Short Communication

Grouping of bread wheat cultivars by seed storage proteins

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Abstract

To determine seed storage protein banding patterns in some bread wheat cultivars and the similarity of banding patterns among different cultivars, an experiment based on seed storage protein electrophoresis (albumin and globulin) was performed. Water and salt soluble proteins were extracted in sixteen wheat cultivars using polyacrylamide gel electrophoresis and banding pattern was obtained. Studied cultivars were Karaj 3, Atila 50, N-8019, Khazar 1, Shahriar, Darya, Chenab, Kouhdasht, Augusta, Toos, Cros shahi, C-845512, Saysons, Ghermezak, Sardari and Tajan. Based on dendrogram, sixteen wheat cultivars were placed in four groups. Cultivars that were placed together in a group were more similar than the others considering morphological characteristics and growth habits. Electrophoretic patterns of seed albumin and globulin proteins in sixteen wheat cultivars showed that these sixteen cultivars are different in terms of protein banding patterns. It means that the albumin and globulin can be used in genetic evaluation to evaluate genetic distances and identify the cultivars. It can also be used for genetic evaluation of seed storage proteins, including investigation of genetic distances and proximity between species and cultivars.

Keywords: albumin, storage protein, electrophoresis, globulin, bread wheat

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Introduction

Wheat (*Triticum aestivum* L.) is the most important crop in the world considering cultivation and consumption. Wheat is the staple food for about 35 percent of the world population and the demand for it has expanded more than any other crop (Rajaram, 2000). Wheat endosperm proteins were among the first proteins to be studied

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when Beccari in 1745 reported the isolation of gluten. Later, storage proteins from rye and barley were isolated. At the beginning of the 20th century, a systematic study was conducted by Osborne (1907) to develop a classification for cereal-seed proteins based on their sequential extraction and differential solubility. He classified wheat proteins into four different groups, albumins (soluble in water and dilute buffers), globulins (not soluble in water but soluble in saline solutions), prolamins (which are soluble in 70–90% ethanol), and glutelins (which are soluble in dilute acid or alkali). Albumin and globulin comprise about 12% and 5% of the total protein in wheat, respectively (Chen and Bushuk, 1970). They are found mainly in the embryo and seed aleurone layer (Payne et al., 1982). Gliadin and glutenin are major storage proteins of the wheat endosperm. Each of them, constitute approximately 40% of total seed protein (Khan and Bushuk, 1979). In the study of wheat proteins, Osborne nomenclature and solubility criteria have been used so much although the solubility of wheat proteins by chromatographic electrophoresis is greatly different (Kelly and Koenig, 1962). Many of albumins and globulins are enzymes or enzyme inhibitors. For example, wheat albumin acts as inhibitor of the alpha-amylase enzyme (Onoshin et al., 2006). Alpha-amylase/trypsin, serpins and purothionins are predominant albumins and globulins. These predominant albumins and globulins serve as nutrient reserves for the germinating embryo. Secondly, they also help in protecting embryo from insects and pathogens before germination (Dupont and Altenbach, 2003). Their molecular weight (MW) in SDS-PAGE is lower than the gliadins (less than 3000 Daltons) (Gianibelli et al., 2001). The molecular weights of albumins and globulins are mostly lower than 25000, although a significant proportion of the proteins have MW between 60,000 and 70,000 (Veraverbeke and Delcour, 2002). Components of albumin family, including 0.19, 0.28, 0.36 and 0.53 are divided based on electrophoretic mobility index. Albumin 0.19 is the most abundant (Onoshin et al., 2006). In amino acid composition, they are different from gluten, in terms of having a lower content of glutamic acid and more lysine. In fact, due to the presence of lysine, this group of proteins has an amino acid composition that is appropriate to the needs of both humans and monogastric animals. Unfortunately, because they are a small part of the wheat endosperm, their presence is not sufficient to overcome the deficiency of lysine in wheat flour (Gianibelli et al., 2001).

Researchers now widely use electrophoresis techniques as a useful tool for separating proteins and other molecules, such as DNA and RNA to study the genetic diversity in plants. Study of genetic diversity and determination of potential genetic resources using customary methods of field is very laborious and time consuming and a large area of land is allocated to it. Scientists have presented electrophoresis method as a powerful technique for detection and differentiation of the seed protein as an alternative to the previous methods (Cooke, 1988). Boulter et al. (1966) studied the application of protein banding patterns in plant systematic patterns. Ladizinsky and Hymowitz (1979) have investigated the seed protein electrophoresis with a new approach and they pointed out some of the benefits of protein banding patterns such as stability of storage proteins. Gardiner and Forde (1988) identified pasture legumes species and cultivars by seed protein electrophoresis. Sengbush (1983) believed that since the morphological characteristics are the final products of the genes and follow a complex biochemical pathway, they are affected by a series of genes and external stimuli. Therefore, it is appropriate to study the seed protein contents that are the primary

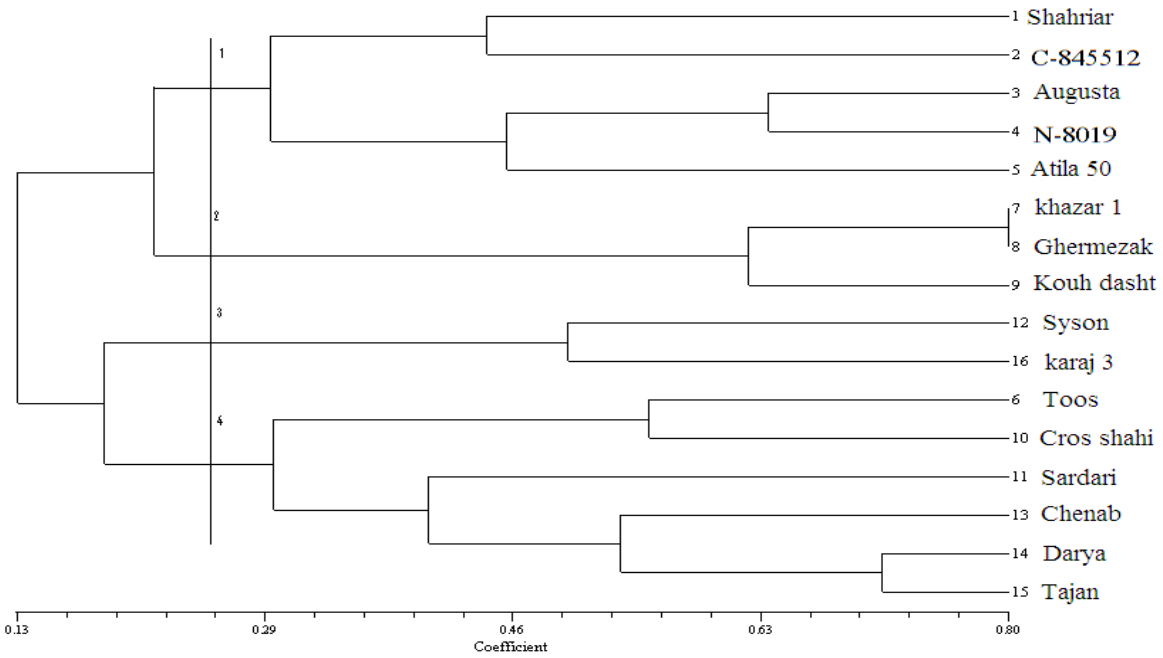

Fig. I. Dendrogram resulted from cluster analysis of electrophoretic data for sixteen wheat cultivars

product of genes. The aim of this study was to determine the banding patterns of seed storage proteins (albumin and globulin) of different bread wheat cultivars and the similarity of banding patterns among them.

Material and Method

Seeds of sixteen wheat cultivars including Karaj 3, Atila 50, N-8019, Khazar 1, Shahriar, Darya, Chenab, Kouhdasht, Augusta, Toos, Crosshahi, C-845512, Sayson, Ghermezak, Sardari and Tajan were obtained for the study. Salt and water soluble proteins were extracted using the method described by Laemmli (1970). In order to extract the desired proteins, the seeds were crushed in a porcelain mortar. Then 0.04 g of the obtained powder from the seeds was mixed with 1500 µL of the extraction buffer to obtain the desired protein extracts. Samples were placed in the freezer for 15 minutes after vortex. Then, they were melted in the laboratory temperature for 15 minutes. This procedure was repeated three times and then the samples were centrifuged at 10,000 rpm for 10 min to obtain clear supernatant. Then 300 µL of clear supernatant was removed from the solution and was mixed with 100 ml (about 3 to 1) staining solution of water and salt. Discrimination of protein subunits was performed based on Lawrence and Shepherds (1981) using one-dimensional SDS-PAGE. The lower and upper gels (separating gel) were prepared with a concentration of 10% and then 30 µL of extracted seeds were placed in the gel wells. Electrophoresis was performed with 30 mA current. Proteins were separated based on differences in molecular weight. Staining was performed overnight. Protein bands were valued based on Payne et al. (1981a). In this catalog, the highest band is named by the first number and subsequent numbers were used for other bands. Dendrogram was constructed from the data using NTSYS software.

Results

Cluster analysis based on water and salt soluble proteins (albumin and globulin)

separated the sixteen cultivars of wheat into four distinct groups. Cultivars in the first group were Shahriar, C-845512, Augusta, N-8019, and Atila 50. The cultivars appeared in the second group were Khazar 1, Ghermezak, and Kouhdasht. The third group included Karaj 3 and Saysons. Finally, the fourth group consisted of the cultivars Crosshahi, Sardari, Chenab, Darya, Tajan and Toos (Fig. I). Cultivars placed together in one group may have more similarity in terms of morphological characteristics and growth habits than the others.

Discussion

Today, evaluation of cultivars for purity using protein markers provides the possibility of obtaining more accurate information. Proteins as primary products of possible genes or QTLs are characterized by genome and governed by genetic factors, reflecting the specialized nature of genetic systems. Therefore, they are used as effective indicators to determine the genotype and evaluate the genetic structure of the species and cultivars (Konarev, 1996).

Electrophoretic pattern of albumin and globulin proteins of wheat seeds in sixteen cultivars (Fig. II) showed that these cultivars are different, in terms of diversity of protein bands, that is albumin and globulin can be used in genetic evaluation to assess the genetic distance and proximity or remoteness of the species and cultivars relative to each other.

Protein polymorphism that has been created under the control of alleles can be clearly visible and identifiable by means of electrophoresis. Among the various proteins, seed storage proteins especially gliadins, have been identified as the best protein indicator for variety identification, identification of biotype and purity of the wheat cultivars due to high levels of polymorphism, ease of extraction and electrophoretic analysis of its component parts (Sozinov, 1979).

Electrophoretic pattern of proteins like albumin in seeds of six cultivars of wheat, two cultivars of barley and one cultivar of corn generally showed that albumin can be used in taxonomic and systematic studies to assess the position of different taxa as a taxonomic indicator and also can be used in genetic evaluation to assess the genetic distance and proximity or remoteness of the species and cultivars relative to each other

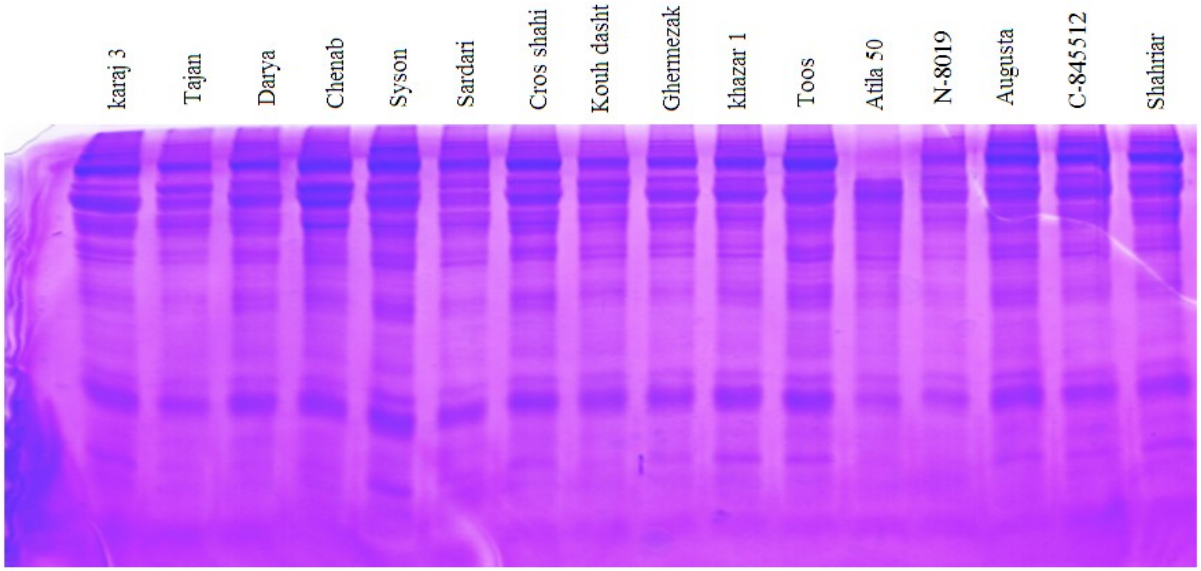

Fig. II. proteins bands from electrophoresis of salt and water soluble proteins in sixteen bread wheat cultivar

(Heidarizadeh, 2006). According to a study conducted on mung bean, selection based on protein patterns of albumin and globulin in the early stages of breeding programs could be effective in increasing the yield and early maturity (Ghavami, 2001). Albumin and globulin have been used in some studies as specific genome markers for chromosomes of wheat (Singh, 2001). High concentration of albumin is inversely related to baking quality. It also affects the quality of the pasta. Beta-amylases which are of the albumin protein have disulfide bonds with low molecular weight (LMW) glutenin subunits. LMW glutenin subunits are associated with weak dough properties (Bushuk, 1994).

The seed storage protein pattern is considered as the genotypic fingerprint. It is, therefore, used for several purposes such as plant variety protection, registration, certification, patents and as a breeding tool especially in flour quality breeding programs.

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