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Study on protein Changes in wheat under drought stress

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Abstract

A study was performed to study the effect of drought stress on protein changes of five wheat genotypes. Wheat genotypes were planted in early November, growing season of 2008-2009. The experiment was carried out in a factotial design with three replications. Two drought stress levels (irrigated and Non irrigated) and five genotype of wheat (F103-L-1-12//PONY/OPATA, F1.158/FDL//BLO/3/SH14414/CROW/4/C ICWH99381-0AP-0AP-OMAR-6MAR, PYN/BAU//VORONA/HD2402, KATILA-13, and SARDARI- HD35/5/DMN//SUT/AG(ES86-7)/3/ICWH99-0552-0AP-0AP-OMAR-3MAR) factorialed in plots as random. Proteins profiling of samples was performed using SDS- polyacrylamide gels. The results showed that. In all genotypes the effects of drought stress on seed storage proteins were significant. Also results shows that, in drought stress treatment the seed storage proteins was more than irrigated treatment. No effects drought stress treatments on protein banding patterns. Also, results indicated that not obvious any new band and not deleted any bands. Therefore we concluded that seed protein bands are very stable in front of environment changes.

Key words: Protein and wheat genotype

Introduction

Water deficit 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). Decrease in growth rate is caused by reduction in radiation use efficiency when drought was imposed at various growth stages, such as tillering, booting, earing, anthesis, and grain development stages (Ashraf., 1998). Wheat (*Triticum aestivum* L.) 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 (Abd-ElHaleemet al., 2009). It is cultivated under rainfed conditions and the area near the tail end of canals where shortage of water is often experienced. The average yield of wheat is quite low in such areas, which is mainly due to shortage of water (Ashraf, 1998). 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). 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

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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). 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. 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). 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). This study was performed to study the effect of drought stress changes in protein of wheat genotypes.

Materials and Methods

In this study five wheat genotypes were planted in early November, growing season of 2008-2009. The soil texture of the research farm was sandy-loam. The experiment was carried out in a factotial design with three replications. Two drought stress levels (irrigated and Non irrigated) and five genotype of wheat (F103-L-1-12//PONY/OPATA, F1.158/FDL//BLO/3/SH14414/CROW/4/C ICWH99381-0AP-0AP-OMAR-6MAR, PYN/BAU//VORONA/HD2402 , KATILA-13, and SARDARI-HD35/5/DMN//SUT/AG(ES86-7)/3/ ICWH99-0552-0AP-0AP-OMAR-3MAR) factorialed in plots as random. The 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. 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

The results showed that, the effect of drought stress treatment on total seed protein was significant, but the other treatments were not significant on total protein. The comparison of the mean values of the seed protein showed that irrigated treatment has the highest seed protein and the Non irrigated treatment has the lowest seed protein and the differences between them were significant. Among the genotypes F103-L-1-12//PONY/OPATA genotype in Non irrigated condition has the highest seed protein and the OR F1.158/FDL//BLO/3/SH14414/CROW/4/C ICWH99381-0AP-0AP-OMAR-6MAR genotype in irrigated condition cultivar has the lowest seed protein. Similar results were reported by Kim et al., 1990; Suoyi Han et al, 2009. The seed storage proteins patterns of five wheat genotypes under Non irrigated and irrigated condition after electrophoresis are shown in Figure 1. In total 21- 30 bands (since below 14kDa until over 78kDa molecular weight band) per genotypes were detected in electrophoregrams. The SDS- PAGE

results revealed no effects treatment (water dificit) on the protein banding patterns but the related Non irrigated bands were chromatic, because they have high 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 in all treatments. 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). Also, we founded that in Non irrigated treatment protein percentage was more than irrigated condition but in irrigated condition total protein and protein yield were more than Non irrigatrd condition.

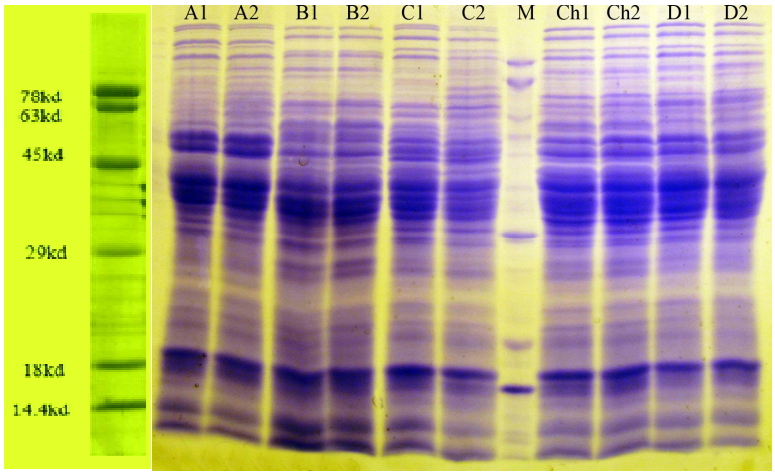

figure 1. Protein banding patterns in wheat genotypes under normal and drought stress conditions

Column name from left to right:
A1 and A2= F103-L-1-12//PONY/OPATA genotype under with and without drought stress respectively
B1 and B2= OR F1.158/FDL//BLO/3/SH14414/CROW/4/C ICWH99381-0AP-0AP-OMAR-6MAR genotype under with and without drought stress respectively
M= Marker
C1 and C2= PYN/BAU//VORONA/HD2402 genotype under with and without drought stress respectively
Ch1 and Ch2= KATILA-13genotype under with and without drought stress respectively
D1 and D2= SARDARI-HD35/5/DMN//SUT/AG(ES86-7)/3/ ICWH99-0552-0AP-0AP-OMAR-3MAR genotype under with and without drought stress respectively.

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