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Protein profile and seeds storage proteins changes in wheat genotypes under control and drought stress conditions

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This paper investigates drought stress (DS) impacts on the protein content and protein banding pattern of five wheat genotypes, planted at the research farm of Razi University, Kermanshah, Iran, in early November, during the 2008-2009 growing season. The study included a factorial design experiment with three replications. Two DS levels, including irrigated and non-irrigated, and five wheat genotypes, including Zarin, CA8055//KS82W409/STEPHENS. Bolani, Shahriar, and WS-82-9, were randomly factorialed in plots. The study used SDS- polyacrylamide gels to profile the samples' proteins. The results indicated the considerable impact of DS on the sample's seed storage proteins: drought stress had a positive relationship with seed storage proteins as an increase in the former induced an increase in the latter. In contrast, increased drought stress did not influence protein banding patterns since increased stress neither formed new bands nor eliminated any current band.

Keywords: Drought stress, Electrophoresis, Protein, Wheat

INTRODUCTION

A critical cereal crop and a major food source for over one-third of the world population, Wheat (*Triticum aestivum* L.) has a far more critical share in providing calories and protein than other similar crops (Khayatnezhad and Gholamin, 2021a, Karasakal et al. 2020b, Huang et al. 2021). It is cultivated under rainfed conditions and near the tail end of canals, often under water-related stresses that result in lower average wheat yield (Khayatnezhad and Nasehi, 2021, Si et al. 2020, Ren and Khayatnezhad, 2021).

Water stress is the most critical and influential environmental type of stress in farming worldwide. Accordingly, plant breeders attempt to find new ways to increase yield under drought (Alayi et al. 2020, Arjaghi et al. 2021). Adequate water at or after the anthesis period not only allows the plant to increase (Aletor, 2021).

However, the photosynthesis rate provides additional time for carbohydrate translocation to grains (Jia et al. 2020, Gholamin and Khayatnezhad, 2020a), improving grain size and increasing grain yield. The decline in radiation use efficiency under drought stress hampers the growth rate. This hindering effect occurs when drought stress is implemented in various stages, such as tillering, booting, earing, anthesis, and grain development (Barth, 2021, Hewitt, 2021).

Various factors influence seed protein content and baking quality highly, including heritage and environmental elements, particularly drought stress and heat stress during the grain-filling period (GFP), and the crops access to nitrogen content (Khayatnezhad and Nasehi, 2021, Karasakal et al. 2020a, Li et al. 2021, Huma et al. 2021, Esmaeilzadeh et al. 2020). Proteomic tools have recently become popular, thanks to the high accuracy and strong performance to detect and monitor protein composition changes (Gholamin and Khayatnezhad, 2020d). Storage proteins can be utilized to develop different methods for studying genetic diversity and dividing plants into varieties (Khayatnezhad and Gholamin, 2021b, Sun et al. 2021, Farhadi et al. 2020). Variations in environmental conditions can rarely influence seed protein, and they have highly stable banding patterns. This stability can be a competent tool for identifying optimum cultivars, even as an effective supplemental identification method, especially in cases of legal disputes over a cultivar's identity or parenting cultivars (Khayatnezhad and Gholamin, 2020a, Gholamin and Khayatnezhad, 2020c, Fataei, 2017, Ghomi Avili and Makaremi, 2020). Seed storage protein is an effective tool to investigate the genetic diversity of wild and cultivated rice (Khayatnezhad, 2012, Si et al. 2020, Fataei et al. 2018). Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is a highly cost-effective, straightforward, and popular biochemical technique to study the germplasm's genetic structure (Zhu et al. 2021, Khayatnezhad and Gholamin, 2021a, Huang et al. 2021, Ren and Khayatnezhad, 2021). The significant independence from the effects of variations in the environment makes profiling seed storage proteins using the SDS-PAGE technology a unique tool for germ plasm's economic characterization (Khayatnezhad and Gholamin, 2012b, Kabir et al. 2021). During the seed development stage, crops store many compounds and specific proteins, but a particular feature in this process is the accumulation of these substances in high amounts (Gholamin and Khayatnezhad, 2020b).

Research farms and independent breeders actively attempt to develop new methods and techniques to enhance seed storage protein (Khayatnezhad and Gholamin, 2020b, Radmanesh, 2021). Proteins are responsible for many essential cellular activities, and protein changing makes possible morphological changes in plants (Muhibbu-din, 2020). Even though protein composition response to environmental factors in mature wheat grain derives from growth-stage changes in protein deposition, researchers have rarely addressed the impacts of water stress and nitrogen fertilizer on grains' protein profiling (Rodríguez, 2021). For gave to highest seed yield in agriculture addition to both nitrogen and phosphate fertilizer is very important (Khayatnezhad and Gholamin, 2012a). For gave the highest seed yield and protein yield in rapseed (Ren and Khayatnezhad, 2021) and maize (Huang et al. 2021, Yin et al. 2021, Sun and Khayatnezhad, 2021, Khayatnezhad and Gholamin, 2021b, Gholamin and Khayatnezhad, 2021) should apply both nitrogen and phosphate biofertilizers. Besides, the results of many studies have indicated the significance of irrigation at branching, anthesis, and pod formation stages and its positive correlation with a higher yield (Omrani and Fataei, 2018). Considering what was mentioned above, this paper deals with the impact of drought on protein content and protein banding pattern in five wheat genotypes.

MATERIALS AND METHODS

Experimental design

During the 2008-2009 period, the researchers planted five wheat genotypes in early November, at the Research Farm of Razi University in Kermanshah (latitude 34°20´ N, longitude 46°20´ E, altitude 1351.6 m above sea level), Iran. The seeds were provided by Dryland Agricultural Research Institute and Agricultural and Natural Resources Research Center. Kermanshah is located west of Iran and has an average annual temperature of 13.8°C and has an annual precipitation of 478 mm. The soil used in the study had a sandy-loam texture, and the experiment had a factorial design with three replications. The samples underwent with and without irrigation stress treatments. The selected wheat genotypes were Zarin, CA8055//KS82W409/STEPHENS. Bolani, Shahriar, and WS-82-9, which were factorialed in plots as random. The researchers used Mancozeb to pretreat the genotypes to ensure a minimum soil-borne disease probability before sowing them in five 3m long rows, spaced 20 cm apart. The final stand density was set to be 400 plants per m2.

Seed protein and electrophoresis

The study used mortar to powder one seed was, and 10mg (0.01g) of this seed powder was poured into a 1.5ml micro-tube. 400µl of the protein 10% glycerol, 5% β-mercaptoethanol, 5 M urea, and 0.0001% bromophenol blue) was added and mixed well by vortexing. The crude homogenates were centrifuged at room temperature at 13000 RPM for 20 min using a micro-centrifuge machine. The supernatant was detached and used for protein profiling. Following Bradford (1976), the study used a dye-binding assay to measure extracts' protein concentration. The supernatant was mixed (4:1) with cracking solution (10 ml containing 1g SDS,0.01g bromophenol blue, 2ml β-mercaptoethanol, 1.5ml 0.5*M* tris, pH 6.8, 5g sucrose, and 6.5 ml water) on vortex mixer followed by heating in a boiling water bath for five minutes to denature the proteins. Follwoing Laemmli (1970), the researchers used SDS- polyacrylamide gels to profile the proteins. An amount of 150 μg of proteins from all samples and protein molecular weight markers were loaded into 10% gels later to undergo electrophoresis at a constant voltage of 100 volts, followed by a 45-minute dyeing stage in coomassie blue G-250. Then, gels were kept in 10% Acetic acid and 40% ethanol solution overnight while being constantly agitated on a shaker. Finally, the gels were rinsed distilled water for a quarter, during which water was changed every one-third of a quarter.

RESULTS AND DISCUSSION

Seed storage proteins

Although inducing DS had a considerable impact on seed protein at the 1% level, other treatments were non-significant. Comparing the seed protein's mean values indicated that the DS-treated cultivar had the highest seed protein, and the control treatment had the lowest with a considerable difference. Among the genotypes treatments, the following genotype represented the best response to DS in terms of seed protein: CA8055//KS82W409/STEPHENS. In contrast, the Bolani genotype in the control condition displayed a disappointing performance in terms of yield, and the difference was notable, which correlated with the reported results of Kim et al. (1990) and Suoyi Han et al. (2009).

SDS-PAGE Protein Analysis

Fig. 1 illustrates five wheat genotypes' seed storage protein patterns under drought stress and control conditions after electrophoresis. Electrophoregrams revealed a total of 22- 29 bands (from below 14kDa until over 78kDa molecular weight band) per genotypes. Based on the SDS- PAGE results, drought stress had a non-significant impact on the protein banding patterns, but the related sever drought stress bands were chromatic since they had the highest protein concentration (Fig.1). These results were correlated with other researches (Gholamin and Khayatnezhad, 2012, Zaeimdar et al. 2019, Khayatnezhad and Nasehi, 2021, Sun et al. 2021, Bi et al. 2021). Nevertheless, the reported results did not show a clear formation of a new band or the removal of the existing bands, indicating that grain protein banding pattern in of more crops is very stable and not sensitive to environmental changes (Gholamin and Khayatnezhad, 2021, Xu et al. 2021, Ma et al. 2021).

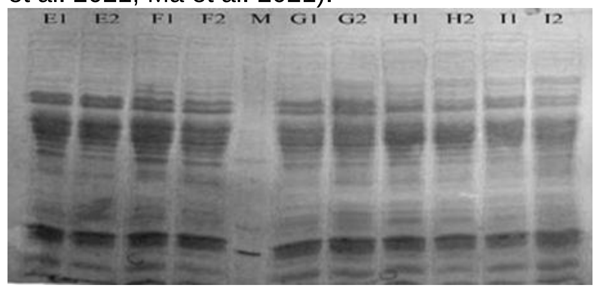

Figure 1: Protein banding patterns in wheat genotypes under normal and drought stress conditions

Column name from the left to right

E1 and E2= Zarin genotype under and without drought stress, respectively

F1 and F2= CA8055//KS82W409/STEPHENS genotype under and without drought stress, respectively

M= Marker

G1 and G2= Bolani genotype under and without drought stress, respectively

H1 and H2= Shahriar genotype under and without drought stress, respectively

I1 and I2= WS-82-9 genotype under and without drought stress, respectively.

CONCLUSION

Drought stress had a positive relationship with seed storage proteins as an increase in the former induced an increase in the latter. In contrast, increased drought stress did not influence protein banding patterns since increased stress neither formed new bands nor eliminated any current band.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Mani Alizadeh conducted, planned, Analyzed the data, wrote manuscript and interpreted the results and involved in manuscript preparation. All authors read and approved the final version.

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