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Effect of water deficit on total protein and protein profile in wheat (*Triticum aestivum* L.) genotypes

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Abstract

Seed protein usually is not sensitive to environmental fluctuations; its banding pattern is very stable which advocated for cultivars identification purpose in crop. This study was planned to study effect of water deficit on protein content and protein banding pattern of wheat genotypes. In this study five wheat genotypes were planted in early November, growing season of 2008-2009. The experiment was carried out in a factorial design with three replications. Two water use levels (with and without irrigation) and five genotype of wheat (SABALAN/4/VRZ/3/OR F1.148/TDL//BLO, HAMAM-4, KAUZ'S'/MACHETE, Atila2/PBW65 and M-79-7) factorialed in plots as random. Proteins profiling of samples was performed using SDS- polyacryl amide gels. The results showed that, No effects Non irrigated treatments on protein banding patterns. Also, results indicated that not obvious any new band and not deleted any bands. Among the genotypes treatments HAMAM-4 genotype in Non irrigated condition had the highest seed protein and the KAUZ'S'/MACHETE genotype in irrigated condition had the lowest seed protein.

Key words: SDS-page, water deficit and wheat

Introduction

Common wheat cultivated under rainfed conditions and the area near the tail end of canals where shortage of water is often experienced. Wheat is the most important cereal crop, it's stable diet for more than one third of the world population and contributes more calories and protein to the world diet than any other cereal crop. The average yield of wheat is quite low in such areas, which is mainly due to shortage of water (Ashraf, 1998). Water stress is the most significant environmental stress in agriculture worldwide and improving yield under drought is a major goal of plant breeding (Cattivelli et al., 2008). Storage proteins in seeds 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). Storage protein is a method to investigate genetic variation and to classify plant varieties (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 (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. 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). Despite the fact that the response of protein composition to environmental factors in mature wheat grain results from changes in protein deposition

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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). 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 rapseed (Kiani et al, 2013) and maize (Beyranvand et al, 2013) should apply both nitrogen and phosphate biofertilizers. 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 aim of this examine is study on effect of water deficit on total protein and banding pattern of it in five wheat genotypes.

Materials and Methods

Plant material

In this experiment Five wheat genotypes were planted in early November, growing season of 2008-2009 at research farm of Razi University, Kermanshah (latitude 34°20´ N, longtitude 46°20´ E, altitude 1351.6 m above sea level), Iran. The seeds were provided by Dry land Agricultural Research Institute, and Agricultural and Natural Resources Research Center, Kermanshah, Iran. Kermanshah is located in west of Iran and has a mean annual temperature of 13.8°C and has annual rainfall of 478 mm. The soil texture of the research farm was sandy-loam. The experiment was carried out in a factorial design with three replications. Two water use levels (with and without irrigation) and five genotype of wheat (SABALAN/4/VRZ/3/OR F1.148/TDL//BLO, HAMAM-4, KAUZ'S'/MACHETE, Atila2/PBW65 and M-79-7) factorialed in plots as random. Seeds were pretreated with Mancozeb to minimize the probability of seed- and soil-borne diseases. The seeds were sown in five 3 m long rows, spaced 20 cm apart in end of November. The final stand density was set to be 400 plants per m^2.

Protein analysis

For protein analysis 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.

Results and discussion

SDS-PAGE Protein Analysis

The seed storage proteins patterns of five wheat genitypes under irrigated and Non irrigated condition after electrophoresis are shown in Figure 1. In total 21- 32 bands (since below 14kDa until over 78kDa molecular weight band) per genotypes were detected in electrophoregrams. The SDS- PAGE results revealed no effects treatment (Non irrigated) on the protein banding patterns but the related severe 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, these results indicated that not obvious any new band and not deleted any bands. These

findings were indicated that grain protein banding pattern in of more crops is very stable and not sensitive to environmental changes (Tanksley and Jones, 1981).

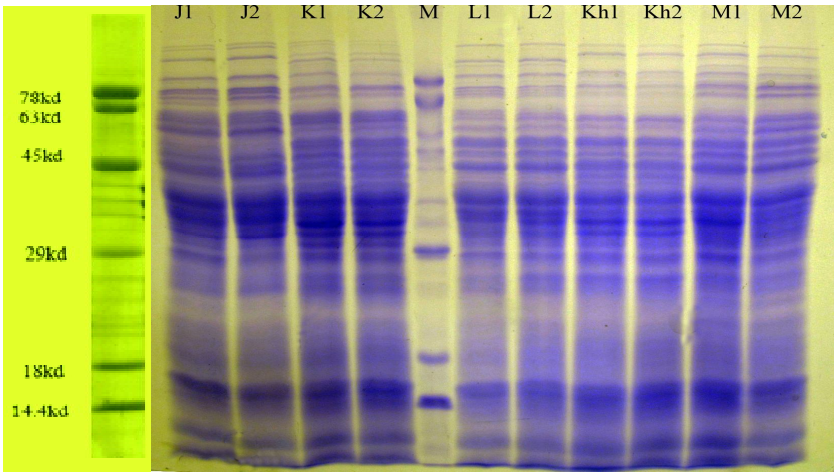

figure 1. Protein banding patterns in wheat genotypes under normal and drought stress conditions

Column name from left to right: J1 and J2= SABALAN/4/VRZ/3/OR F1.148/TDL//BLO genotype under with and without drought stress respectively, K1 and K2= HAMAM-4genotype under with and without drought stress respectively, M= Marker, L1 and L2= KAUZ'S'/MACHETE genotype under with and without drought stress respectively, Kh1 and Kh2= Atila2/PBW65 genotype under with and without drought stress respectively, M1 and M2= M-79-7genotype under with and without drought stress respectively

Total protein analysis

The effect of iriigation treatment on seed protein was significant. The comparison of the mean values of the seed protein showed that Non stress treatment had highest seed protein and the irrigated treatment had the lowest seed protein and the difference was significant. Among the genotypes treatments HAMAM-4 genotype in Non irrigated condition had the highest seed protein and the KAUZ'S'/MACHETE genotype in irrigated condition had the lowest seed protein and the difference was significant. Similar results were reported by Kim et al., 1990; Suoyi Han et al, 2009.

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