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Seed protein changes in chickpea (*Cice arietinum* L.) under application of K fertilizer and irrigation

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ABSTRACT

A field experiment was performed in order to evaluate the effects of irrigation and application of K fertilizer on seed storage proteins in chickpea, ILC-482 cultivar. Experiment was performed in factorial using randomized complete block design with three replications. In this experiment, two factor consist of irrigation in three levels (I1= non irrigation and I2= irrigation in flowering stage), K fertilizer in six levels (K1=0, K2=25kg/ha, K3=50kg/ha, K4=75kg/ha, K5=100kg/ha and K6=125kg/ha) was studied. The results showed that the effect of irrigation on seed storage proteins and protein yield was significant. Also results showed that effect of K fertilizer on seed storage proteins and protein yield was not significant. In non irrigation treatment seed storage proteins was increased compared to irrigation treatment and protein yield decreased. Also, results showed that no effects treatments (irrigation and K fertilizer) on protein banding patterns. Also, results indicated that not obvious any new band and not deleted any bands in all treatments.

Key words: K fertilizer, Seed storage protein

INTRODUCTION

Chickpea is the third most important food legume after peas and soybean in the world. Due to high protein content, it has become an important component of human diet in developing world. (FAO, 2003). It is cultivated on a large scale in arid and semiarid environments, and has considerable importance as food, feed and fodder. 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,

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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 nitrogen fertilizer on protein profiling of grains (Iqbal et al, 2005). 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). For give to highest seed yield and protein in agriculture addition to both nitrogen and phosphate fertilizer is very important (Shaban, 2013a,b). For give the highest seed yield and protein yield in barley (Azimi et al, 2013) and maize (Beyranvand et al, 2013) should apply both nitrogen and phosphate fertilizers. Therefore this study was planned to examine effect of drought stress and K fertilizer on protein content 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 factorial design with three replications. In this experiment, two factor consist of irrigation in three levels (I_1= non irrigation and I_2= irrigation in flowering stage), K fertilizer in six levels (K1=0, K2=25kg/ha, K3=50kg/ha, K4=75kg/ha, K5=100kg/ha and K6=125kg/ha) was studied. Plants The plots were fertilized with, P 2 O5 at the rate of abave as basal application. The seeds were sown in rows on April 14, 2010. Each ILC-482 cultivar of chickpea 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 3 cm depth in each row. The crop field was weeded twice to control weeds.

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.

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)

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.

RESULTS AND DISCUSSION

SDS-PAGE Protein Analysis

In this experiment seed storage proteins patterns ILC-482 cultivar of chickpea under irrigation and K fertilizer after electrophoresis are shown in Figure 1. In total 28- 32 bands (since below 14kDa until over 78kDa molecular weight band) per treatments were detected in electrophoregrams. The SDS- PAGE results revealed that no effects treatments (irrigation and K fertilizer) on the protein banding patterns but in non irrigation treatment (1, 2, 3, 4, 5, and 6) columns were chromatic, because they have highest protein concentration (figure 1) compared to irrigation treatments (7, 8, 9, 10, 11, and 12) columns. In each level of K application all bands were similar that shows the protein was not affected by application of K fertilizer. 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 in all treatments. These findings were indicated that grain protein banding pattern is very stable and not sensitive to environmental changes and nutrition (Tanksley and Jones, 1981).

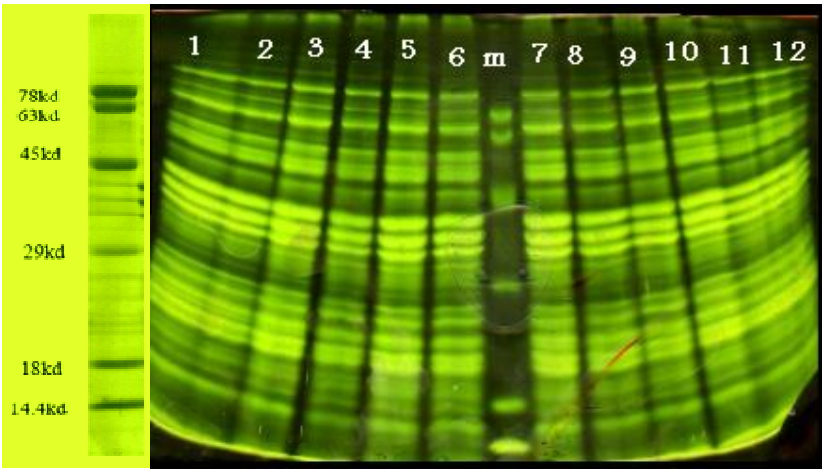

Figure 1. Protein banding patterns in chickpea under irrigation and application of K fertilizer

Column name from left to right:

1,2,3,4, 5, 6=Application of K fertilizer treatments in irrigation treatment with K_1=0, K_2=25kg/ha, K_3=50kg/ha, K_4=75kg/ha, K_5=100kg/ha and K_6=125kg/ha respectively.
m= Marker
7, 8, 9, 10, 11, 12,= Application of Kfertilizer treatments in non irrigation treatment with K_1=0, K_2=25kg/ha, K_3=50kg/ha, K_4=75kg/ha, K_5=100kg/ha and K_6=125kg/ha respectively.

Seed storage proteins

The effect of irrigation treatment on seed protein was significant at 1% level. The comparison of the mean values of the seed protein showed that non irrigation treatment has the highest (1.66mg/ml) seed protein and the irrigation treatment has the lowest seed protein (1.22mg/ml) and the difference is significant. Among the K fertilizer treatments application of 125kg/ha treatment has the highest (1.48mg/ml) seed protein and the control treatment has the lowest seed protein (1.17mg/ml) and the difference is not significant. Similar results were reported by Ottman et al. 2000.

Protein yield

The effect of irrigation and K fertilizer treatments on protein yield were significant at 1% level. The comparison of the mean values of the protein yield showed that irrigation treatment has the highest (411.3 kg ha^{-1}) protein yield and the non irrigation treatment has the lowest protein yield (264.2 kg ha^{-1}) and the difference is significant. Among the K fertilizer treatments, the highest protein yield (321.2 kg ha-1) was belonged to the 125 kg ha^{-1} treatment and the lowest protein yield (219.6 kg ha-1) was belonged at control treatment and the difference was significant. These results were in agreement with the findings of Ottman et al. 2000;. Rharrabti et al. 2001.

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