Chapter

Quality Protein Maize: An Alternative Food to Mitigate Protein Deficiency in Developing Countries

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Abstract

Maize (*Zea mays* L.) plays a significant role in human nutrition and animal feed. After the discovery of opaque-2 mutants in maize, that produces with enhanced levels of lysine and tryptophan. Quality protein maize (QPM) holds superior nutritional value and is essentially exchangeable with normal maize. The increasing use of maize as feed, increasing interest of the consumers in nutritionally enriched products and rising demand for maize seed are the core driving forces behind emerging importance of maize crop in India. Protein malnutrition is a serious global issue demanding huge resources on healthcare. The problem can be addressed to a considerable extent by shifting to quality protein maize diet. The development of QPM hybrids through advanced breeding approach like molecular marker-assisted breeding was adopted. It could solve the issue related to protein deficiency in developing countries.

Keywords: maize, opaque-2, QPM, protein deficiency, marker-assisted breeding

1. Introduction

Improvement of protein quality of maize incorporated the mutant gene called opaque-2, thus leading to the development of quality protein maize (QPM). Several natural mutants, which confer the highest lysine and tryptophan levels, had been identified in the 1960s and 1970s, i.e. opaque-2, opaque-6, opaque-7, floury-2 and floury-3 [1]. QPMs are having more quantity of lysine and tryptophan and lesser quantity of leucine and isoleucine. Baby corn, sweet corn, popcorn, waxy corn and high oil corn were targeted to develop quality protein maize [2]. QPM hybrids with different kernel colors have been developed and are released in India for their cultivation in various agroclimatic conditions. The technology involved in the production of QPM and normal maize are the same, but QPMs should be grown separately to maintain its purity.

In the QPM, recessive opaque-2 (o2) allele has been successfully utilized in the conversion breeding program for increasing the quality of protein in maize [3]. Primarily, maize varieties with o2 mutation were not chosen by farmers and consumers, because of opaque endosperm. Opaque-2 mutant is susceptible to pests and diseases, and it also undergoes grain breakage during milling [4]. Endosperm modifier genes, which present hard endosperm in the o2 background, were developed at the International Maize and Wheat Improvement Center (CIMMYT), Mexico [5], and University of Natal, South Africa [6]. This leads to the development of nutritionally enriched hard endosperm maize, widely known as 'quality protein maize' [3].

2. Nutrition deficiency and related challenges

With the increasing world population, enhancing the production of food and nutritional quality of staple crops is the strategy to address the emerging food crises [7]. A food crisis causes multidimensional effects on human nutrition, and it causes malnutrition. It also has effects on the supply of food quantity and quality of food. In the last two decades, these problems have been tried to be solved to reduce the proportion of the world's malnourished population [8]. Protein deficiency malnutrition has emerged as a major nutritional problem, particularly in the developing countries [9]. In the developing countries, cereals play an important source of dietary protein for humans, which comprise 70% of the protein intake [10]. Maize is the world's third primary cereal crop, which is an important protein source used as food and feed for humans and animals and also used in corn starch industry, corn oil production, etc. [11]. QPM has more quantity of carbohydrates, fats, proteins, vitamins and minerals. It is also called as a 'poor man's cereal crop'. In developing countries like Africa and Latin America, as the animal protein is very limited and expensive, which results in being unavailable to a vast sector of the population, maize grains provide about 15–56% of total daily calories in people's diets [12]. Nearly 9.09 million hectares were allocated to cultivate maize, which produces nearly 24.26 million tons in India and can be cultivated throughout the year [13]. Maize proteins consists just 1.81 and 0.35% of lysine and tryptophan content, respectively, which is very low compared with the Food and Agriculture Organization (FAO) recommendation. From the human nutrition perspective, lysine and tryptophan are the most considerable limiting amino acid in the maize endosperm protein. Thus humans and other monogastric animals should include other alternative sources of lysine and tryptophan in their healthy diets [14]. Babies fed on normal maize without any protein supplements suffer from malnutrition and develop Kwashiorkor disease [15]. In this context, the International Maize and Wheat Improvement Center (CIMMYT) and the International Institute of Tropical Agriculture (IITA) are developing varieties to improve the protein quality of maize by incorporating the opaque-2, along with modifier genes, thus increasing the amount of lysine (>4.0%) and tryptophan (>0.8%) contents in the whole grain compared with normal maize [16]. Maize cultivars containing high yield with increasing levels of lysine and tryptophan and having the kernel structure of conventional maize have the potential to reduce the malnutrition [14].

3. Storage proteins in QPM

The mature maize kernel consists of a germ, pericarp and endosperm. An endosperm consists of 90% starch which is a source of concentrated energy and 10% protein which include albumins, globulins, zein and glutelin out of which zein consists 50–70% of total proportion [10]. Zeins are the important storage proteins; these forms as deposit on rough endoplasmic reticulum-delimited protein bodies (PBs) [17]. During the maturation of kernel, these protein bodies become densely packed between starch grains in the vitreous regions of the endosperm [18]. Zeins are a group of four structurally distinct alcohol-soluble proteins (α-zein, β-zein, γ-zein and δ-zein) [17] present only in seeds' endosperm and playing a key role in storing and supplying N, C and S to the germinating seedling [12]. Among those zein proteins, α-zeins and δ-zeins are deposited in the central region, and γ-zeins and β-zeins were deposited in the outer region of protein bodies [19]. The zein fractions are rich in cysteine and methionine amino acids, and it also consists of glutamine, leucine and proline and is completely devoid of two important essential amino acids lysine and tryptophan, whereas other proteins consist of these amino acids in large quantities [20]. The zein synthesis serves as a model system to study coordinated genetic regulation of several genes expressed at very high levels at a specific developmental stage. Suppression of zein fraction without drastically altering the contribution of other fractions could be, thus, seen as a feasible approach to bring about improvements in the amino acid balance in maize grain [12].

3.1 Zein gene

Zein is a class of prolamin proteins that are mainly present in maize. All the zein polypeptides are products of different structural genes [21]. Most of the prolamin genes have a promoter element called the endosperm or prolamin box. The promoter element is present about the 300 base pairs upstream of the translation start codon and has a conserved 15-bp element that contains the 7-bp endosperm motif (TGTAAAG) [22]. This endosperm motif acts as a tissue-specific enhancer in Mr. 22,000 gene promoters [23].

Genetic analysis of o2 modifiers revealed several quantitative trait loci (QTLs) dispersed on the chromosomes. These identified QTLs were correlated with the 27-kDa γ-zein gene expression and protein quantity in QPM [24]. The 27-kDa γ-zein gene expression is not under the control of the o2 protein [25]. The o2 modifier genes involved in the 27-kDa γ-zein gene expressions are observed in two different QTLs. The first of these is associated with increased expression [26]. Single copy of γ-zein genes encodes the 50, 27 and 16-kDa proteins, which were observed in the B73 genome [27]. Based on the allotetraploidization and protein-sequence similarity, both 27 and 16 kDa γ-zein genes originated from a common progenitor [28]. It is about 20–25% of total zeins; the low abundance 50-kDa γ-zein gene has low similarity with other two γ-zein genes [27]. The γRNAi and βRNAi were involved in maize kernel opacity to increase the intensification. It reveals that opacity was not involved in reducing the thickness of the opaque-2-mutated endosperm; it is due to partial arrangement of starch granules in the endosperm [29]. Although discrete protein bodies were observed in endosperm cells, honeycomb-like masses of protein bodies were observed. It indicates that different zeins have played an important role in the endosperm development.

4. Nutrition analysis of QPMs

Generally, quality of protein nutrition was estimated by composition of amino acids, digestibility and amino acid requirement to consume the protein. The QPMs are reported to have increased levels of lysine and tryptophan in the endosperm protein, which enhances the biological value of protein similar to the milk protein. It has brought about great hope in the effort to improve human nutrition [30]. Firstly there is a significant difference in the QPM kernel when compared to normal maize kernel. Kernel hardness was determined by calculating floatation index where it is 57% for QPM, whereas for normal maize, it is 19.7%. The whole kernel protein was 13.15% in QPMs with contribution of 8.6 and 13.88% from endosperm and germ, whereas it is 9.25% in normal maize with contribution of 7.9 and 1.28% from endosperm and germ, respectively [18]. An improvement of protein quality has been correlated with the presence of the opaque-2 mutant gene [31]. Crude protein of QPM was higher than the normal maize, and the proportional contribution of the germ is lower in QPMs than with normal varieties. These structural and biochemical changes that happen in the kernel lead to the modifications of the protein profile, both in content and structure, and therefore on the functionality of the protein extracted from QPM [30]. Based on the chemical component analysis, QPM whole kernels showed highest protein content compared with normal maize [32].

5. Efforts in enhancing QPM production

5.1 Genetics of QPM

QPM contains the mutation at opaque-2 loci, which changes the protein composition of the maize endosperm, resulting in increased concentrations of lysine and tryptophan [33]. The increase in concentration (60–100%) of these two essential amino acids increased the biological value of QPM (80%), when compared to normal maize (40–57%) [34]. The biological value of cow milk protein was about 90%, whereas QPM has about 80% value [35].

QTL mapping of o2 modifiers insights that it encodes that the 27-kDa - zein protein and it is observed on chromosome-7 long arm [36]. The function of the 27-kDa zein protein in the formation of vitreous endosperm was revealed when the protein quantity increased threefold in QPM compared with soft opaque-2 mutant [37]. An increase in the number of zein proteins and their compaction between starch grains is partially involved in endosperm modification in QPM [38]. The o2 modifier genes have complexity in inheritance [12]; it reveals that several other loci control the formation of a vitreous kernel in QPM. For identifying the other factors linked to the endosperm modification, [39] performed a proteomic study of the non-zein proteins, and it was observed that the quantity of a starch synthesis enzyme and the amylopectin branching structure are changed in QPM. It is supported that QPM starch expands more than normal maize. It reveals that suppression of the opaque endosperm in QPM was associated with the starch grain properties.

Maize protein quantity can be enhanced with the opaque-2 (o2) mutation, which increases the lysine and tryptophan levels by decreasing the synthesis of zeins. The QPM utilization mainly restricts due to chalky and soft texture kernels [3]. The quality protein maize was developed based on introgression of opaque-2 QTLs, called o2 modifiers which convert to hard and vitreous endosperm [40]. QPM development has significantly improved the status of nutrient-deficient people who suffer from malnutrition and protein energy deficiency in the developing countries [41].

6. Breading efforts in QPM

Although QPM breeding has been practiced for more than 60 years, genetic mechanism and genetic components controlling endosperm modification are not clearly understood. Opaque-2 (o_2) modifier loci have been distributed on six chromosomes [26]. The opaque-2 modification is positively correlated with 27-kDa γ-zein in an F_2 population and recombinant inbred lines (RILs), which are produced through crosses between QPM and an o2 mutant as parents [42]. Gene silencing or deletion of γ-zeins eliminates 27-kDa γ-zein expression, and it eliminates the formation of vitreous endosperm [43]. Zein proteins are stored at rough endoplasmic reticulum-retained protein bodies in the endosperm [44]. For protein body formation, 27-kDa γ-zein, 16-kDa γ-zein and 15-kDa β-zein plays an important role in initiation and stabilization [19]. Zein gene knockout studies in QPM showed irregular, clumped protein bodies in lesser number and an opaque phenotype [29].

Worldwide different agricultural research centers are showing significant progress in increasing the lysine and tryptophan content in the whole grain [16]. Maize varieties' improvement and QPM conversion programs, a multi-trait selection procedure using independent selection levels has been employed to increase grain yield, resistance to pest and diseases, accumulate modifiers and improve other important traits in which QPM germplasm is defective [4]. In QPM breeding program, protein and tryptophan analysis in germplasm is an important step [45, 46]. A broad range of the CIMMYT's maize populations have been converted to QPM. This germplasm is reported to have high potential for QPM cultivar development [47, 48]. QPM with high protein quality and grain yield could be accepted by the farmers [1]. QPM germplasm has been widely used for the development of QPM cultivars with high grain yield in African countries [16]. The important problem in QPM breeding is abiotic stresses. Water stress and soil infertility are the most important stresses that reduce maize productivity in developing countries. It affects major maize yield loss in African countries [49]. High land usage affects the soil fertility and decreases the nitrogen content in the soils [50]. Global climate change could influence the soil fertility and water holding capacity, and it also affects the maize production [51].

Worldwide, a large number of normal maize hybrids have been released and commercialized. But the QPM-based germplasm is quite narrow, and significantly small numbers of genetically diverse QPM hybrids are available. Nearly 12 QPM hybrids have been released in India, compared to greater than hundred normal maize hybrids [52]. In this context, it is necessary to develop various QPM varieties across the world. Conversion of QPM through conventional breeding takes at least 10–15 years. Conversion of elite normal maize hybrids into QPM hybrids requires lesser time, initially due to tested combining ability, heterosis and adaptability of the released hybrids [53]. Opaque-2 recessive allele introgression through conventional backcross breeding of 6–7 generations is required. Through marker-assisted advanced backcross breeding, time could be significantly reduced to two backcrosses [54, 55].

The opaque-2 mutation in maize inspired the research interest, with wishes to significantly increase the nutritional status of maize consumers in developing countries. QPM, which has high lysine and tryptophan, holds the security of improving the nutritional condition of children whose main staple food is maize. It is an alternative food for protein supplement in the diet. QPM has been an alternative to the people who are using synthetic lysine and tryptophan.

6.1 QPM genotypes for stress conditions

Under stress conditions, the quality of the QPM protein does not vary, but the modifications of endosperm and the content of the proteins vary greatly. To enhance the yield of QPMS under different stress conditions is the major constrain for the breeders. Drought stress affects on QPM yield mainly in grain-filling stage [56]. Some studies reported that the supply of selenium to the plant could reduce the negative effects of the water stress conditions and is considered as the cost-efficient approach to improve the quality and yield of maize [57]. Supplying nitrogen and sulfur results in the enhanced growth and yield of QPMs [58]. Some

QPM have the potential to resist some biotic stresses that are caused by some diseases and pests, but the development of QPMs that has resistance to pest or diseases that attack the grains got more importance. Thus the CIMMYT developed the QPM varieties that are resistant to some viruses and are distributed to the National Agricultural Research System (NARS) breeders that are present at different countries in 2002 [59]. During the breading process of QPMs, multiple genes are involved in enhancing the yield of grains, whereas nonadditive gene actions are highly involved for inheritance of the trait. QPM hybrids that are evaluated under salt-, drought- and *Striga*-affected conditions showed nonadditive gene action [60]. Different varieties of QPM genotypes that adapt to the environmental conditions of sub-Saharan Africa were developed by the CIMMYT (2005), and thus great benefits for children have been documented [61]. QPM hybrids could help the poor people for elevation of malnutrition in developing countries.

7. Conclusion

There is a need for the development of QPM hybrids in developing countries for protein energy source. All the agricultural research institutes have started this QPM improvement work. Through conventional breeding methodologies, the international maize research center research team has slowly improved the original opaque-2 problems. Marker-assisted breeding is an alternative method to improve the QPM production and productivity in the developing countries.

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Conflict of interest

The author declares that there is no conflict of interest on this book chapter.

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