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Original Research Article

Isolation and Characterization of Storage Protein - Zein in Quality Protein Maize (QPM)

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A B S T R A C T

The nutritional quality of normal maize is poor because of predominance of zein. In the present study three genotypes normal, chalky *Opaque*-2 and QPM) was used for characterization of storage protein-zein. The α, β and γ-zein protein fractions were extracted from seed endosperm. The results of α, β and γ-zein polypeptide bands were found in all the three genotypes. Prat *et al.*, (1985) reported that γ--zein having high cysteine content and it involved in disulfide interactions that influence kernel hardness in maize. The polypeptide band of γ-zein at 27 kDa was observed more intense in QPM, followed by normal and least in Chalky Opaque-2 endosperm. This γ-zein may responsible for in kernel vitreosity and hardness in maize QPM genotypes.

Keywords

Chalky *Opaque*-2, maize, quality protein maize, γ-zein

Introduction

In maize (*Zea mays* L.), nearly 50% of the protein in the seed is contributed by the alcohol-soluble proteins known as zeins. The zein are synthesized in the endosperm between 12-50 days post-pollination and accumulated in membrane bound organelles called protein bodies (Alan *et al.*, 1991) Maize plays a very important role in human and animal nutrition. In India more than 60 per cent of the maize produced is directly or indirectly consumed as a human food. Nutritional quality of normal maize proteins is poor because of predominance of zein, which is deficient in two essential amino acids namely lysine and tryptophan. Its continuous consumption as a staple food may be responsible for pellagra, a tryptophan- niacin deficiency disease (Gopalan *et al.*, 1975). However, the discovery of opaque-2 gene (Mertz *et al.*, 1964), in maize led to a major break-through in realization that the quality of normal maize protein could be improved by genetic manipulation. In the presence of opaque-2 and floury-2 genes zein synthesis is decreased thereby increasing the percentage of lysine and tryptophan contents (Nelson, 1969, Paulis *et al.*, 1969, Jones *et al.*, 1977, Gupta *et al.*, 1979, Gupta *et al.*, 1980, Seva Nayak and Gupta 2017). Opaque-2 varieties were developed in India these cultivars have not been accepted by the farmers and consumers because they have a soft, chalky endosperm texture resulting in lower grain

yield and higher susceptibility for developing ear rots disease and stored grain pests. Keeping this in view efforts were made for improving the agronomic performance of opaque-2 genotypes by using recurrent selection for opaque-2 modifies genes. Maize breeders and biochemist jointly at Indian Institute of Maize Research, in India and at the International Maize and Wheat Improvement Center (CIMMYT) in Mexico converted Chalky Opaque-2 maize into hard endosperm Opaque-2 varieties that have high nutritional quality, superior grain yields, traditional appearance, conventional hardness (Gupta *et al.,* 1979, Singh *et al.,* 1973, Singh *et al.,* 1974, Singh *et al.,* 1976, Singh *et al.,* 1985, Zhou *et al.,* 2016) resistance to disease and pest. (Prat *et al.,* 1985, Gayral *et al.,* 2017) reported that γ-zein having high cysteine content and it involved in disulfide interactions that influence kernel hardness in maize. The present investigation was undertaken to study the fractionation of storage protein-zein in quality protein maize

Materials and Methods

Seed Materials

Seeds of three maize varieties, namely Navjot (Normal), *Opaque*-2 (Chalky *Opaque*-2) and Shakti-I (Quality Protein Maize (QPM) (Fig-1a,b,c) were collected from the Indian Institute of Maize Research, Indian Agricultural Research Institute, New Delhi.

Sample preparation

Kernel of all the three varieties, used in the present study, were soaked in distilled water at 4^0C for an hr, then the pericarp was removed, and the endosperm and embryo of each varieties were separated with scalpel, and the endosperms of each varieties were collected and dried at room temperature. The dried endosperms ground to 100-mesh and flour was defatted with hexane by Soxhlet Apparatus for 6 hr.

Extraction of Zein fraction for SDS-PAGE

Extraction of zein sub-fraction for SDS-PAGE was done according to Esen (1986 and 1987). Fig-2 shows the flow chart of the procedure. 1gm of defatted sample was taken in 150 ml of conical flask then 10 ml of 60% 2-PrOH/1% 2-ME was added [(1:10 ratio sample (gm) solvent (ml)] and shaken for 18 hr. in water bath-cum-shaker (30^oC) then centrifuged at 12000 g (4^oC) for 10 min. The supernatant was collected in a 25 ml of conical flask. This supernatant designated as whole zein. Then pellet was discarded and added 30 ml of 2-PrOH (100% v/v) to the whole zein. The resulting solution was left standing overnight (20 hr.) at 4^oC.Then centrifuged at 12000 g (4^oC) for 10 min the pellet was stored for β and γ-zein extraction, and supernatant was collected then added to it 70 ml of distilled water to make it 30% 2-PrOH, the supernatants, referred to as solubility fraction-1 (ASF-1 or α-Zein). In addition 1.0 ml (0.01 vol./ml) of 3 M NaAc (pH 6.0) was added to make the solution of 30 mM NaAc/30% PrOH of final concentrations for α-Zein purification. The solution was left for standing over night at 4^oC, and then centrifugation was carried out at 5000 g for 10 min. The supernatant was discarded; the pellet was washed with 5 ml of distilled water followed by centrifugation. The α- zein Pellet was dissolved in 5 ml of 60% tertbutanol, and then freeze dried for SDS-PAGE. The stored β and γ-zein pellet from 12000 g centrifugation was washed twice with 5 ml of 90% 2-PrOH/0.5% 2-ME for 10 to 15 min, followed by centrifugation at 5000 g for 5 min. Then the pellet was

solubilized in 5 ml of 60% 2-PrOH/2% 2-ME and left for overnight at room temperature. The solution of 30% 2-PrOH was made first by adding 10 ml of distilled water and 0.15 ml of (0.01 v/ml) 3M NaAc (pH 6.0) was added to make to the solution 30 mM NaAc/30% PrOH final concentrations, for β- and γ-zein separations. The resulting 30 mM NaAc /30% PrOH /2% 2-ME solution was allowed to stand at 4^{o}C over night and centrifuged at 5000 g for 10 min. The supernatant designated as ASF3 or γ-zein.Its volume was reduced to about one half in dialysis bag by solid sucrose, then dialyzed against H_2O and freeze-dried for SDS-PAGE. The pellet, designated ASF2 or β-zein was washed twice with 30 mM NaAc/30% 2-PrOH /0.5 2 -ME (pH 6.0) for 10 min. followed by centrifugation. It was then suspended in 5 ml of 60% tert butanol and freeze dried for SDS-PAGE.

SDS-polyacrylamide gel electrophoresis of storage protein-zein

Storage protein was electrophoresed on 12 per cent SDS-Polyacrylamide gel using 0.025 M Tris glycine buffer (pH 8.3) containing 0.1 per cent sodium dodecyl sulphate (SDS) (Laemmli, 1970).

Results and Discussion

The result of α, β and γ-zein polypeptide bands are shown in (Fig-3). The polypeptide band of γ-zein at 27 kDa was observed more intense in QPM, followed by normal and least in Chalky Opaque-2 endosperm. (Fig-3). Paiva *et al.,* (1991) also reported more intense of γ-zein polypeptide at 27 kDa in QPM than normal and Chalky Opaque-2 maize endosperm. The polypeptide band of α-zein at 22 kDa was found more intense in normal followed by QPM endosperm and least in Chalky Opaque-2 endosperm. The β-zein showed two polypeptide bands at 18 and 17 kDa. The polypeptide band of β-zein at 18 kDa was found more intense in QPM than normal and Chalky Opaque-2 endosperm. Second polypeptide band of β-zein at 17 kDa was found more intense in normal and QPM than Chalky Opaque-2 endosperm.

Wua *et al.,* (2010) reported that, γ-zeins is essential for endosperm modification in quality protein maize and Seva Nayak and Gupta (2017) reported that QPM variety Shakti-1 having the high level of lysine and tryptophan contents, conventional kernel hardness and vitreousness and also shown that to contain relatively high amounts of γ -zein in the endosperm. The γ -zein was found in very small amounts in the endosperm of Chalky Opaque-2, which have a very soft endosperm texture. While the nature of the γ-zein with kernel vitreosity is unclear, it seems that to have both a high lysine and tryptophan content and a hard, vitreous, kernel the seed must also have a high level of γ -zein. Considering γ -zein localize at the periphery of the protein bodies (Lending and Larkins 1989) and its high Cysteine content (Prat *et al.,* 1985). This γ-zein may be involved in disulfide interactions that influence kernel hardness in QPM genotypes.

Earlier studies (wallace *et al.,* 1990) also reported that the mechanism by which *Opaque-2* modifier converts the starchy endosperm of *Opaque-2* mutants to a hard, vitreous phenotype is not understood. The major biochemical difference between QPM and standard *Opaque-2* mutants is a two to threefold increase in γ-zein protein and mRNA (Wallace *et al.,* 1990, Geetha *et al.,* 1991). It has been demonstrated that the degree of modification (vitreousness) and the increased amount of γ-zein are highly correlated and dependant on the dosage of modified genes (Lopes and Larkins1991).

Fig.1a Seeds of Normal showing completely light passage

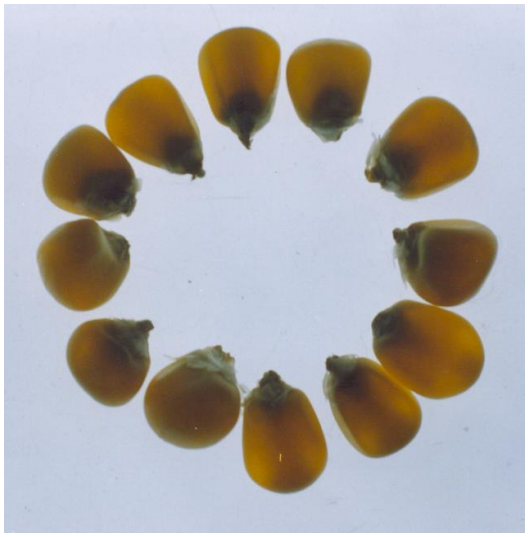


Fig.1b Seeds of Chalky Opaque-2 showing no light passage

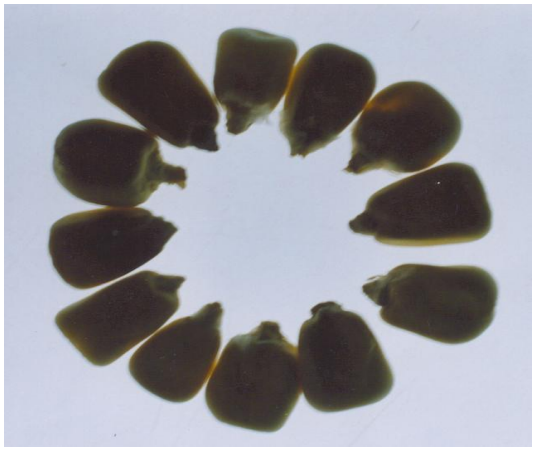


Fig.1c Seeds of QPM showing partial light passage

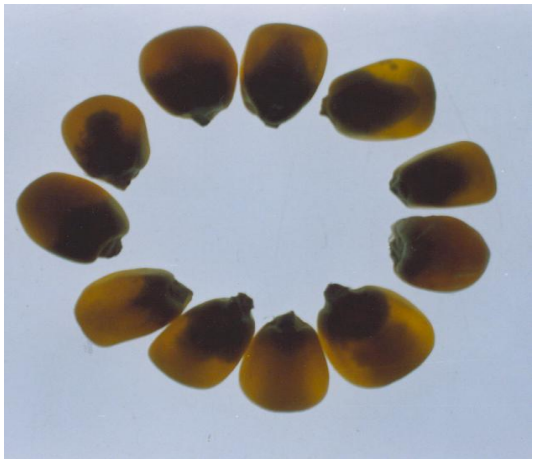
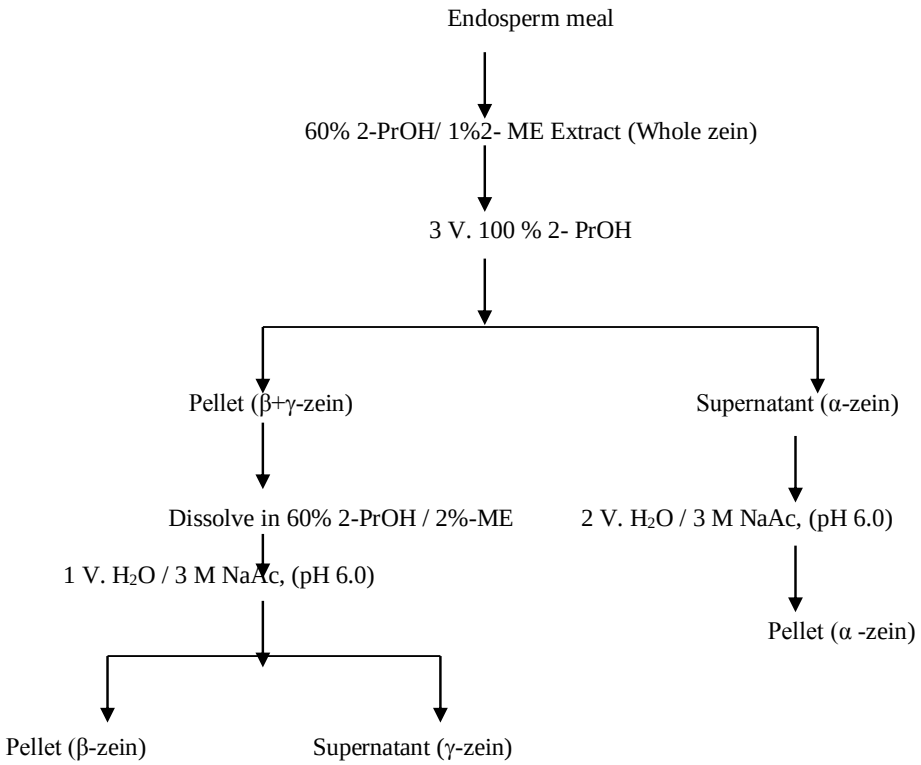


Fig.1 Flow sheet diagram showing the fractions of zein



(*Source: Esen, A. 1986 and 1987*)

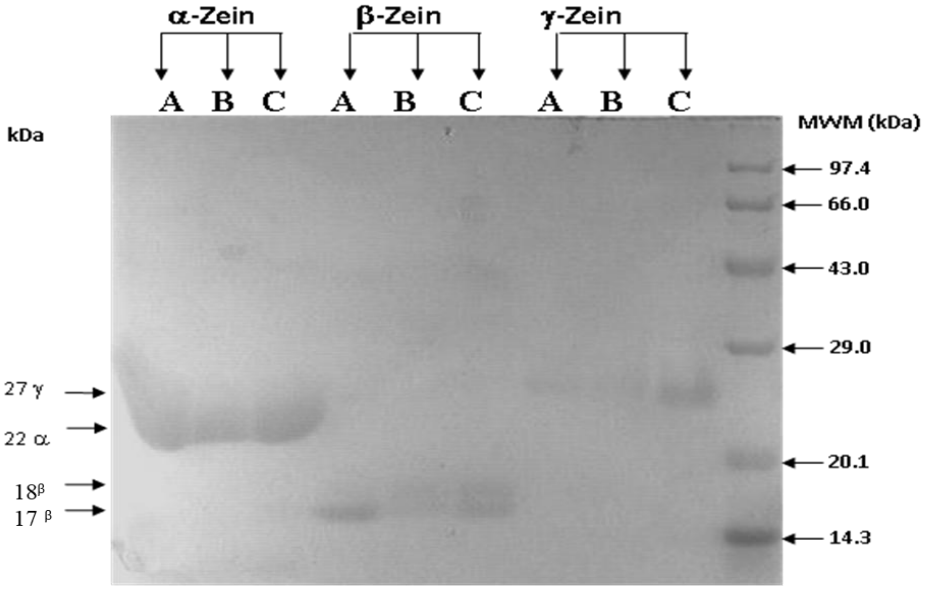


Fig-3 Electrophoretic pattern (SDS-PAGE) of Zein fractions α, β and γ extracted from - Normal, Chalky Opaque-2 and QPM endosperm.
A: Normal; B: Chalky Opaque-2 and C:QPM
MWM: Molecular Weight Marker

Thus, it appears that γ-zein protein is involved in some way in the formation of vitreous endosperm, which is generally related to hardness (Watson, 1987). The α-Zein (22kDa) was observed more intense in normal followed by QPM and least in Chalky Opaque-2. Mauricio and Brian (1991) also reported that 22 kDa of α-zein is almost absent in Chalky Opaque-2 and QPM as compared to normal maize genotype. Apart from α and γ-zein, β-zein at 18 kDa was found more intense in QPM than normal and Chalky Opaque-2. The second polypeptide of β-zein at 17 kDa was found more intense in normal and QPM than Chalky Opaque-2 endosperm. These changes showed that the nature of QPM endosperm zein is being changed tremendously and modifier gene playing significant role in modifying endosperm from Chalky to vitreous and hard type.

The γ-zein was observed more in QPM followed by normal and least in Chalky Opaque-2.This γ-zein may be responsible for in kernel vitreosity and hardness in QPM genotypes

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