Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(2): 3486-3492
Received: 05-01-2018
Accepted: 07-02-2018

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Quality protein maize: An overview

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Abstract
Maize (*Zea mays* L.) is a staple food for millions of people all over the world. Breeding for improved protein quality in maize began in the mid-1960s with the discovery of mutants, such as *opaque-2*. The homozygous *o2* (*opaque-2*) mutant causes a decrease in the production of endosperm alpha-zein protein and an increase in the proportion of non-zein proteins that naturally contain higher levels of lysine and tryptophan. Maize with improved protein quality due to its superiority in lysine and tryptophan content which occurred due to *opaque-2* gene is referred to as Quality Protein Maize (QPM). As many of the negative effects of *o2* modifying genes such as poor pest resistance and starchy endosperm mutants were reported, breeders started working to identify genotypes that restore the vitreous endosperm phenotype in *o2* background. The most effective *o2* modifier genes provided the background for QPM. Marker-assisted selection (MAS) for QTLs in breeding could be undertaken in maize. The accuracy of QTL mapping can be improved by increasing population sizes and the number of testing environments, but these same techniques also improve conventional phenotypic selection. Therefore, MAS for polygenic traits is mainly restricted to situations where phenotypic selection cannot be easily implemented. The use of molecular markers offers great savings as it is not necessary to phenotypically screen the progeny for the desired character so we don't have to wait for plant to reach certain maturity level but they can be screened at young stage and also the number of backcross to elite parental lines can be reduced.

Keywords: marker-assisted selection (MAS), molecular breeding, opaque-2 gene, quality protein maize, quantitative trait loci (QTL).

Introduction
Maize (*Zea mays* L.) is very important food and animal feed crop in many parts of the world. It is one of the most important cereals in the world. A small percentage of the crop is eaten as fresh maize, which is boiled or roasted as a snack; kernels are stripped from the cob, dried, and then stored or sold for further processing (Nuss and Tanumihardjo, 2011) [24]. It is high yielding, easy to process, readily digestible and cheaper than other cereals. It is a good source of many nutrients including thiamine, pantothenic acid, folate, dietary fibre, vitamin, phosphorous and manganese (Nedi *et al.*, 2016) [22]. It is also a versatile crop growing across a wide range of agro-ecological zone. Corn's contribution to heart health lies not just in its fibre but in the significant amounts of folate that corn supplies. It also contains cryptoxanthin, a natural carotenoid pigment which has the potential to reduce lung cancer. The phenolic content in maize helps in free radical scavenging activity. Historically, demand for the grain was driven by the poultry and starch industries. But with changing food habits, the demand for food additives derived from maize is also growing. The demand for starch is strong and is growing 10 to 12 per cent every year due to rising consumption in the food and pharma industry (Murdia *et al*., 2016) [21]. After its introduction from New World explorers in the 16th century, maize quickly rooted itself as a main ingredient in local cuisine due to its relatively high grain yield, low labour requirements, and favourable storage characteristics. Nearly one-half a millennium later, maize has made a distinct imprint across African landscapes with nearly 95% of harvests used for human consumption (Nuss and Tanumihardjo 2011) [24]. Several million people in the developing world consume maize as staple food and derive their nutritional requirement from it. With high content of carbohydrate, fats, proteins, some important vitamins and minerals, maize has acquired a stature as a "Poor Man's Nutricera" however, one of the important characteristic of this cereal is low quality of storage protein due to low concentration of lysine, tryptophan and sulphur containing amino acids (Murdia *et al.*, 2016) [21]. Maize can be grown over a range of agro-climatic zones and this quality makes it a versatile crop. Maize is suitable to be grown in diverse environmental conditions which is not possible for any other crop. It is grown from 58°N to 40°S, from below sea level to altitudes higher than 3000 m, and in areas with 250 mm to more than 5000 mm of rainfall per year and with a growing cycle ranging from 3 to 13 months (CIMMYT, 2000) [5].

Storage Proteins In Maize

Several million people, particularly in the developing countries, derive their protein and calorie requirements from maize. With high content of carbohydrates, fats, proteins and some of important vitamins and minerals, maize acquired a well-deserved reputation as a poor man's nutria-cereal. Animal protein, of course being of higher quality, is scarce and expensive, thereby unavailable to a vast sector of the population. Therefore, this vast segment of human population depends upon cereals for their nutrition and livelihood (Murdia *et al.,* 2016) [21]. The maize grains contain endosperm which is rich in starch (71%). Protein from cereals including normal maize, have poor nutritional value because of reduced content of essential amino-acids such as lysine and tryptophan leading to harmful consequences such as growth retardation, protein energy mal-nutrition, anemia, pellagra, free radical damage etc. As a consequence, the use of maize as food is decreasing day by day among health conscious people (Graham *et al.,* 1990) [15]. The germ proteins are superior in quality and quantity. The endosperm of maize contains a group of four structurally distinct hydrophobic, alcohol soluble proteins called Zeins, encoded by specific class of structural genes that belong to a large gene family clustered in several genomic regions. Zeins are classified into four types based on structure i.e. α, β, γ, and δ zeins which are deposited in protein body (Esen, 1987) [8]. These four types of Zeins α, β, γ and δ aggregate in distinctive spatial pattern within the protein body. The most abundant proteins in the grain endosperm are zeins particularly, alpha zein, which are poor in lysine and tryptophan. Zeins usually account for 50 % to 70% of the endosperm protein and are characterized by a high content of glutamine, leucine and proline (Tasai, 1979) [31]. Since zeins are essentially devoid of lysine and tryptophan, they dilute the concentration of these essential amino acids from other types of endosperm protein; collectively called non-Zeins, consisting of enzymes, structural polypeptides and membrane associated proteins. In normal maize consists of various endosperm storage proteins such as:-Albumin (3%): water soluble, Globulin (3%): salt soluble, Zeins (60%) or Prolamine: alcohol soluble and Gluteins (34%) alkali soluble. In contrast, the germ protein is predominantly in the form of albumin (60%) while having a relatively small alcohol fraction (Schnieder, 1995) [28]. All fractions other than Zeins are balanced in amino acid content and are quiet rich in lysine and tryptophan. Suppression of lysine deficient gene fraction without drastically altering the contribution of other proteins could be seen as a feasible approach to bring about improvement in the amino acid balance in maize grain (Wall and Paulis, 1978) [37].

Qpm and Its Biological Value

Quality protein maize (QPM) is a maize variety that possesses significantly higher levels of two essential amino acids, lysine and tryptophan as compared to Normal Maize (NM) varieties. The higher levels of lysine and tryptophan are due to the presence of the opaque-2 gene in a homozygous recessive state which contributes to doubling the biological value of maize (Vivek *et al.,* 2008) [36]. Breeding for improved protein quality in maize began in the mid-1960s with the discovery of mutants, such as *opaque-2,* that produce enhanced levels of lysine and tryptophan, the two amino acids deficient in maize endosperm proteins. However, adverse pleiotropic effects imposed severe constraints on successful exploitation of these mutants (Krivanek *et al.,* 2007) [18]. QPM holds superior nutritional and biological value and is essentially interchangeable with normal maize in cultivation and kernel phenotype (Panda *et al.,* 2010) [26]. QPM is hard kernel *o2* varieties (Villegas *et al.,* 1992) [34]. The presence of *opaque*-2 (*o2)* gene in the homozygous recessive state (*o2o2*) is contributing to doubling the biological value of maize (Bressani, 1992) [3]. *o2* gene in recessive state is the pre-requisite for the entire process of obtaining high lysine/tryptophan maize. It is nutritionally enhanced maize that was developed by researchers from CIMMYT using two genetic systems, opaque gene and gene modifiers. The use of these two genetic systems overcame the highly complex problems that were inherent in the original soft endosperm opaque.

The biological value of protein is estimated on the average proportion of absorbed protein (nitrogen) that is needed to provide the necessary amino acids for metabolic function or for maintenance and growth. Biological value is closely related to protein quality, which in the case of maize is limited mostly due to low concentrations of essential amino acids. Several studies on children and adults have found that subjects eating QPM had significantly higher nitrogen retention than those who ate normal maize (Bressani, 1991) [4], indicating that QPM protein is more "bio-available". The biological value of QPM is about 80% which is more than wheat and rice and approximately double of normal maize and similar to the biological value of milk - 90% (FAO, 1992) [9]. Several researchers demonstrated the superior protein quality and protein digestibility of QPM over normal maize. A minimum daily intake of approximately 125 gm of *opaque-2* (*o2*) maize might guarantee nitrogen equilibrium. This could not be obtained by using even twice the amount of normal maize. The other nutritional benefits of QPM include higher niacin availability due to a higher tryptophan and lower leucine content which also helps to combat pellagra. High quality protein maize can be transformed into edible products without compromising in its quality or acceptability (De Bosque *et al.,* 1988) [7]. Further, high quality protein maize can be transformed into edible products without deterioration of its quality or acceptability, and can be used in conventional and new food products. Graham *et al.* (1989) [14] stated that '*To anyone familiar with the nutritional problems of weaned infants and small children in the developing countries of the world, and with the fact that millions of them depend on maize for most of their dietary energy, nitrogen and essential amino acids, the potential advantages of quality protein maize are enormous. To assume that these children will always be given a complementary source of nitrogen and amino acids is a cruel delusion*'. Gevers (1989) [11] indicated the potential utility of high-lysine maize in feeds for monogastric animals, and how QPM could bring in significant immediate rewards through direct industrial exploitation.

Opaque-2 and The Development Of Quality Protein Maize (Qpm)

The guiding principle in developing competitive QPM genotypes was combining the nutritional advantages offered by the *o2* mutation with the *o2* modifiers that contribute to the genetically complex endosperm modification trait. To achieve this goal, a conservative approach was initially adopted in relation to biochemical characteristics, to strike a proper balance between protein content and quality. Since the *o2* gene boosts lysine levels two-fold, efforts were devoted to maintenance rather than further enhancing the levels of lysine at protein levels of 9–10% in the whole grain. This approach greatly facilitated breeding agronomically superior QPM

genotypes, with a specific emphasis on alleviation of key problems related to grain texture of *o2* genotypes (Prasanna *et al.*, 2001) [27]. Mertz (1962) [20] proposed that maize kernels with lower zein content might accumulate non zeins which were known to have high lysine and tryptophan. This notion made Nelson (1965) [23] to propose that opaque and floury endosperm mutants might have reduced zein content. Together Nelson and Mertz demonstrated that *o2* had less zein protein and nearly double the lysine and tryptophan content of normal maize and therefore it might be useful to improve nutritional quality. In 1970s and 1980s the CIMMYT(Centro Internacional de Mezoramiento de Maiz Y Trigo) or International Maize and Wheat Improvement Centre in Mexico, established and extensive program to develop higher lysine corn from *o2* genotypes led by Mertz's student Dr. S.K. Vasal in association with Dr. E.Villegas and were awarded world food prize in 2000. Segregation and analysis of kernels with a range of endosperm modification began at CIMMYT as early as in 1969 by John Lonnquist and V. L. Asnani Modified kernels were classified into different categories and laboratory analyses were carried out to study the effects of the degree of modification on biochemical characteristics (Villegas *et al.*, 1984) [33]. Currently the only recessive gene of starchy endosperm mutants to be isolated is *o2*, which encodes a defective basic domain leucine zipper transcription factor. *o2* regulates expression of the 22kDa α zeins and several other genes including lysine ketoglutarate reductase (LKR). An *o2* mutant typically shows a marked increase in non-zein protein which in association with the decrease in lysine poor α-zein protein, leads to greater percentage of lysine and tryptophan. The loss of LKR activity results in increase level of free lysine. Several SSR markers, closely linked to more than ten gene modifiers, have been identified up to now (Wu *et al*., 2002) [40]. These genes influence 27kD gamma-zein content, elongation factor eEFI1A content and enzyme activities which control important metabolic steps in amino acid synthesis and lysine degradation. The identified markers are being introduced in breeding programs (Danson *et al*., 2006) [6].

Genetic Manipulation of Protein Quality

Opaque-2 has grain protein in the endosperm nearly twice as compared to normal maize. *o2* mutation was first described by Jones and Singleton in the early 1920s but its nutritional significance was discovered by Mertz *et al.* (1964) [20] Purdue University, U.S.A. This discovery was followed by mutation for floury (fl-2) which can also alter endosperm nutritional quality. The mutants derive their name from soft floury opaque endosperm. The development of high lysine/tryptophan maize involves manipulating three distinct genetic systems:

1. The recessive allele of the *opaque-2* gene (*o2o2)*
2. Modifiers/enhancers of the *o2o2* – containing endosperm to confer higher lysine and tryptophan
3. Genes that modify the *opaque-2*- induced soft endosperm to hard endosperm.

The presence of the *o2* gene in different maize genotypes changes the relative share of protein fractions and therefore alters protein quality (Lazic-Jancic, 1986) [19]. The homozygous *o2* mutant causes a decrease in the production of endosperm alpha-zein protein and a corresponding increase in the proportion of non-zein proteins that naturally contain higher levels of lysine and tryptophan (Gibbon and Larkins, 2005) [12]. Therefore, in a given quantity of protein from *o2o2* maize, the proportion of non-zeins is higher, which predisposes *o2* maize to have higher lysine and tryptophan concentration. In order to confer higher levels of these amino acids, the presence of another set of genes called enhancers is required. It has been shown that an increased level of gamma zein likely contributes to the recovery of a hard endosperm phenotype, given that the *o2*- modified grains (hard endosperm) have approximately double the amount of gamma zein in the endosperm as the *o2*-only mutants (Wallace *et al*., 1990) [38]. In another words, proportion of gamma zeins generally increase in *o2* germplasm, during the recovery of hard endosperm.

***Opaque-2* Modifing Genes**

Because of poor pest resistance and processing characteristics of starchy endosperm mutants, breeders worked to identify genotypes that restore the vitreous endosperm phenotype in *o2* background. The most effective *o2* modifier genes provided the background for QPM. These modifiers effectively suppress the starchy *o2* phenotype. Inheritance of these modifier genes is complex and is likely to involve several loci, which complicate the genetic analysis of QPM. Genetic map of *o2* modifiers revealed linkage with the locus encoding the 27kDa γ-zein storage protein in a region near the end of the long arm of chromosome 7. A possible role for the 27kDa γ-zein in the formation of vitreous endosperm was supported by the observation that this protein was increased 2-3 folds in QPM compared with soft *o2*. One of the most prominent changes in modified *o2* (m*o2*) endosperm was the amount of starch synthesis enzyme, and subsequent analysis of starch structure showed that amylopectin branching is altered in QPM. Some studies have demonstrated a positive relationship between the levels of 27 kDa γ-zein and kernel hardness of QPM genotypes (Wallace *et al.*, 1990 [38] and Wu *et al.*, 2010) [41]. Because 27 kDa γ-zein appears to facilitate PB formation, it was postulated that an increase in the number of PB and their compaction between starch granules is responsible for endosperm modification in QPM. To find other factors contributing to the vitreous phenotype in QPM, Gibbon *et al.* (2003) [13] conducted a proteomic analysis and found a higher accumulation of granule bound starch synthase in CM105 m*o2* (QPM) compared with its unmodified *o2* counterpart. They also observed that starch granules in CM105 m*o2* associated with one another and the spaces between them were filled in vitreous areas of the kernel, which suggested a mechanism that complements that postulated for the 27 kDa γ-zein. The consequence of this difference is that QPM starch swells more than normal and starch granule in mature endosperm were associated with one another. The starch granules in an *opaque* kernel (*opaque*) have spaces between them, and they show no evidence of prominent contacts between one another. However, starch granules in the endosperm of a m*o2* kernel (modified) have material that completely fills the spaces between them in the vitreous region of the endosperm (located in the lower part of the image); granules in the starchy regions also have substantial contacts between them.

Utilization of *Opaque-2* and Its Challenges

The discovery of high lysine mutation in maize aroused great hope and considerable interest world wide and many believed that it would soon lead to development of nutritionally enhanced cereals. In initial stages both *o2* and *fl2* genes were used singly or in combination with each other. But as some undesirable effects of *fl2* gene were discovered, its use slowed down and discontinued. The euphoria over the discovery of

o2 and its direct utilization in breeding programs was soon tampered with the realization that pleiotropic effect of this mutation-soft endosperm with damaged kernel and inferior food processing could not be easily tackled (Toro *et al.*, 2003) [30]. These features result in a soft, chalky endosperm that dried slowly making it prone to damage, a thick pericarp, more susceptibility to diseases and pests, higher storage losses and also affects harvest ability. Since the kernel weight is reduced due to less density per unit volume as starch is loosely packed with lot of air spaces, there is corresponding decline in the yield (Singh and Venkatesh, 2006) [29] which can be almost to the tune of 10 percent or above. Especially in developing countries, where farmers are accustomed to hard flint and dent grains, the kernel appearance of such mutants made it less ideal for large scale use and adoption in target areas (Nedi *et al.*, 2016) [22]. The mutations that alter grain protein synthesis cause changes in texture of grains. The early opaque-2 (o2) mutants had reduced levels of α-zeins resulting in small unexpanded protein bodies (Geetha *et al.*, 1991) [10], whereas, o15 that reduces -zeins leads to smaller number of protein bodies. Other mutations such as floury-2 *(fl-2), Mucronate (Mc)* and defective endosperm (De B30) result in irregularly shaped protein bodies. In order to overcome these apparent limitations for large scale use of such mutants, efforts were directed towards identification of alternative mutants that did not carry such disadvantages.

The search then continue to detect new mutants which resulted in *opaque-6, opaque-7, opaque-11, floury-1, floury-3*, and other mutants but none offered any additional advantage over *o2* in maize breeding programme. Under the dynamic leadership of Dr. S.K. Vasal, researchers at CIMMYT, and University of Natal, RSA, various endosperm modifier genes were identified that could favorably alter the grain characteristics to overcome an important obstacles in popularization of high lysine *o2* maize.These modifier genes do not have any effect of their own and interact to improve the kernel hardiness and appearance and increase kernel weight and density. A large number of reports followed wherein varying degree of endosperm modification were observed (Paez *et al.*, 1969) [25]. This end up with the idea that such endosperm modifier gene could be used along with *o2* gene either singly or in combination with other mutant such as sugary2 (*su2*) in order to have acceptable characteristics in the final product.Such combination resulted in maize line that possessed high lysine, vitreous grain and better protein digestibility. A major challenge with QPM is the dissemination of the material into the farmer's field. The dissemination and adoption of QPM is still lagging behind normal endosperm maize especially in regions such as sub-Saharan Africa where it is needed most including Ethiopia (Aman *et al.*, 2016) [1]. Unfortunately, in the early 1990's the CIMMYT QPM breeding program was discontinued and as such the critical step of promoting this improved material was also severely limited. Since the late 1990's however, the Nippon Foundation of Japan and then later the Canadian International Development Agency (CIDA) have funded the continued improvement and promotion of QPM in several developing countries (Nedi *et al.*, 2016) [22].

Qpm Germplasm and Donor Stock

Initial efforts towards development of QPM donor stock were difficult. Selection for kernel modification had to be practiced at all stages, while simultaneously maintaining protein quality. Two approaches were efficiently used in developing QPM donor stock (Vasal, 1999) [32]:

1. The first was intra population selection for genetic modifiers in *o2* background exhibiting a higher frequency of modified *o2* kernels.
2. The second approach involved recombination of superior hard endosperm *o2* families. Selection for modified ears, showing high frequency of modified kernels with good protein quality was practiced for 3-4 cycles. By mid 1970s a high degree of endosperm modification was achieved in these materials and genotypes were ready for utilization as QPM donor stock.

The development of QPM donor stock led to large scale QPM germplasm development efforts in different genetic backgrounds representing tropical, sub-tropical and highland maize germplasm involving different maturities as well as grain colour and texture. Potentially useful normal maize populations were identified for QPM conversion program. Due to the complexity and nature of modified phenotype trait, it was realized that a standard backcross program will not work; therefore modified backcrossing cum selection program was done. Many advanced maize populations in CIMMYT maize program were converted to QPM using this procedure. Several different ways was used in forming and improving these broad base QPM gene pools however, these two procedures were used in CIMMYT's work on QPM:

1. Recombination of hard endosperm *opaque-2* inbred or non-inbred families selected independently in each of the *o2* population. Controlled population by hand or alternatively half sib isolation can be used.
2. Crossing of normal genotypes to one or more QPM donors followed by advancing F1 crosses to F2 controlled by sib mating or in an isolation by planting balanced bulk and/or individual ears in a half sib recombination block.

As QPM program succeeded in developing huge volume of germplasm it was important to plan strategy that will help in reducing germplasm volume and also permit systemic and efficient handling of this valuable germplasm. Knowledge of the germplasm as well as colour and maturity considerations was the principle guiding factors in this merging process.

An initiative on QPM hybrid breeding at CIMMYT was made in 1985, as the QPM hybrids offer several advantages in relation to

a) Exploitation of heterosis
b) Ease in maintaining seed purity in contrast to open pollinated variety (OPV).
c) Uniformity and Stability in kernel modification in hybrids
d) Requirement for minimum protein quality monitoring as long as the purity of parental line is ensured.

Breeding Qpm

For breeding QPM mainly three components are required i.e. good promising non-QPM germplasm, good QPM donors (having good penetrance, expressivity and no linkage drag) and good testers for testing its combining ability. Breeding programs should start by converting elite non QPM inbred and open pollinated varieties (OPV) to QPM by backcrossing or by pedigree method. Regardless of the breeding method and approach 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 light 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. percentage of tryptophan and protein in a sample, through laboratory analysis Vivek *et al*. (2008) [36]. The most important aspect in developing acceptable QPM lines is to combine the nutritional advantage offered by the *o2* mutation with the *o2* modifier along with acceptable agronomics traits like grain yield, resistance against disease and grain virtuousness. During 1980 CIMMYT took initiative to convert a number of non QPM genotype to QPM genotype. They followed a modified back crossing – cum–recurrent selection. Thereafter CIMMYT initiated development of QPM hybrid in 1985. After three decades of intensive research the team led by Vasal developed a number of QPM lines / hybrids that revolutionized maize cultivation around world. This contribution later recognized by conferring the "World Food Prize" on vassal and villages in 2000.India is one of the first few countries to focus on *o2* maize 14 and has released three *o2* composites, namely Shakti, Ratan and Protina in 1970 followed by one modified superior *o2* composite Shakti1 in 1997 (Table. 1).

Table 1: QPM cultivars released for commercial cultivation in India

Cultivar	Pedigree	Year	Centre
Shakti	Composite	1971	AICRP
Rattan	Composite	1971	AICRP
Protina	Composite	1970	AICRP
Shakti 1	Composite	1997	DMR
Shaktiman1(Hybrid)	(CML 142 × CML 150) × CML 186	2001	RAU, Dholi
Shaktiman2 (Hybrid)	CML 176 × CML 186	2004	RAU, Dholi
HQPM 1 (Hybrid)	HKI193-1 × HKI 163	2005	CCS HAU, Karnal
Shaktiman3 (Hybrid)	CML161 × CML 163	2006	RAU, Dholi
Shaktiman4 (Hybrid)	CML161 × CML 169	2006	RAU, Dholi
HQPM 5(Hybrid)	HKI 163 × HKI 161	2007	CCS HAU, Karnal
HQPM 7(Hybrid)	HKI 193-1 × HKI 161	2008	CCS HAU, Karnal
Vivek QPM 9(Hybrid)	VQL 1 × VQL 2	2008	VPKAS,Almora
HQPM 4(Hybrid)	HKI-193-2 x HKI-161	2010	CCS HAU, Karnal

Light Table Selection

Light table selection (selection for desired level of modification/opaqueness in the kernel) is done to pick out kernels with the *o2o2* recessive genotypes by using the degree of opaqueness as an indirect measure or secondary trait. Due to segregation of genes for endosperm hardness varying levels of opaqueness are observed on a light table. A kernel with *o2o2* genotype (soft endosperm) is seen as complete opaqueness, while kernels with *O2o2* heterozygous or *O2O2* dominant genotypes (hard endosperm) are translucent. Gradation in the opaqueness is visually assessed on a 1 to 5 scoring scale. The scores are as follows:

Type 1: not opaque, Type 2: 25% opaque

Type 3: 50% opaque, Type 4: 75% opaque

Type 5: 100% opaque

Less opaqueness implies higher/more action of modifiers. Types 1 to 3 would be 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 o*2o2* 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 generation.

Qpm Quality

The samples are usually first sent to the laboratory for protein content and tryptophan analysis at the F_3 or F_4 stage (before the first test cross). Both lysine and tryptophan concentrations are increased in QPM, but only tryptophan is analyzed on routine basis, because lysine and tryptophan are highly correlated and, normally, the value of lysine is four times that of tryptophan. Due to the well-established relationship between these amino acids in the protein of *opaque-2* maize endosperm (Hernandez and Bates, 1969 [17]; Villegas *et al*., 1992) [34], tryptophan can be used as a single parameter for evaluating the nutritional quality of the protein. When interpreting the results of laboratory analysis for making selections, the protein, tryptophan and quality index (QI - tryptophan to protein ratio in the sample) have to be above the acceptable limits described in Table 3 (Vivek *et al*, 2008) [35].

Relationship between Quality Index, Protein Quantity, and Protein Quality

Quality Index (QI)

The quality index system is the tryptophan-to-protein ratio in the sample, expressed as a percentage as shown in table 2. For example: Tryptophan in sample = 0.08%, Protein in sample = 10% and QI = 100*0.08/10, which is = 0.8.

Table 2: Showing protein, lysine and tryptophan (Vivek *et al*., 2008) [35]

		QPM	Non-QPM (%)
In protein	Protein	>=8	>=8
	Lysine	4	2
	Tryptophan	>0.65	< 0.60
		Whole grain	Endosperm
In sample	Tryptophan	> 0.075	>0.07
	Quality index	> 0.8	>0.7

The plant whose trait is required in another plant is generally made male and the one who receives is the female. The map distance and the order of markers on linkage group can be calculated using the BC1 algorithm of the MAPMAKER/Exp30. The selection of BC_1 individual can be carried out on the basis of: (i) Selection of heterozygotes for o *opaque-2* gene, specific for SSR markers (ii) Double recombinant was selected from selected plants.

Molecular Marker Assisted Selection (Mas) For Improvement Of Quality Maize Protein (Qpm)

The development of QPM requires manipulation of various genetic systems such as *o2* endosperm modifiers and amino acid modifier and as such conventional breeding procedure are quite laborious and result sometime frustrating.It is very tedious to continuously select for optimum level of one trait while maintaining desire level of other. The conventional backcross strategy for conversion of normal maize to QPM suffer from two problem one that being a recessive trait which needs selection has to be carried out at each backcross in order to fix the recessive *o2* allele, prior to selection for endosperm modification thereby extending the time period for line conversion. Moreover quality trait such as grain protein content cannot be selected prior to seed formation making screening very difficult besides low cost and reliable method of screening are not available.

Marker assisted selection (MAS), also known as marker assisted-breeding, (MAB), is the use of molecular markers as tools in a plant or animal breeding programme. MAS makes easy to select and breed individuals with the desired traits for the next generation. MAS can improve the accuracy and speed of the selection process in a breeding programme. MAS can thus be defined as a laboratory process that uses genetic testing to dispense with testing the performance of every individual in the field. MAS use genotyping tests that can be carried out at an early stage in the breeding programme. This enables the identification of individuals with the desired trait(s) and selecting without waiting for plants to mature for assessment in the field. MAS is an appropriate technology for the trait such as high lysine in endosperm in maize and can be a cost effective procedure for selecting *o2* locus in breeding programme with sequencing of maize genome being finished a large number of marker system are now available that are associated with o2 and endosperm modification phonotype.

An appropriate application of such markers greatly enhances the efficiency of selection for improvement of grain protein in maize besides cutting down at cost and time. Both foreground and background MAS can be effectively employed for selecting *o2* phenotype ensuring maximum recovery of recurrent problem. Babu *et al*. (2005) [2] used MAS for development of QPM parental line of vivek-9 hybrid and developed QPM in less than half the time required through conventional breeding. Danson *et al.* (2006) [6] used various markers to introgress *o2* gene into herbicide tolerant elite maize inbred lines. They found that using markers for QPM and endosperm modification can greatly enhance the selection efficiency for isolating fully modified kernel in QPM background reviewed enthusiasm in our endeavor to make QPM of that real potential use.

Development of Qpm Hybrid Through Mas

As compared to the period required for development QPM hybrid through conventional method of back crossing, MAS was the method of choice, as few molecular markers was already known with in the *o2* gene and these markers were capable of detecting the *o2* gene in heterozygous state. To convert normal maize hybrid into a promising QPM hybrid viz. Vivek Maize Hybrid (Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora). This hybrid was developed by crossing two inbreds *viz.*, CM 212 and CM145, which were used as recipient of *o2* gene. Donor inbred line CML 180 and CML 170 were obtained from CIMMYT. Gupta (2012) [19] converted CM 212 and CM 145, the normal maize inbred into QPM inbred, VQL 1 and VQL 2 respectably.VQL 1 showed 92.0%, of the recipient genome (CM212), whereas VQL 2 possessed 94.44% genome of the recipient parent (CM 145). The two converted QPM inbreds were crossed to recover QPM hybrid, Vivek QPM show 41% increase in tryptophan and 30% increase in lysine over original hybrid.

QTLs and Associated Molecular Marker

Various molecular marker methods allow the organism DNA sequence to be mapped onto regions of their genome. Using these maps, putative DNA sequences affecting traits of interest can then be detected by testing for statistical associations between the DNA sequence variants (of a particular marker) and any trait of interest. Though not necessarily genes themselves Quantitative Trait Loci (QTL) are stretch of DNA that are closely linked to the genes that underlie. Information about QTLs can be used in a number of ways to increase heritability and favorable gene action. QTLs and their associated molecular markers often account for a greater proportion of the additive genetic effects than the phenotype alone. Furthermore, knowledge of genetic architecture can be exploited to add or delete specific alleles that contribute to breeding value. When either genetic linkage or epistasis among loci with antagonistic effects on a trait limits genetic gain, QTL information can be used to break these undesirable allelic relationships. When QTLs have successfully been identified and validated, they can be applied to a breeding programme to select for the traits of interest.

The basic steps in a MAS breeding program are:

1. Screen the population for traits of interest
2. Construct genetic linkage maps
3. Identify the molecular markers that are linked to the trait of interest
4. Test the applicability and reliability of the markers in predicting the traits (marker validation)
5. Develop a high throughput and reproducible genotyping facilities for screening large number of samples in a time and cost effective manner
6. Implement MAS into the breeding program (monitor the introgression of desirable traits in the program)

Conclusion

One of the current grand challenge in plant biology remains identifying those gene combinations that lead to significant crop improvement. Efforts are being made by the NARS for conversion of elite, local inbred lines into QPM versions. Recent research on improved protein quality and modification of kernel texture in QPM has created the opportunity for rapid progress in understanding the mechanisms underlying these traits. Integrating molecular marker technologies such as MAS into breeding strategies could become increasingly important in the coming years. With the development and access to reliable PCR based markers such as SSRs and SNPs, in several crop plants, efficiency of genotyping large populations or breeding materials has significantly increased.

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