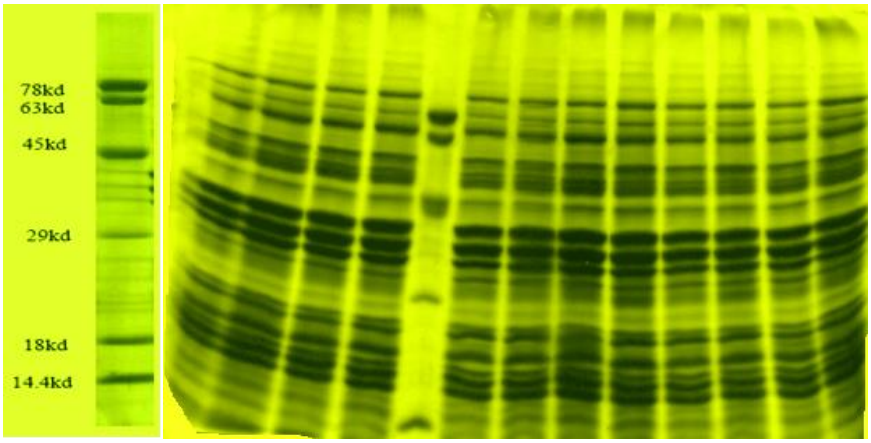

Figure 1. Protein banding patterns in chickpea cultivars under drought stress and Zn fertilizer

Column name from left to right:

1,2,3,4= Sever drought stress treatment (S2) 5= Marker (m)

6,7,8,9= Moderate drought stress treatment (s1) 10,11,12,13= No drought stress treatment(S0)

1,6,10= GREET cultivar 2,7,11= Flipe cultivar;

3,8,12= Bivanij cultivar 4,9,13= Arman cultivar

Table1. Analysis of variance for grain protein in chickpea cultivars under drought stress and nitrogen Zn fertilizer starter

		Means of square	
Source of variation	Degree of freedom	seed Proteins	protein yield
repetition	2	0.109	30956
Drought stress	2	0.205**	827050*
Error (Ea)	4	0.023	18996
Zn fertilizer	1	0.022	8024
variety	3	0.026	84199**
Zn fertilizer* stress	2	0.005	14386
Zn fertilizer* variety	3	0.109	9889
variety* stress	6	0.03	0.03
stress* variety* Zn fertilizer	6	0.095	3695
Error (Eb)	42	0.431	14584
CV		12.55	32.58

*and **: Significant at 5% and 1% probability levels, respectively

Table2. Mean comparisons for grain protein in chickpea cultivars under drought stress and nitrogen Zn fertilizer starter

Drought stress	Seed Proteins(mg/m	Protein Yield(kg/ha)
No stress	1.34b	457.5a
Medium stress	1.43ab	263.6b
Sever stress	1.55a	192c
LSD	0.122	110.47
Zn fertilizer		
No fertilizer	1.37	308.62
Used of fertilizer	1.44	329.73
LSD	0.087	49.47
varieties		
Arman	1.47	348.6a
bivanij	1.4	362.8a
Flipe	1.49	217.1b
GREET	1.46	348.1a
LSD	0.12	69.97

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

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