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Variation of storage proteins and isozymes within maize inbred lines

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Abstract

Seed storage proteins of ten maize inbred lines were investigated by sodium dodecyl sulfate polyacrylamide gel electrophoresis. In addition, fourteen isozyme loci representing nine isozyme systems were analysed. Salt-soluble protein fraction contained a large number of proteins (30 - 40 bands) of different sizes with genotypic differences among the ten inbred lines. The methionine-rich 10 kD zein showed differential expression in the ten inbred lines with different migration rates on the SDS-PAGE. This polypeptide was completely absent in the inbred line G221D. Among nine of the inbred lines, eight of 14 isozyme loci were polymorphic and six were monomorphic resulting in seven unique fingerprints.

Additional key words: isozyme polymorphism, maize genotypes, SDS-PAGE, *Zea mays* L., zein.

Introduction

The storage proteins of maize seed have been classified according to their solubility into several fractions: albumins, globulins, prolamins and glutelins. Albumins and globulins are the protein fractions soluble in water and salt solutions. The prolamin proteins of maize seed are traditionally called zeins (Osborne 1987). Analysis of these proteins by SDS-polyacrylamide gel electrophoresis typically reveals a mixture of polypeptides ranging in size from 27 to 10 kD. Landry and Moureaux (1970) separated the glutelin fraction (*i.e.* proteins remaining in the residue after extraction by water, salt solutions and alcohols) into three components. These components are; zein-like which is soluble in alcohol plus β-mercapto-ethanol, glutelin-like soluble in alkaline buffer with β-mercaptoethanol and glutelin residue soluble in alkaline buffer with β-mercaptoethanol plus sodium dodecyl sulphate detergent. The zein-like fraction had its name by Sodek and Wilson (1971) because of its high content of zein-like polypeptides, although it also contained polypeptides different from classical zein. This fraction was also named alcohol-soluble reduced glutelin (ASG) with a subsequent division into water-soluble and water-insoluble fractions (Paulis and Wall 1977).

In addition to seed protein electrophoretic profiles, isozyme markers have been widely used in determining genetic variability in many plant species and in characterizing and identifying inbred lines and cultivars, (Goodman and Stuber 1980, Cardy and Kannenberg 1982, Smith *et al.* 1985, Smith and Smith 1987, Smith 1988).

The present work is an attempt to separate the salt soluble proteins and zeins of the maize seed storage proteins from ten Egyptian maize inbred lines, and to assess the genotypic variation among these lines on the basis of the electrophoretic analysis of the seed storage proteins and isozymes.

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Abbreviations: ACP - acid phosphatase; ADH - alcohol dehydrogenase; β-GLU - β-glucosidase; GOT - glutamic-oxalacetic transaminase; IDH - isocitrate dehydrogenase; MDH - malate dehydrogenase; 6-PGD - 6-phosphogluconate dehydrogenase; PGM - phosphoglucomutase; PHI - phosphohexose isomerase.

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Materials and methods

Seeds of ten Egyptian maize (*Zea mays* L.) inbred lines (Table 1) were kindly provided by the Maize Research Section, Agricultural Research Center, Giza, Egypt.

Table 1. Maize inbred lines examined in the present study.

Inbred	Germplasm source
G12	American Early Dent
Rg15	synthetic Laposta × Ci64 (Sc14)
G336	H3091469, Mexico (locally bred)
G221D	Early White composite
In171	CM 104, India.
Rg58	synthetic Laposta × G307 (Sc14)
Rg59	synthetic Laposta × G307 (Sc14)
Rg60	synthetic Laposta × G307 (Sc14)
Rg61	synthetic Laposta × G307 (Sc14)
Rg62	synthetic Laposta × G307 (Sc14)

Salt soluble proteins and zeins were extracted from finely ground meal using a modified procedure of Landry and Moureaux (1970). One part of the meal was mixed with five parts of 0.5 M NaCl and the mixture was stirred for 30 min at 4 °C, then centrifuged at 5 000 *g* and the extract saved. The residue was treated again with the same volume of 0.05 M sodium phosphate, pH 7.8, containing 0.5 M NaCl and 0.01 M EDTA and stirred for another 30 min at 4 °C to extract salt-soluble proteins. Zein fraction was extracted by treating the residue with five volumes of 70 % isopropanol and 0.5 % β-mercapto-ethanol at room temperature.

Samples for electrophoresis were prepared by precipitating salt-soluble proteins with five volumes of cold acetone at -20 °C for 2 h. The zein fraction was precipitated from alcoholic solution by addition of two volumes of 4 % NaCl. Pellets obtained after centrifugation at 7 500 *g* for 20 min were dissolved in 25 - 50 mm^3 of sample buffer (0.125 M Tris/HCl, pH 6.8, 2 % (m/v) SDS, 10 % (m/v) sucrose, 1 % (v/v) β-mercaptoethanol, 0.1 % (m/v) bromophenol blue) and denatured by heating at 80 °C for 3 - 5 min. 12 % polyacrylamide slab gels (0.1 × 18.0 × 18.0 cm) were prepared and equal amounts of proteins were loaded. SDS-polyacrylamide gel electrophoresis of proteins was performed according to Laemmli (1970).

Starch gel electrophoresis was performed for the detection of nine isozyme systems according to Stuber *et al.* (1988). The enzymes analyzed were acid phosphatase (ACP), alchol dehydrogenase (ADH), β-glucosidase (β-GLU), glutamic-oxalacetic transaminase (GOT), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), phosphoglucomutase (PGM), 6-phospho-gluconate dehydrogenase (6-PGD) and phosphohexose isomerase (PHI).

Results and discussion

Salt soluble proteins and zeins were extracted and analyzed using SDS-PAGE. The salt-soluble protein fraction of the ten inbred lines revealed great variability reflecting the genotypic differences (Fig. 1). This fraction

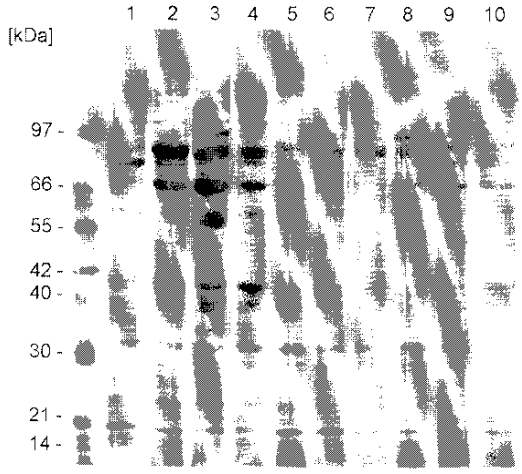

Fig. 1. SDS-PAGE of salt-soluble proteins extracted from maize inbred lines G12, Rg15, G336, G221D, In171, Rg58, Rg59, Rg60, Rg61, and Rg62.

was composed of 30 - 40 bands with a number of major bands ranging from 4 to 13 in Rg58 and G336, respectively. Four major bands of about 57, 28, 23 and 14 kD, were present in all the lines. Genotypic variability expressed as variation in protein banding pattern was more prominent in two regions spanning a molecular mass range of 66 - 45 kD and 29 - 22 kD. Inbred lines Rg61 and Rg62 showed almost similar banding patterns, they differed only in a few minor bands. This could be due to the fact that these two inbred lines were developed from the same germplasm source. Although lines Rg58, Rg59 and Rg60 are also related to Rg61 and Rg62, however, they exhibited great heterogeneity in their protein banding patterns. This might suggest that Rg61 and Rg62 are more closely related than the other three inbred lines. Similarly, the protein profile of Rg15 showed considerable variations when compared to the previous Rg lines although they share a considerable part of their genetic background (Table 1). Each of the remaining lines revealed a characteristic banding pattern reflecting its genetic background. Line G336 was unique in its banding pattern as it contained the highest number of major bands.

Table 2. Allele designation at 14 isozyme loci in nine maize inbred lines.

Inbred line	MDH			6-PGD		PHI	ACP	β-GLU	PGM		IDH		GOT	ADH
	1	2	3	1	2	1	1	1	1	2	1	2	1	1
Rg15	6	6	16	3.8	5.5	4	4	7	9	4	4	6	4	4
G336	6	6	16	3.8	5.5	2	2	2	9	4	4	6	6	4
G221D	6	6	16	3.8	5.5	2	2	2	9	4	8	6	4	4
In171	6	3.5	16	3.8	5.5	4	2	2	9	4	4	4	4	4
Rg58	6	6	16	3.8	5.5	2	2	7	16	4	4	6	4	4
Rg59	6	6	16	3.8	5.5	2	2	7	16	4	4	6	4	4
Rg60	6	3.5	16	3.8	5.5	4	4	1	9	4	4	4	4	4
Rg61	6	6	16	3.8	5.5	4	2	9	9	4	4	6	4	4
Rg62	6	6	16	3.8	5.5	4	2	9	9	4	4	6	4	4

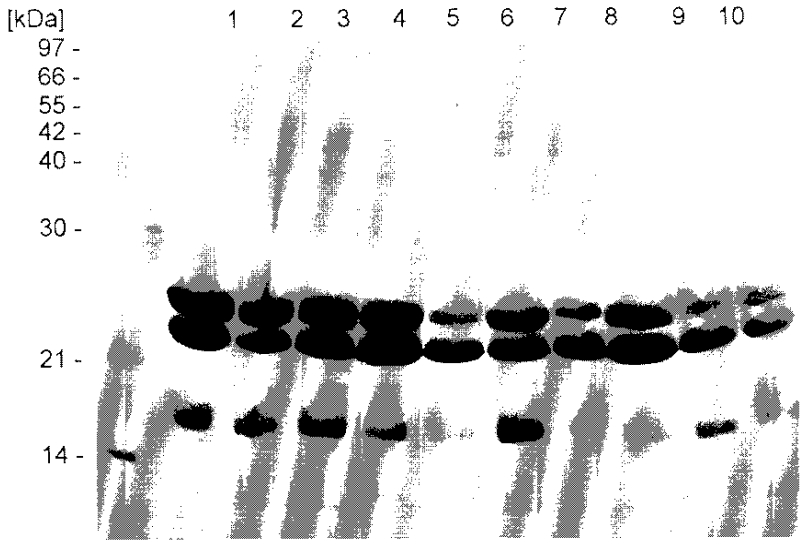

Fig. 2. SDS-PAGE of zein extracted from maize inbred lines G12, Rg15, G336, Rg58, Rg59, Rg60, Rg61, Rg62, G221D and In171.

The electrophoretic analysis of zein fraction (Fig. 2) revealed two major zein polypeptides (approx. 27 and 23 kD) were prominent in all the lines, in addition to two diffused bands (approx. 19 and 16 kD). Moreover, a very low molecular mass polypeptide (10 kD) appeared in all the lines except G221D. These data indicated that the zein storage protein consists mainly of two major subunits (27 and 23 kD) which is in good agreement with the results of Ganchev *et al.* (1979), Abe *et al.* (1981) and Landry and Sallantin (1983). The 10 kD zein polypeptide showed differential expression in the ten inbred lines with different migration rates on the SDS-PAGE. This

polypeptide was completely absent in the line G221D. This protein called Δ-zein by Thompson and Larkins (1994) is exceptionally rich in the sulfur amino acids, methionine (23 %) and cysteine (4 %) and undoubtedly constitutes an important source of sulfur storage for the seeds.

The results of the present study emphasize the special usefulness of two maize storage protein fractions, *i.e.* salt-soluble protein fraction and zeins, for the detection of genotypic variability and inbred line identification. In addition, variations in the sulfur-rich zein fraction is of potential importance in manipulating genes controlling the nutritional quality of maize proteins.

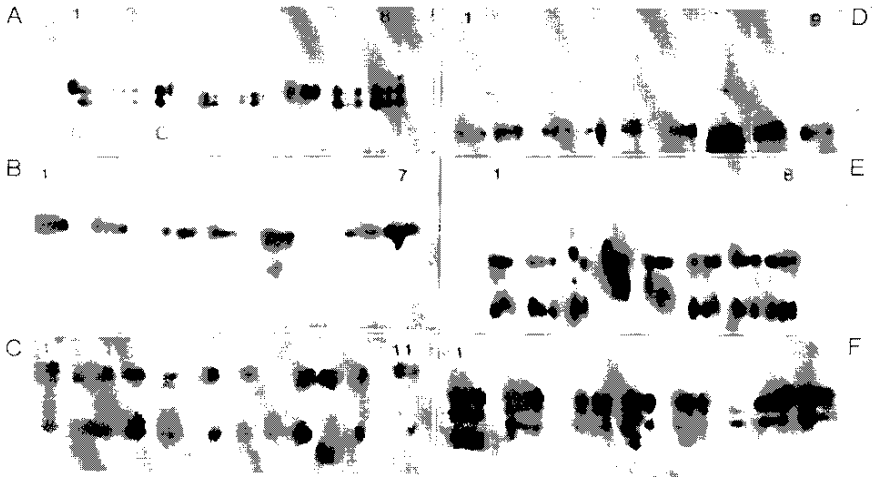

Fig. 3. Zymogram of seven of the studied isozyme systems.
A) IDH, *lanes*: *1* - control, *2* - G336, *3* - control, *4* - Rg58, *5* - Rg59, *6* - Rg60, *7* - Rg61, *8* - Rg62.
B) PGM, *lanes*: *1* - Rg15, *2* - G336, *3* - G221D, *4* - In171, *5* - Rg58, *6* - Rg60, *7* - Rg61.
C) GOT, *lanes*: *1* - control, *2* - Rg58, *3* - Rg59, *4* - Rg60, *5* - Rg61, *6* - Rg62, *7* - In171, *8* - G221D, *9* - G336, *10* - Rg15, *11* - control.
D) ACP, *lanes*: *1* - control, *2* - G336, *3* - G221D, *4* - In171, *5* - Rg58, *6* - Rg59, *7* - Rg60, *8* - Rg61, *9* - Rg62.
E) 6-PGD and PHI, *lanes*: *1* - control, *2* - In171, *3* - control, *4* - Rg58, *5* - Rg59, *6* - Rg60, *7* - Rg61, *8* - Rg62.
F) MDH, *lanes*: *1* - Rg60, *2* - Rg59, *3* - Rg58, *4* - control, *5* - In171, *6* - G221D, *7* - G336, *8* - Rg15.

The survey conducted on isozyme polymorphism among nine of the inbred lines showed that out of 14 isozyme loci analyzed, eight were polymorphic and six monomorphic. Of the eight polymorphic loci, seven were dimorphic (MDH-2, PHI-1, ACP-1, PGM-1, IDH-1, IDH-2 and GOT-1) and one tetramorphic (β-GLU-1) (Table 2, Fig. 3).

The different combination of the alleles at the 14 loci gave seven unique fingerprints with two pairs of inbred lines sharing the same fingerprint (lines Rg58 and Rg59; Rg61 and Rg62). The lines having similar fingerprints are closely related (Table 1). Therefore, further analysis using additional enzyme systems could be used to discriminate between the above lines. However, based on SDS-PAGE of the salt-soluble protein fraction, lines Rg58 and Rg59 had different protein profiles, whereas, lines Rg61 and Rg62 had nearly identical ones. Thus, both methods complement each other in characterizing or fingerprinting maize inbred lines.

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