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Full Length Article

Diversity in Seed Storage Proteins in Maize Genetic Resources: I. Variation in Alcohol Soluble Zein Protein Fraction

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Abstract

Being predominant protein of maize seed, its nutritional quality is dependent on composition of zein polypeptides. The major storage protein of maize seed is alcohol soluble zein, which accounts for more than 50% of total seed storage protein. The variation of zein fraction of seed storage protein in maize genetic resources was assessed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Variation in terms of absence and presence, intensity and molecular size was observed in zein polypeptides. On the basis of variation in banding patterns of zein polypeptides, the maize genetic resources were classified into five variation groups which highlight the wide variation in zein polypeptides among maize genetic resources. The group 2 exhibited high accumulation of 70 and 60 kDa zein polypeptides and group 3 exhibited high accumulation of 25 and 20 kDa zein polypeptides as compared to group 1, 4 and 5.These maize genetic resources might have significant implications for nutritional quality improvement of maize.

Keywords: Maize germplasm; Seed storage protein; Zein protein; Maize genetic resources

Introduction

Maize (*Zea mays* L.) is the world's third leading cereal crop, after wheat and rice (Sleper and Poehlman, 2006). It provides bulk of raw material for the livestock and many agro-allied industries in the world (Bello *et al*., 2010; Randjelovic *et al.,* 2011) and contains carbohydrates, protein, fat, vitamins (A, B, E) and also some important nutrients for metabolism (Orhun, 2013).

Maize seed consists of two types of protein i.e., zein and non-zein protein. The term zein is used for prolamins in maize which is alcohol soluble protein and could be extracted with ethanol (Wallace *et al*., 1990; Lawton, 2006). Zein is major seed storage protein of maize (Freitas *et al*., 2005) and consists of one major and three minor classes and these four classes constitute approximately 50-70% of maize endosperm (Vasal, 1999). The non-zein protein consists of globulins (3%), glutelins (34%) and albumins (3%). Zein is specific to maize endosperm (Prasanna *et al*., 2001) and not present in any other part of plant.

As maize is deficient in essential amino acids such as lysine, tryptophan and methionine due to higher proportion of prolamins therefore, the nutritional profile of maize is poor (Sofi *et al*., 2009). To improve the nutritional value a natural mutant, opaque 2 (o2) was found which contains higher non-zein protein and also a higher amount of lysine but lower amount of zein (Wu *et al*., 2010).

However, to broaden the genetic base for nutritional quality improvement of maize seeds it is imperative to assess the diversity of zein polypeptides in maize germplasm.

Germplasm are a vital source of gene and play key role in crop improvement. The genebank of Plant Genetic Resources Institute has preserved the germplasm of maize collected from various agro-ecological and geographical zones of Pakistan. But so far these germplasm are not characterized for variation in zein polypeptides. Therefore, current study assessed the diversity of zein polypeptides in maize germplasm and found wide variation in zein polypeptides. The maize germplasm were classified to various groups on the basis of variation in zein protein. The variation found in zein polypeptides in maize accessions will be quite helpful for nutritional quality improvement of maize.

Materials and Methods

Plant Materials

The plant materials used in the present study were 50 accession of maize germplasm acquired from the genebank of Plant Genetic Resources Institute, National Agricultural Research Centre, Islamabad. The detailed information about plants materials are given in Table 1.

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Table1: List of maize accessions used in the present study and their collection sites

Accession no.	Province
014856	Punjab
014857	Punjab
014858	Punjab
014860	Punjab
014861	Punjab
014862	Punjab
014864	Punjab
014865	Punjab
014868	Punjab
014869	KP
014871	Balochistan
014872	Balochistan
014873	Balochistan
014876	Balochistan
014890	Balochistan
014905	Balochistan
014908	Balochistan
014917	Balochistan
014918	Balochistan
014919	Balochistan
014922	Balochistan
014925	Balochistan
014927	Balochistan
014929	Punjab
014930	Sindh
014931	Sindh
014932	Sindh
014933	Sindh
014935	Sindh
014944	AJK
014949	AJK
014957	AJK
014960	AJK
014963	AJK
014971	AJK
014992	AJK
014994	AJK
014997	AJK
014998	AJK
014999	AJK
015004	AJK
015005	AJK
015008	AJK
015014	Punjab
015015	Punjab
015016	Punjab
015018	N/A
015019	N/A
015023	Gilgit
015025	Gilgit

Extraction of Zein Proteins

About 3-4 seeds were taken, crushed and ground to fine flour. About 100 mg (0.1 g) of flour was taken in 1.5 mL eppendorf tube. About 500 µL (100% cold acetone) was added and vortexed (2 min). Then sample tubes were placed on vibrator at 4ºC for 1 h and centrifuged (12000 rpm, 10 min). After centrifugation, the supernatant was removed and 1 mL 0.5M NaCl was added to the sample tubes and vortexed. Again the sample tubes were placed on vibrator (4ºC, 1 h) and centrifuged (12000 rpm, 10 min). Then the supernatant was removed and1 mL 70% ethanol and 5% mercaptoethanol was added. The samples were vortexed and incubated (3 h, 60ºC). After centrifugation (12000 rpm, 10 min) about 30 µL supernatant in a fresh tube was taken and mixed with 30 µL of extraction buffer (4.5% SDS, 1.8 M Tris, 5% 2-marcaptoethanol, 10% glycerol and Dye 0.05 g) for sample preparation. The samples were boiled (5 min), centrifuged (1 min) and vortexed (15 s).Then the samples were subjected to SDS-PAGE analysis.

Electrophoresis

Zein protein were analyzed on 10% polyacrylamide gel using SDS-PAGE mini gel apparatus AE-6530, Atto Japan according to the procedure described by Khan *et al*. (2010). Electrode buffer (0.025 M Tris, 0.129 M Glycine, 0.125% SDS) were used for electrophoresis. Electrophoresis was done at 100 V until the blue dye reached the bottom of gel.

Fixing, Staining and Destaining

After electrophoresis, the gel was placed in a fixing solution (acetic acid-methanol-water 10:10:80) for 1 h. Then the gel was stained in staining solution (Methanol- acetic acid – water –coomassie brilliant blue 44:6:50:0.2 g) for 40 min. The gel was destained in destaining solution (Methanol-acetic acid-water 20:5:75) until the blue background disappeared and the bands become visible.

Results

Zein protein extracted from the maize kernel was detected on the basis of molecular weight into six different size (70, 60, 25, 20, 15 and 10 kDa) bands. The zein protein was classified into five different groups on the basis of variation in size, intensity and presence/absence of bands (Fig. 1). We classified maize germplasm into various groups based on variation in zein polypeptides (Fig. 3).

The group 1 includes maize accessions with 70, 60, 25, 20, 15 and 10 kDa molecular weight bands. In this group, 10 kDa band found was absent in remaining four groups. It contains a high intensity band of 25 kDa and medium intensity band of 70, 60, 20, 15 and 10 kDa. The group 2 includes those accessions, which contain 70, 60, 25 and 20 kDa molecular size bands. In this group, 70 and 60 kDa bands were of high intensity and bands of 25 and 20 kDa of medium or light intensity. In this group, 15 kDa and 10 kDa band were absent and present in group 1. The group 3 includes those accessions which contain 70, 60, 25 and 20 kDa size bands but this group is opposite to group 2, because it contains high intensity bands of 25 and 20 kDa and medium or light bands of 70 and 60 kDa. In this group, 15 and 10 kDa bands were absent and majority of accessions belong to this group. The group 4 includes accessions containing 70 and 60 kDa size bands; however,

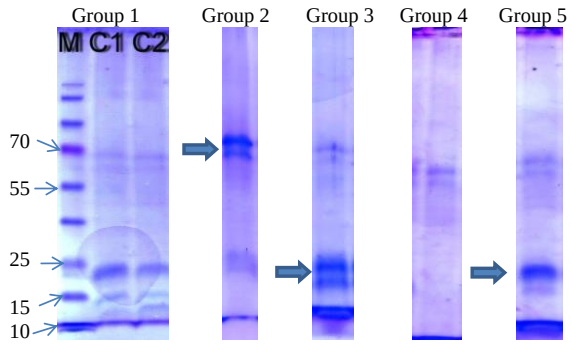

Fig. 1: M=Protein ladder, C1= Check 1 (Azam), C2= Check 2 (Ev1097). Groups1-5 represents various zein variations groups. Arrow indicates the high intensity band.

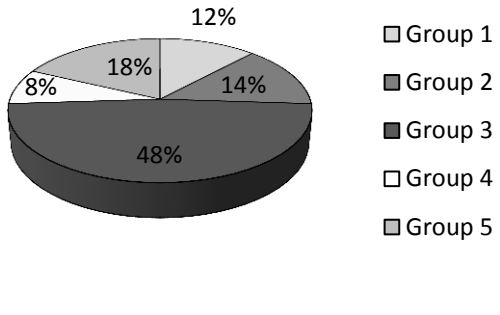

Fig. 2: Frequency distribution of zein variation groups in different maize accessions.

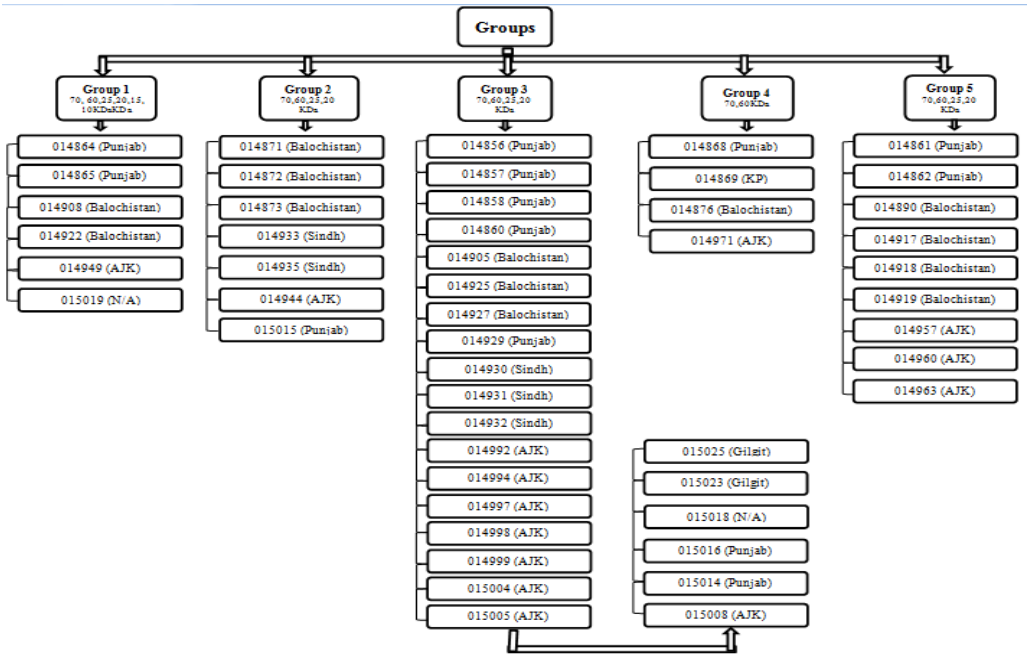

Fig. 3: Groups on the basis of variation inzein protein with respective molecular sizes of zein proteins detected in each group. The maize accessions falling in various groups are indicated with their respective provinces

in this group 25, 20, 15 and 10 kDa size bands were absent. It contains medium size bands of 70 and 60 kDa. The group 5 includes 70, 60, 25 and 20 kDa size bands. In this group, 25 kDa band is of high intensity than the remaining three bands (70, 60 and 20 kDa). This group contains medium intensity bands of 70, 60 and 20 kDa. The bands of 15 and 10 kDa were absent in this group.

The frequency distribution revealed that maximum accessions fall in group 3 (48%) and minimum accessions fall in group 4 (8%). The remaining accessions fall in group 1 (12%), group 2 (14%) and group 5 (18%) (Fig. 2).

Discussion

Seed storage proteins are less affected by the environment and its analysis using SDS-PAGE are considered an important tool to assess the diversity among crop species (Khurshid and Rabbani, 2012). In the present study, 50 accessions and two checks (Azam and Ev1097) of maize were studied and classified into five groups on the basis of variation in zein protein. Variation in terms of absence and presence, intensity and molecular size was observed in zein polypeptides. The classification of zein polypeptides into five distinct variation groups highlights the wide variation in zein polypeptides among maize genetic resources. The group 2 and 3 exhibited high accumulation of zein polypeptides as compared to group 1, 4 and 5. These maize genetic resources might have significant implications for nutritional quality improvement of maize. Lysine is essential amino acid which is needed for human and animal health. As maize is deficient in essential amino acids such as lysine due to higher proportion of prolamins (Zein)

therefore, the nutritional profile of maize is poor (Sofi *et al*., 2009). To improve the nutritional value a natural mutant, opaque 2 (o2) was found which contains higher non-zein protein and also higher amount of lysine but lower amount of zein (Wu *et al*., 2010). In the above reports, it was found that the lysine content increases with the decrease of zein content. As the zein content is low in group 4 therefore, there might be possibility of high lysine content in these germplasm.

Beside this, we found novel bands of 60 kDa and 70 kDa which are not previously reported (Freitas *et al*., 2005). Therefore, more thorough study is needed to further analyze the lysine content of maize germplasm falling in group 4 and characterize the novel bands observed in this study which might have significant implications for nutritional quality improvement of maize.

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