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Research Review Articles

Breeding quality protein maize (*Zea mays*): Genetic and analytical perspective

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ABSTRACT

Maize is one of the prominent cereal crops locally as well as internationally for both biological and industrial processing. The nutritional value of maize grains is graded to be poor due to limiting essential amino acids mainly lysine and tryptophan. A series of events beginning by the discovery of the *opaque-2* mutation until the development of enhanced protein quality cultivars paved the way for the role of amino acid modifiers genes and proteins. Quality Protein Maize (QPM) is the improved variant of the normal maize with enhanced protein quality. Protein body (PB) formation and the association of the molecular structure of the starch and PB has been a study of concern. Various changes in biochemical pathways leading to the changed zein and non-zein proportions have been studied using transgenic technology which forms an important component of maize protein. Maintaining the quality parameter of QPM inbreds, hybrids and populations so developed is a scientific concern to the speedy and precise determination of quality parameters and further maintaining these parameters using standard breeding methods. The role of biochemist and biochemical techniques are of great significance in maize quality protein research since high throughput and precise nutrient profiling of breeding lines, populations, inbred lines and hybrids support a sound QPM development programme. Various advanced techniques have been developed so far, for the protein isolation till detection and quantification of protein.Among these, the most advanced is the Ultra Performance Liquid Chromatography(UPLC) technique which is highly accurate and can be used efficiently for amino acid profiling of the maize germplasm, breeding lines and hybrids to support the decision making the process in QPM breeding programme. Thus, this review gives us an overview of the breeding methods and biochemical techniques utilized in the development of QPM in the current year 2019 and beyond.

Keyword: Marker-assisted selection, Opaque-2, Protein body, QPM, UPLC

Maize, commonly referred to as corn, is considered a unique plant and is the staple food for millions of people. Unlike the other major cereal crops, typical monoecious and protan dry nature of maize favors cross-pollination and exploitation of heterosis along with economical and sustainable hybrid seed production using male sterility or manual detasselling approaches, broad morphological variation, and genetic plasticity and diversity. In terms of utilization, maize is again unique since maize green plant is used as fodder, immature ears are used as baby corn and matured grain is used from human consumption to poultry and animal feed, and diverse processed edible and industrial products. Maize can take advantage of sunlight better than most other major cereal crops and grows more rapidly because of the size and distribution of its foliage (Kumar *et al*. 2013). Besides the morphological benefits, the main parameter of utmost importance is the yield. Compared to other cereal crops, the higher yield of maize is possible because of the low position of the ear, where it can capture a greater proportion of the nutrients, unlike other cereals whose seeds are found high up on the plant stalk. The ear is covered with a husk leaf, thereby shielding the kernels from pests and accidental dispersal, in contrast to other cereals where individual grains are covered with bractea.

Maize is also unique in nutritional value as various biofortification programs were included for the enhancement of already present quality proteins (lysine and tryptophan) (Tufchi *et al*. 2015, 2017, Chaudhary *et al*. 2018), provitamin A (Rashmi *et al*. 2014, Tufchi *et al*. 2015) and iron-zinc (Kumar *et al*. 2018) using conventional as well as molecular approaches.

Maize proteins

The mature maize kernel contains an embryo and a much larger endosperm, both of which are surrounded by the seed coat. The second-largest chemical component after starch (70-75%) in maize kernel is the protein content which varies on an average from 8-13%. The distribution of the two essential amino acids, i.e. lysine and tryptophan are variable as the germ is sufficient while the endosperm is

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deficient in these amino acids, besides having the majority (6.5%) of the maize protein in the endosperm. Zeins are the seed storage proteins of maize which are encoded by specific classes of structural genes that belong to a large gene family clustered in several genomic regions. They form accretions called protein bodies in the rough endoplasmic reticulum and are more numerous in endosperm regions, with high zein content in horny endosperm and low in the floury endosperm. Zeins have been classified as α- (22 and 19 kDa), β- (14 kDa), γ- (27 and 16 kDa), and δ- (10 kDa) zeins on the basis of the degree of relatedness of their primary gene and amino acid sequences. The α-zeins are by far the most abundant fraction and comprise up to 80% of the total zeins. In normal maize genotypes, zeins are characterized by a high content of glutamine, leucine and proline but are devoid of lysine and tryptophan. Suppression of lysine-deficient zein fraction without drastically altering the contribution of other fractions could be, thus, seen as a feasible approach to bring about an improvement in the amino acid composition in maize grain.

Events that enhanced the biological value of maize protein

In 1962 Edwin Mertz proposed that *opaque-2* (*o2*) mutant maize with decreased zein had increased lysine and tryptophan content which may be because of more accumulation of non-zeins in the kernel endosperm. Later, Oliver Nelson suggested that the *opaque* and *floury* endosperm mutants might have reduced zein content, based on the knowledge that seed with low protein content in the Illinois long-term selection population had a soft, starchy endosperm. Both the scientists together then quickly demonstrated that *o2* had less zein protein and nearly double the lysine and tryptophan content of normal maize and, therefore, might be useful to improve its nutritional quality. Almost a decade, backcrossing was used for mass conversion of standard maize inbred lines into *opaque-2* variants along with the parallel study of their combined abilities for yield and other important agronomical traits. These research programs were intensive in the countries in which maize was an economically important crop. A very potent program in this field was also developed at Maize Research Institute "ZemunPolje", where a significant number of commercial *opaque-2* inbred lines and hybrids with improved nutritional and biological value were developed. Unfortunately, because of undesirable pleiotropic effects of the *opaque 2* genes which included chalky endosperm, most of these programs were stopped and only a small number of crop research institutes continued this work. During the 1970s and 1980s, the CIMMYT in Mexico established an extensive program to develop high lysine corn using *o2* allele. The project was led by Dr S K Vasal, who, in association with Dr Evangelina Villegas, selected simultaneously for *o2* genotypes with normal endosperm texture and increased the levels of essential amino acids.The initial efforts were tedious, because they required selecting the seed manually and performing microanalysis of lysine content but they were able to convert the floury soft endosperm kernels into harder types, increase grain yield potential to the level of the normal maize types, endow the *opaque-2* maize with disease and insect resistance and with utilization and storage qualities similar to those of superior normal maize.The new, normal-looking, normal-tasting *opaque-2* type maize was renamed "Quality Protein Maize" or QPM (Vivek *et al*. 2008). Extermination of the negative effects of the *opaque-2* gene while maintaining its superior nutritional quality lead to the development of commercially accepted QPM.

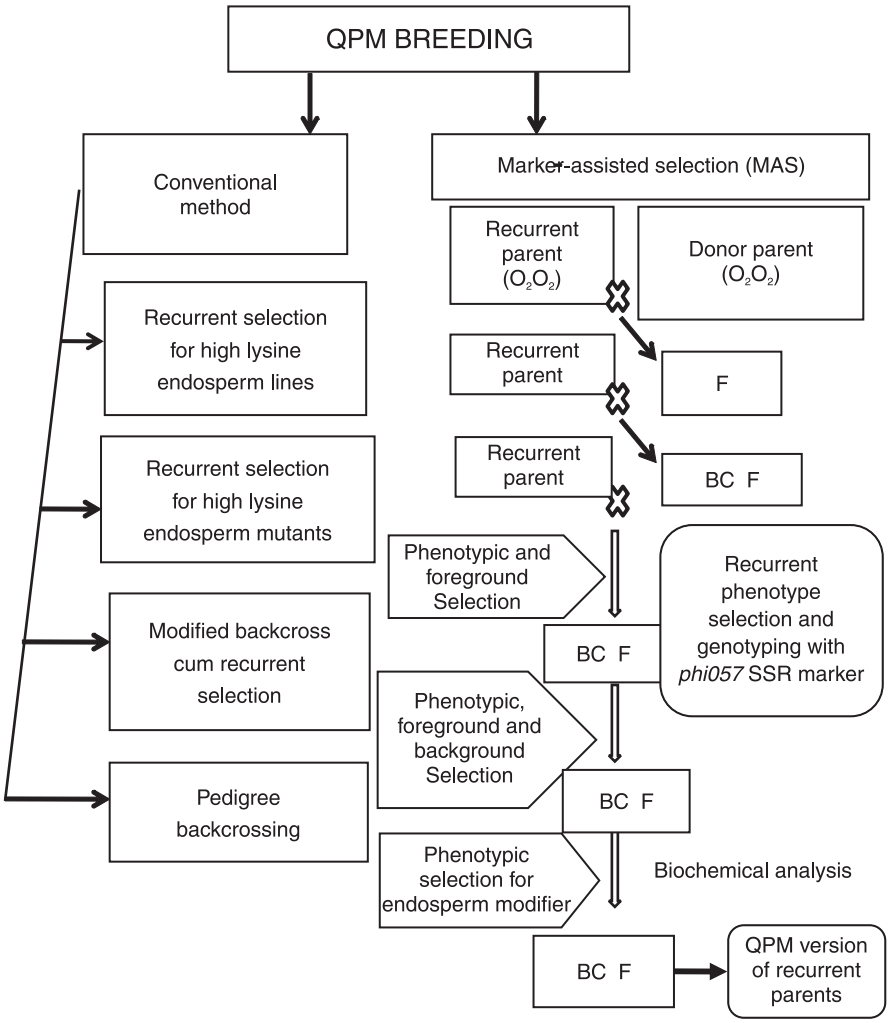

Fig 1 Classical breeding and molecular marker-assisted breeding for QPM development.

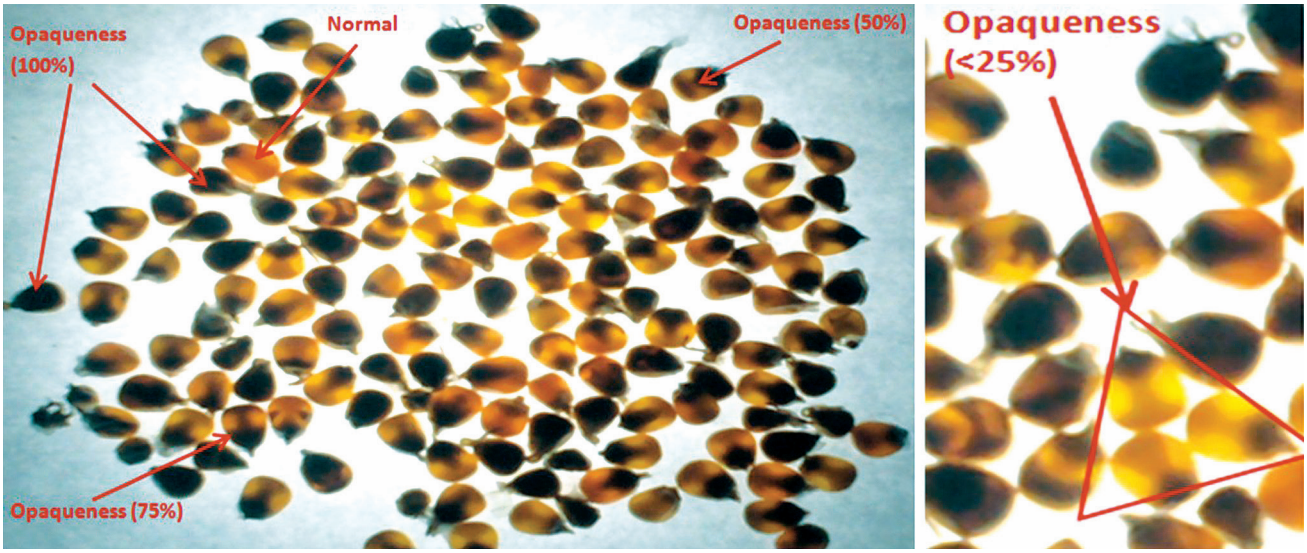

Fig 2 a. Light table screening; b. Gradation based on opaqueness as Type 2 = 25% opaque.

Approach to QPM breeding

The objective of the QPM breeding program was to develop QPM inbred lines and open-pollinated varieties (OPVs) from elite non-QPM source germplasm using backcrossing and pedigree methods. There are two possible approaches to QPM breeding, one is the classical breeding (CB) approach and the other is the molecular marker-assisted breeding (MAB) approach (Fig 1). Regardless of the breeding methods and approaches used, there are two unique and essential steps in the development of QPM germplasm. The first is to simultaneously identify segregants in a family or population having the *o2* allele in the homozygous recessive (*o2o2*) condition with a hard endosperm. The conventional approach for this task uses lightbox table and the molecular approach involves the use of both molecular markers and light table. The second step is to identify and confirm QPM quality, i.e. the percentage of tryptophan and protein in a sample, through laboratory analysis (Vivek *et al.* 2008). There is a need for monitoring both protein content of grains as well as tryptophan and lysine while breeding or selecting for QPM. This will ensure desirable levels of these amino acids in the protein of the evolving genotypes, besides its effect differed significantly for the QPM varieties concerning all agronomic characters when grown under different locations.

The degree of opaqueness is graded from 1-5 scores. The scores are: Type (modification score) 1: not opaque (Normal); Type (modification score) 2: 25% opaque (Fig 2b); Type (modification score) 3: 50% opaque; Type (modification score) 4: 75% opaque; Type (modification score) 5: 100% opaque.Types 1 to 3 are considered QPM, provided their protein quality is verified. It is recommended to select only types 2 and 3 in a conventional breeding approach. Type 2 kernels should be selected only in advanced generations because *O2O2* or *O2o2* genotypes may have a small degree of opaqueness and the presence of *o2o2* genotypes in early generations is the priority. Type 3 is recommended for selection in early generations as it is a compromise between the guaranteed presence of *o2o2* (high priority) and good modification (which can be improved in subsequent generations).

Since the development of molecular marker techniques in the 1980s, the genetic background of many agronomically important traits in maize has been dissected. MAS is gaining considerable importance due to the efficiency and identification of precise genomic regions of interest (foreground selection) and the recovery of the recurrent parent genome (background selection) (Babu *et al*. 2005). It has recently become possible to use MAS to accelerate selection for the *opaque-2* allele in QPM breeding programs. Three SSR (*Simple Sequence Repeats*) markers namely *phi57*, *phi112* and *umc1066* have been identified for monitoring the inheritance of *opaque 2* genes (Danson *et al.* 2006). These markers are located within the *opaque-2* gene, indicating a very high correlation between marker data and phenotypic expression. The *phi112* marker is dominant and therefore identifies normal (*O2O2*) and heterozygous (*O2o2*) genotypes. The breeder can assume that all other genotypes are of desired homozygous recessive type (*o2o2*). The other two markers namely *phi57* and *umc1066* are co-dominant and can identify all three possible genotypes at *O2* locus. Various non-QPM lines were successfully introgressed with opaque-2 using these markers (Babu *et al.* 2005, Tufchi *et al*. 2015, 2017). However, without concurrent selection for amino acid modifiers, protein quality can drop considerably. Many studies hypothesized the importance of amino acid modifier genes in the modification of altered amino acids profile. Along with the relative levels of lysine and tryptophan content in the grain endosperm (Krivanek *et al.* 2007) altered amino acid profiles such as increased histidine, arginine, aspartic acid and glycine content while a reduced glutamic acid, alanine and leucine content were observed in *o2* maize. The decrease in leucine is considered particularly desirable asit makes leucine–isoleucine ratio more balanced,

which in turn helps to liberate more tryptophan for niacin biosynthesis, and thus, helps to combat pellagra.

Validation of crude protein and limiting amino acids

Validation of enhanced limiting amino acids mainly lysine and tryptophan with total protein content are to be done at every step in the conventional method which necessitates the need for the biochemical analysis of each sample for development of QPM germplasm. The biochemical analysis for total protein content in the endosperm and tryptophan concentration in endosperm protein in each class of kernel modification is to be carried out according to standard procedures. In short, the samples are de-germed after removing the pericarp and finely ground. The resulting flour is defatted and total nitrogen content is determined by Kjeldahl procedure (AOAC 1965) and percentage of protein is calculated by multiplying the N content with a factor of 6.25. The Kjeldahl method was introduced in 1883 and consists of three main steps: digestion, distillation, and ammonia determination (titration being the primary method). The Kjeldahl method uses sulfuric acid, a variety of catalysts, and salts to convert organically bound nitrogen in the samples to ammonium with its subsequent measurement**.** However, successful analysis requires proper sampling and sample handling, which depend on the type of material. The Kjeldahl procedure has several variants, based mainly on sample size and apparatus required. Several rapid and accurate instrumental methods have been gradually introduced that have some advantages compared to older techniques if a large number of samples are to be run. Thus, extracted nitrogen from Kjeldahl can be determined by several other methods, i.e., spectrophotometric, potentiometric with ion-selective electrode, flow injection analysis, ion chromatographic, and chemiluminescent methods. Quality control is essential for accurate and precise measurements of nitrogen by the Kjeldahl method. The Dumas method or the nitrogen combustion method is an attractive alternative to the widely used Kjeldahl assay. Numerous recent technical breakthroughs have improved the method's accuracy, and the adoption of several automated features has made the method easy to use. The basis of the Dumas method is the conversion of all the nitrogen forms in the sample to the nitrogen oxides through combustion at 800-1000^{0}C, reduction of these forms to nitrogen gas and subsequent measurement by use of a thermal conductivity detector. The Dumas method requires less than 5 min per sample, can be semi-automated, avoids the use of corrosive and hazardous chemicals, and is a relatively safe procedure (Jung *et al.* 2003). Despite some negative factors (i.e. it is hazardous, lengthy, and labour intensive), the Kjeldahl method and its variants with instrumental finish remain as accurate and reliable methods (Saez *et al*. 2013).

After the determination of protein content, it is essential to estimate the content of limiting amino acids in maize to identify QPM line. Lysine and tryptophan concentration in maize kernels of agronomically advanced QPM lines are highly correlated. A 3:1 ratio of lysine to tryptophan has been reported in normal and quality protein maize (Vivek *et al*. 2008). Therefore, in a QPM breeding program, only one of these amino acids is typically monitored, tryptophan being the more commonly measured of the two due to lower laboratory costs (Krivanek *et al.* 2007). Rapid and reliable determination of lysine content is one of the major limiting factors for QPM breeding programmes, worldwide. Lysine measurements made by conventional amino acid analysis are expensive and slow, making them prohibitive for most breeding programmes. Therefore, such programmes have traditionally relied on indirect measurement of lysine based on colorimetric analysis (Jung *et al.* 2003) or by indirectly inferring lysine content through colorimetric analysis of tryptophan content. A colorimetric method based on acetic acid has been used to analyze tryptophan concentration in maize grains for more than 30 years at CIMMYT and several institutes with QPM breeding programs around the world (Vivek *et al.* 2008). Alternative semi-quantitative methods using tryptophan mutant fungi or bacteria (Saez *et al*. 2013) have also been developed, but they are laborious and limited by microorganism growth rate. Recently an accurate, reliable, and inexpensive method for tryptophan analysis in whole-grain maize flour to support QPM research efforts around the world came into existence (Nurit *et al.* 2009). Tryptophan reacts with glyoxylic acid in the presence of sulphuric acid and ferric chloride, producing a coloured compound that absorbs at 560 nm. A series of experiments varying the reagent concentrations, hydrolysis time, and length of the colorimetric reaction resulted in an optimized protocol which uses 0.1 M glyoxylic acid in 7 N sulfuric acid and 1.8 mM ferric chloride, and 30 min reaction time. This method produced stable and reproducible results for tryptophan concentration in whole-grain maize flour and was validated by comparison with data obtained using an acetic acid-based colorimetric procedure (r^2 = 0.80) and high-performance liquid chromatography (HPLC) (r^2 = 0.71) (Nurit *et al.* 2009).

A breakthrough was made in the 1990s through the development of an enzyme-linked immune sorbent assay (ELISA) that provided a more objective and rapid means of estimating lysine content in maize endosperm. The origin of lysine-containing proteins in cereal grains is usually determined by extracting the flour with different solvents. SDS-PAGE of these fractions revealed that the majority of the non-zein proteins and nearly 80% of lysine in the endosperm proteins were recovered in the soluble protein fraction. Habben *et al.* (1995) developed an ELISA using EF-1*a* antiserum, to measure the level of this protein in maize genotypes.The study revealed a remarkably high positive correlation (r^2 = 0.92) between lysine levels in the endosperm and EF-1*a* content. The maize EF-1*a* has been recentlycharacterized and its relationship to protein quality in the endosperm demonstrated. The concentration of EF-1*a*, thus, appears to provide a useful index of the lysine content in the cereal grain proteins. The ELISA for EF-1*a* provides a sensitive, efficient, less laborious, and inexpensive method

Table 1 Standard criteria for selection of QPM and non-QPM maize lines

Type of material	Parameters (%)	QPM	Non-QPM
Endosperm	Protein	>=8	>=8
	Lysine	4	2.5
	Tryptophan	>0.65	<0.65
In sample	*Whole grain*	*Endosperm*	
Tryptophan	>0.075	>0.07	
Quality index	>0.8	>0.7	

of monitoring the lysine content of maize kernel, and is more amenable to automation than non-zein quantification. Several countries in the QPM Research and Development Network facilitated by CIMMYT, have begun to utilize the ELISA in rapid screening of QPM breeding materials for protein quality but due to cost-effectiveness and easy analysis still, the colorimetric method is worldwide used in the development of QPM lines.

Eventually, it becomes apparent that the simple genetic nature of *o2* maize has been converted to a classic polygenic trait about QPM and must be manipulated as such in breeding programs. If lysine or tryptophan levels are not frequently monitored during the breeding process the additional gains in protein quality may be lost even though the *o2o2* genotype is maintained (Krivanek *et al.* 2007). Hence, it is advised that the whole grain analysis on early segregating generations and endosperm analysis on elite germplasm and final products should be done. Besides the quantification of the tryptophan content, another important parameter quality index (QI) is to be determined. QI is the tryptophan-to-protein ratio in the sample, expressed as a percentage. Relationship between QI, protein quantity, and protein quality is essential to categorize QPM from non-QPM lines. In most of the cases, there is a negative correlation between protein quality and quantity (% protein) and QI.The QI does not indicate the quantity of protein. Hence, when interpreting lab results for making selections, it is essential to make sure that the protein, tryptophan, and QI are above the acceptable limits (Table 1).

Based on results using the glyoxylic acid method breeders consider QPM samples with values >0.07% for tryptophan in the sample and with quality index > or equal to 0.7% (Vivek *et al.* 2008). For elite and pre-commercial breeding material, it is recommended to analyze tryptophan, lysine, and protein to ensure the superiority of the material. In a comparative study of normal and QPM maize the lysine levels in normal and QPM maize average 2% and 4%, and tryptophan average 0.4% and 0.8% of total protein in whole grain flour, respectively (Nurit *et al.* 2009). However, lysine levels vary across genetic backgrounds from 1.6 to 2.6% in normal maize and 2.7 to 4.5% in their *o2* converted corresponding genotypes, and tryptophan range varies from 0.2 to 0.5% in normal maize and 0.5 to 1.1% in QPM counterparts (Nurit *et al.* 2009). Besides the colorimetric method more recently HPLC technique has been used for the full profiling of the amino acids in the maize endosperm besides the limiting lysine and tryptophan.

Amino acid profiling using the Ultra performance liquid chromatography (UPLC) helps in the quantification of the amount of limiting amino acids with much accuracy and that too in limited period. Besides, its amount of other amino acids is also quantified simultaneously. Automation in biochemical analysis has led to a much faster rate of QPM lines identification and in multi-dimensional profiling of germplasm utilizing nutritional profile generation. Quantification of amino acids mainly lysine is done using AOAC standard method. In an important study separation and analysis of amino acids were done with a Beckman 6300 Amino Acid Analyzer equipped with a high-performance cation-exchange resin column, and amino acid detection done with a post-column ninhydrin derivation. The analysis revealed tryptophan levels were negatively correlated with endosperm translucence, a measure of kernel hardness. Besides it, the amino acid levels of the inbred lines were significantly correlated with those of the hybrids, although the predictive value was low (R^2= 0.13 and 0.27 for methionine and tryptophan, respectively) (Sáez *et al.* 2013). As in the previous section of the review we

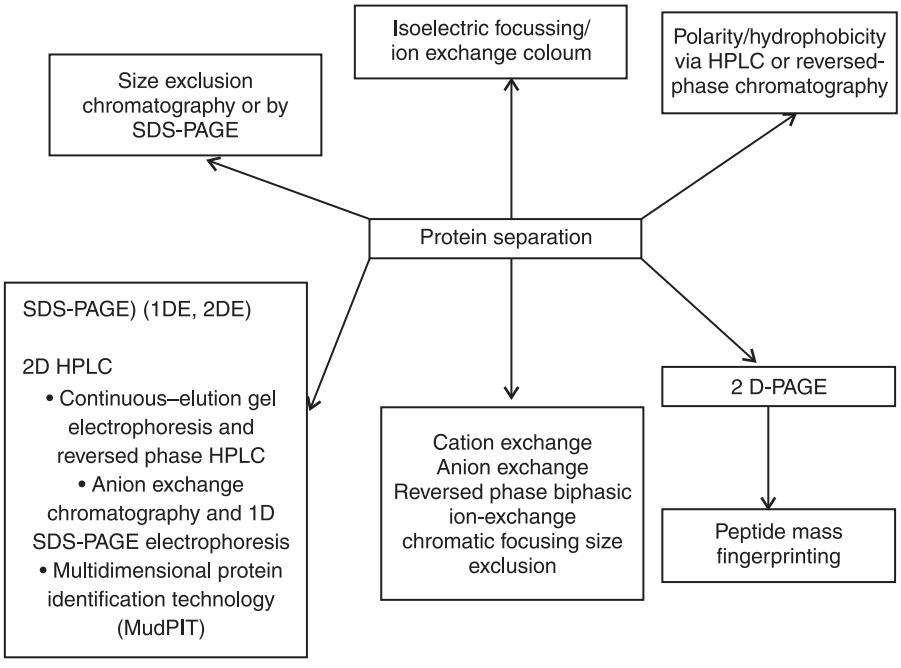

Fig 3 Biochemical techniques involved in protein separation.

discussed about the genes and proteins mainly zeins which play a vital role in improving the quality of the QPM maize and thus necessitate the need for the expression studies of QPM germplasm which will unlock many challenging tasks pertaining to the interaction of Protein Bodies (PB) and starch. The (PBs) cluster formation, its characteristic phenotype along with the effect of endosperm modifiers and amino acid modifiers will be clearer if studies using advanced biochemical techniques are done (Fig 3).

Zein isolation, purification and separation

The extraction and purification of zein have been the subject of many investigations since its discovery by Gorham in 1822. Osborne (1897) and Osborne and Mendel (1914) performed the first systematic study of solvents and conditions for extraction of zein; they concluded that it was soluble inrelatively strong alcohol, acetic acid, phenol, and dilute alkali solutions. Since then, the most commonly used zein solvents have been 60 to 70% solutions of either ethanol or 2-propanol. Zein is not a homogeneous protein species; rather it is amixture of several groups of proteins with similar solubility behaviour. Moureaux and Landry (1968) and Paulis *et al.* (1969) independently discovered the presence of an additional protein fraction extractable with alcohol if a reducing agent is included in the medium. This fraction is referred to as glutelin-l , alcohol-soluble reduced glutelin, zein-2 and zein-like. Amino acid analysis data showed that the fraction extracted with alcohol under reducing conditions had the same predominant amino acids as zein but it had higher amounts of histidine, arginine, proline, glycine and methionine and lower amounts of leucine, isoleucine, aspartic acid, and phenylalanine than zein. Separation of zein into three distinct fractions by adding water stepwise to solutions in alcohol or methyl cellosolve was reported by Watson *et al.* (1936) and Gortner and MacDonald (1944). However, Scallet (1947) showed that the three fractions were not homogeneous when analysed by moving boundary electrophoresis. McKinney *et al*. (1958) isolated zein from maize gluten as two separate solubility fractions: alpha-zein, soluble in 95% ethanol and constituting 80% of total zein, and beta-zein, soluble in 60% ethanol but not in 95% ethanol. The occurrence of two such distinct solubility fractions was later confirmed. Different amino acid composition, relative solubility and mobility of the zeins allow their easy separation on SDS-polyacrylamide gel electrophoresis based on their apparent molecular masses. Based on electrophoretic analysis of whole, α-, and β-zein fractions with and without reduction and alkylation conclusion was derived that β-zein was a disulfide-linked aggregate of components present in native zein and thus did not contain components unique to it. Paulis (1981) extracted the native zein with 70% ethanol/0.5% sodium acetate and α-and, β-zeins according to the procedure of Turner *et al.* (1965) from maize endosperm meal. Extractability, solubility, amino acid and electrophoretic analyses showed that α-zein constituted 35% of total zein, and included two prominent bands with molecular weights 22 and 24 kD, respectively, and had an amino acid and polypeptide composition similar to that of whole zein. As for, β-zein, it failed to enter polyacrylamide gel without reduction but, after reduction, entered the gel and displayed three predominant size components with molecular weights of 24, 22, and 14 kD. It also contained more histidine, arginine, proline, and methionine than did α-zein which Paulis in 1981 attributed to the presence of the 14 kD component in β zein. Alcohol soluble reduced glutelin, or zein-2, was separated into two subfractions, water-soluble and water-insoluble, by dialysis against water. The same fraction was also separated into five subfractions by ion-exchange chromatography. Two of these subfractions, 4 and 5, and a protein isolated by Wilson *et al*. (1981) and Landry *et al.* (1983), reduced-soluble protein, are now known to be the same as the water-soluble alcohol-soluble reduced glutelin isolated byWilson (1991).

UPLC/HPLC for the analysis of maize zeins

HPLC/UPLC is the current more advanced liquid hromatographic technique used for protein separation besides the electrophoretic separation using 2D gel electrophoresis. Now a day's capillary electrophoresis is also an attractive option for protein separation. Using such techniques amino acid profiling can be a comparative method to distinguish QPM lines from non-QPM. Currently, seven different modes of HPLC are employedfor the separation of proteins. These are adsorption chromatography, ion-exchange chromatography, size-exclusion chromatography, affinity chromatography, hydrophobic phase chromatography, normal phase chromatography, and RP chromatography. The choice of chromatographicmode has the greatest impact on the separationand resolution of the proteins of interest. In particular, three of these modes of chromatography havebeen mainly used for the analysis of cereal proteins: ion-exchange HPLC (IE-HPLC), RP-HPLC, and size-exclusion HPLC (SE-HPLC). RP-HPLC is the technique most widely employed for the analysis of maize proteins. In most cases, conventional RP octadecyl silica columns (C-18) were used with IDs ranging from 2.1 to 4.6 mm, although a C-18 capillary column with an ID of 0.3 mm has been employed for the nanogram scale separation of a crude extract of acid-soluble proteins from maize kernels. Replacement of conventional analytical scale chromatography by capillaryliquid chromatography (0.05-0.3 mm ID capillary columns) brings some benefits to the separation process, such as higher sensitivity and lower sample and mobile phase consumption (Ro *et al.* 2004). Even though more sophisticated supports such as monolithic or perfusion columns are being used in the separation of amino acids, peptides, and proteins in very short analysis times, no articles were found dealing with the application of these new supports to the separation of maize proteins. Only the separation of soybean proteins from maize proteins has recently been reported using perfusion chromatography but the separation of maize proteins was not the aim of this work (Castro *et al*. 2005). Regarding the mobile phase, water/ACN (Acetonitrile) mixtures have always been used

for the analysis of maize proteins. The separation of proteins by RP-HPLC requires the presence of an additive acting as an ion-pairing agent. This agent is added to increase the hydrophobicity of proteins by forming ion pairs with the charged groups and, besides, enabling the unfolding of the proteins and thus making the buried hydrophobic groups of the proteins accessible. As a consequence, the interaction of the proteins with the hydrophobic stationary phase becomes possible and hence also their separation. In all the publications reviewed, 0.1% TFA (Trifluroacetic acid) was the additive used in the separation of maize proteins by RP-HPLC. Investigation of maize endosperm protein composition, distribution and quantification of the proportions of zeins by reversed-phase high-performance liquid chromatography (RP-HPLC) revealed that in *o2* lines, the proportion of α-zeins was reduced by 50% compared with non-mutant lines.The proportions of the β and γ zeins in all the non-*o2* mutants were similar to those in non-mutant corn lines while total amount of alcohol-extractable protein was higher in normal corn lines than in non-*o2* mutants, indicating that the number and/or the size of protein bodies in these opaque mutants may be smaller (Ro *et al.* 2004). The common mode of detection was ultraviolet detection at wavelengths of 210 to 220 nm (absorbance of peptidebonds), 254 nm (absorbance of phenylalanine residues), and 280 nm (absorbance of tryptophan and tyrosine residues). Mass spectrometry has become an alternative method for the detection of peptides and proteins and offers unique advantages of high molecular specificity and detection sensitivity. MALDI-TOF MS has been used for the detection and characterization of zein fractions (Adams *et al.* 2004, Castro *et al.* 2005).

Conclusion

In the developing field of proteomics, the rapid separation, identification and characterization of proteins from complex samples is a challenging goal to ensure study of how changes in protein expression and their post-translational modifications (PTMs) can be correlated to a change at the genomic level (Tyers and Mann 2003). Proteomics has thus emerged as one of the most important "post-genomics" approaches to better understand gene and protein function since the completion of the sequencing of the genome (Wang *et al*. 2003). Future studies may involve the use of advanced techniques for Protein isolation, separation, identification, structure and detection with quantification of maize zeins and other unexploredprotein fractions that may influence the quality of QPM maize.

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