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Quality protein maize: The story of Bio-fortification

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Abstract
Maize is one of the most important staple crops to the world especially in the areas like Sub Saharan Africa and South East Asia. Despite of that much importance in the daily diet of some part of the population, it lacks Tryptophan and Lysine, which causes malnutrition, disease like Kwashiorkor and impaired development of skeleton. So, to eradicate this problem the process of bio-fortification is needed. CIMMYT plays a great role in that, to produce the maize varieties with good content of lysine and tryptophan along with other desirable agronomic characters like hard kernel and good yield potential. This process of bio-fortification is the result of using the mutant *opaque 2*. This new type of maize is also very easily replaceable because of its same phenotypic appearance to the normal maize. It also shows good result in the nutrition profile of the children in places like Ghana and Ethiopia.

Keywords: Protein, maize, story, Bio-fortification, Tryptophan

1. Introduction
Maize (*Zea mays* L.) is called as the queen of cereals because of its extensive uses as human food and animal feed (corn meal) (Gupta *et al.* 2009) [18]. It is a C4 crop which belongs to the Gramineae family. Cereals like maize is often become the main source of nutrition, especially in the areas with poor financial condition or underdeveloped *viz.* Africa, Latin America and some parts of Asia (Micic *et al.* 2013) [21]. In the countries of sub-Saharan Africa (SSA) more than 50% land of cereals is under maize cultivation (Masuka *et al.* 2017) [33]. For the people of eastern and southern Africa maize is responsible for 43% of the total protein and 45% of total calories in daily diet (Shiferaw *et al.* 2011) [52]. 5% lysine is optimum for cereals for the nutrition purpose but cereals (e.g., maize) contain only 1.5-2% lysine in general (Young *et al.* 1998). Not only as human food, maize is also a important crop for the livestock. In the developed countries 78% of animal feed comes only from maize (Sofi *et al.* 2009) [53]. The daily diet of an African is so much dependent on maize, for them one fifth of daily calories are dependent on maize alone (Krivanek *et al.* 2007) [26].
For the improvement of the nutritional quality of maize and to manage the food security, CIMMYT was conducting research on this for decades. They have developed quality protein maize (QPM) which possess two-fold of lysine and tryptophan and better in biological value than the non QPM genotypes (Wegrey *et al.* 2014). Quality protein maize was evolved in around late 1960s (Prasanna *et al.* 2001) [44], the newly developed maize contains almost 70-100% more lysine or tryptophan content than the normal maize (Bjarnason *et al.* 1992) [56]. Researchers were found to increase the lysine and tryptophan content of maize opaque 2 (*o2*) gene mutations are needed (Mertz *et al.* 1964) [36]. Increases in the content of that amino acid can double the biological value of protein (Bressani, 1992). Although, there is a problem with this allele is that, due to this opaque 2 gene maize grain yield was reduced (grain weight reduced upto 15-20%) and it is also susceptible to many diseases and insects. These opaque 2 also causes dull and chalky maize kernals, which problem is solved by CIMMYT (Vasal 2001) [44]. The intake of QPM instead of normal maize is so much effective for the human and livestock to meet the daily need of nutrition uptake especially for the protein (Mbuya *et al.*, 2010; Rajendran *et al.* 2014) [35, 46]. The bio-fortified QPM is easily replaceable to the normal maize in field as well as the food habit of the people of area like Sub Saharan Africa (Nuss and Tanumihardjo, 2011) [41] because of its same phenotypic appearance and the agronomic performance in the field condition (Bello *et al.* 2014) [2]. In accordance to the report of Mbuya *et al.* (2010) [35] in Mexico, Central America and China QPM reduced the protein-energy undernutrition (PEU). Not only in the nutrition level but also QPM resulted 10% more yield than the non QPM varieties in China (Mbuya *et al.* 2010) [35].

It has been reported that the opaque 2 protein (QPM protein) responded well to the diet of kwashiorkor affected children in the Ghana and Ethiopia (Krivanek *et al.,* 2007; Nuss and Tanumihardjo, 2011) [26, 41]. On one research in rats, the group of rats fed with 90% QPM for 28 days gained 97g on an average in respect to 27g in case of non QPM fed rats Krivanek *et al.* 2007; Boateng *et al.*, 2012) [26]. 100g QPM is enough to for children and 500g is adequate for the adults to maintain the requirement of lysine as well as the daily protein level of the human body (Nuss and Tanumihardjo, 2011) [41].

2. Breeding strategies for the development of QP

2.1. By using mutants

The potential of mutants for increasing the amino acid content like lysine can be useful, comes under the radar after the discovery of Purdue University of mutant alleles like *opaque 2* (*o2*) and *floury 2* (*fl2*) in the early 1960's (Mertz *et al.*, 1964; Nelson *et al.*, 1965) [36, 40]. These mutants increase not only the lysine and tryptophan content but they also decrease the leucine, glutamic acid and alanine, where decrease in leucine content is a desirable character because it balanced the leucine-isoleucine ratio. Although those mutants degrade the kernel quality as well as the agronomic performance in terms of the yield and resistivity to some diseases like ear rot (Vasal SK, 2000) [55]. During that decade they also found some other mutants like opaque 7, opaque 6, floury 3 but those mutants does not give any expected result in terms of quantity or quality. The breeders of Centro Internacional de Mejoramiento de Maiz y Trigo or CIMMYT had done recurrent selection for high Lysine content but failed to get appreciable results (Vasal SK *et al.*, 1980) [56].

2.2. By using double mutants

After so much of exploration in single mutant, scientists of CIMMYT move towards the use of double mutant. They use *floury 2/opaque 2* double mutant combination, which shows vitreous kernel but it was reported that it shows vitreous kernel but only at rare case. The *sugary 2/opaque 2* mutant combination also shows vitreous kernel, less ear rot, promising kernel appearance, better digestible protein but those double mutant cultivar results smaller kernel size and reduced the economical yield upto 15 to 25% (Vasal SK, 2000; Vasal SK *et al.*, 1980) [55, 56]. The combined use of opaque 2 and the genetic modifiers of the opaque 2 gene double mutant gives the best result among the other double mutant (Vasal SK, 2000) [55].

To increase the grain yield of that they took their main focus towards increasing the germ size. By increasing the germ size, they targeted increase in protein quantity and the quality of the maize. In that case they face a challenge that increase in the germ size of maize kernel alone is quite difficult without affecting the other important traits. Selection for that character is done only if the selection process is done simultaneously with the other important traits (Vasal SK., 1980) [56].

2.3. By developing QPM donor stocks

Development of QPM donor stocks which contain modified kernel along with good protein quality was became the main aim. In the process of QPM donor stocks they used two basic approaches; first one is selection in intrapopulation for the genetic modifiers in opaque 2. They have chosen Four tropical populations and a highland population for getting the higher modified *opaque 2* kernels. For the first cycle they used full sib pollinations. The second cycle was done by the modified ear to row system which is suggested by Lonnquist (Lonnquist JH *et al.,* 1964) [28]. The second approach for the development of QPM donor stocks was recombination of superior hard endosperm of *opaque 2* families. Selection for modified ear and kernel with good protein quality for three to four generations. For the development of large-scale germplasm of QPM donor stocks of different genetic backgrounds like the germplasm of tropical, subtropical and highland areas they didn't go for conventional backcross method. The used method was termed as 'modified backcrossing cum recurrent selection' in CIMMYT (Vasal SK *et al.*, 1980; Vasal SK *et al.*, 1984) [56, 58]. This new process made many QPM from the many advanced maize populations on giving importance kernel modification, high yield and happening of lesser ear rot disease and rapid drying. After all this hard work they got success in developing the QPM with good kernel appearance, less ear rot along with high yield (Vasal SK *et al.*, 2000) [55]

3. Nutritional bio fortification

Normally maize grain contains a good amount of starch (71%), which is present in the endosperm. A protein called Zein is present in its embryo and endosperm part, which is soluble in alcohol (Prassana *et al.* 2001). Some hydrolytic enzymes are present in the outermost layer (aleurone layer) of the grain, where the protein (Zein) is present in the vitreous region (Gibbon and Larkins, 2005). Zein proteins are mainly in the form of albumin, glutelin, globulin and prolamin (Shewry and Halford, 2002) [51].

3.1. Improving protein quality in QPM

Protein quality of the QPM is improved by using new approach- increasing the lysine content of the protein. This approach gives an opportunity that it has no bad effect in the texture of the grain during the incident of o2 maturation. Some proteins which are lysine rich those are protein Z (7.1%), β amylase (5%), chymotrypsin inhibitors CI-1 (9.5%) and CI-2 (11.5%) (Sofi *et al.* 2009) [53]. Increasing of maize grain protein by using potato pollen is also done, it increases 50% of maize protein and lysine (Yu *et al*. 2004).

Increasing the amount of amino acids is also an approach for increasing the protein content of Maize though increasing the free amino acids offers a very less proportion of the increase in grain protein. In the higher plants such as Maize the essential amino acids like lysine, methionine and threonine are synthesized from aspartic acid (Zhu *et al.* 2007) [70]. A promoter named globulin-1 which is present in the embryo and the aleurone layer of the maize regulate gene *Corynebacterium* DHPS results in increase the free lysine 50-100% (Mazur *et al*. 1999) [34]. In contrast when this gene is expressed by glutelin-2 promoter in the endosperm, it doesn't show any increase the free amino acids. A bacterial insensitive called E. *coli* or *Corynebacterium* DHPS in the Arabidopsis plant resulted 12-fold increase in the lysine content of grain (Zhu and Galili, 2003) [71]. In the year of 2006 Monsanto releases a variety with high lysine content which is also developed by using feedback insensitive DHPS gene globuline-1 gene promoter driven *Corynebacterium*.

It has been observed that there is a pleiotropic effect of the gene opaque-2. It was also observed that alteration in the composition of amino acid affects the starch organization of kernel which makes it softer and unpleasant in taste (Tripathy, 2019) [54]. As a solution of this problem, there is a set of modifier genes (QTLs), which improves the kernel hardness (Moro *et al.,* 1995) [37] and also make that more vitreous (Burnett and Larkins, 1999; Ortega and Bates, 1983; Bjarnason *et al.*, 1976) [6, 42, 3] with accumulation of protein (Zhou *et al.,* 2016) [69]. The modified opaque 2 mutants increased the γ-zeins to 27 KD (2 to 3 times than previous) and decreased α-zeins at 22KD (Geetha *et al.*, 1991) [14]. After that a series of amino acid modifier genes found that can improve the lysine and tryptophan content (Krivanek *et al*., 2007) [26].

3.2. Marker assisted selection for breeding

There is always have an increasing demand in case of cereals, to meet this ever-increasing demand of maize grain a breeding strategy is needed (Ribaut *et al.* 2003) [47]. The stream plant biotechnology offers a process named Marker assisted selection (MAS) to develop new varieties with good quality and higher yield potential (Varshney *et al.* 2012). Using of only conventional approach for improving the new QPM cultivars is very much time taking process and also a very hectic task to do because of the involvement of recessive genes like *opaque 2* and *opaque 16*. Introgression of *o2* gene and *o16* gene by backcrossing is not only a six-generation long process but also it demands a check after each generation due its recessive nature and there is also a need of lysine and tryptophan biochemical test in each of the generation which is laborious as well as time consuming (Collard *et al.* 2005) [8].

For those non neglectable reasons for selection of *o2* gene Marker assisted selection (MAS) is more suitable in recent condition (Dreher *et al.* 2003) [12]. For the improvement in grain protein of maize application of markers offers great efficiency to the selection process along with low costing and less time. Vivek-9 hybrid of QPM is developed by using the marker assisted selection process (Babu *et al.* 2005) [1] and this is developed in half of the time conventional breeding strategy takes. In the research of Danson *et al.* (2006) [1] for making an elite variety of QPM which is herbicide tolerant they used different types of markers for the introgression of *o2* gene and got efficient result in the selection procedure.

There are mainly two steps involved for Marker assisted selection, those are: (1) foreground selection and (2) background selection. The first step is for targeting the gene with markers and in the second step for the recovery of recurrent parental genome (RPG), has to target the markers which are uniformly distributed (Hospital *et al.* 1992) [19]. The first step foreground selection helped in the identification of the gene of interest where the rate of recovery of the RPG by two backcrosses is done in the second step background selection (Visscher *et al.* 1996; Hospital *et al.* 1992; Frisch *et al.* 1999) [59, 19, 13]. There are many DNA based markers are available but Simple Sequence Repeat (SSR) is always preferred for its low cost along with the effectiveness. It is a codominant type of marker which is uniformly distributed inside the plant genomes (Powell *et al.* 1996). Three gene based SSR markers (phi112, phi057, umc1066) were used for the characterization of the *o2* gene (Schmidt *et al.* 1987; Kassahun and Prasanna 2003 and Motto *et al.* 1988) [50, 24].

Vivek hybrid-9, an early maturing normal hybrid is converted from normal maize to QPM by using MAS later which is named Vivek QPM 9 (Shown in figure below) (Gupta *et al.* 2013) [17]. This Vivek QPM 9 contains 41% more Tryptophan and 30% more Lysine than the Vivek hybrid-9 (Hossain *et al.* 2019) [62]. Three widely used maize hybrid also reconstituted and improved their lysine content upto 48-74% and tryptophan upto 55-100% by using MAS, from the year 2017 those are also been marketed as commercial variety with name Pusa HM 4 improved, Pusa HM 8 improved and Pusa HM 9 improved (Yadava *et al.* 2017) [62]. Introgression of *o2* allele into the inbred lines is also used for the improvement of new QPM hybrid is also been reported in different countries of the world (Kostadinovic *et al.* 2016; Jompuk *et al.* 2011; Magulama and Sales 2009; Danson *et al.* 2006; Manna *et al.* 2006) [25, 23, 29, 1, 31]. In China a half folds of lysine content increase were reported by pyramiding of *o2* and *o16* genes in the new progenies (Zhang *et al.* 2010, 2013; Yang *et al.* 2005) [67-68]. The renowned institute of India IARI also come with some QPM commercial hybrids by using inbred lines like HQPM 1, HQPM 4, HQPM 5 and HQPM 7 by the introgression of pyramided *o2* and *o16* gene (Sarika *et al.* 2018) [49].

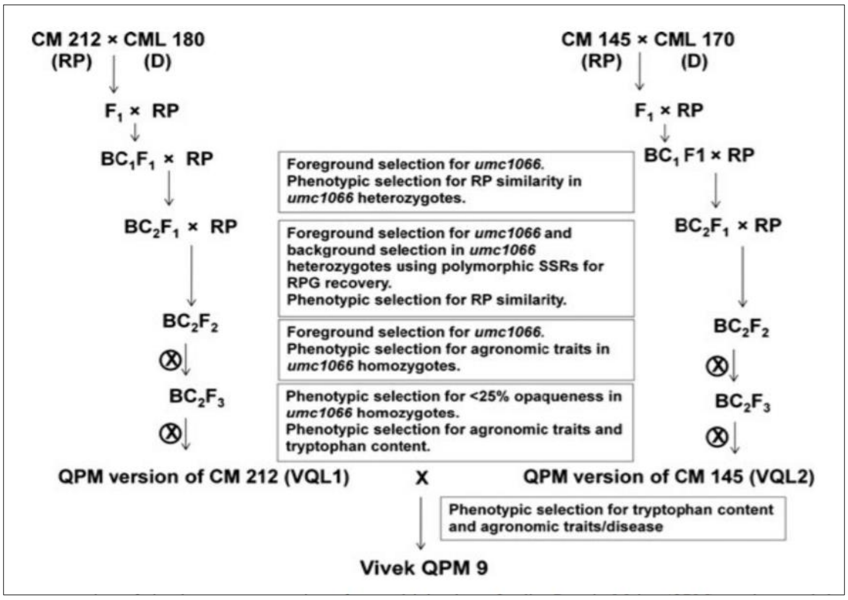

Fig 1: A diagrammatic representation of developing QPM hybrid Vivek QPM 9. (Source: Gupta *et al.* 2013) [17].

Table 1: Evolution of QPM genotypes by using Marker assisted selection in different parts of the world.

Sl. No.	Country	Introgressed gene	Inbred or hybrid	References
1.	India	opaque 16	Inbred	Sarika *et al.* 2017 [48]
2.	China	opaque 16	Inbred	Yang *et al.* 2013 [63]
3.	India	opaque 2	Inbred	Babu *et al.* 2005 [1]
4.	Thailand	opaque 2	Inbred and hybrid	Jompuk *et al.* 2011 [23]
5.	Kenya	opaque 2	Inbred	Danson *et al.* 2006 [9]
6.	Uganda	opaque 2	Inbred	Manna *et al.* 2006 [31]
7.	India	opaque 2, opaque 16	Inbred and hybrid	Sarika *et al.* 2018 [49]

3.3. Introgression of micronutrients

For the improvement of provitamin A (pro A) to improve its nutritional benefit MAS has been used, it results increase of 1-2 ppm to about 8.15 ppm (Zunejare *et al.* 2017). In India world's first commercial pro-A riched QPM cultivar was released which named as Pusa Vivek QPM-9 improved (Yadava *et al.* 2017) [62]. In another research *o2, crtRB1* and *lcyE* genes are introgressed in some QPM hybrids (HQPM 1, HQPM 4, HQPM 5, HQPM 7) for improving the pro A around 9.0-12.9 ppm. After Vitamin E also introgressed to those pro A enriched parental lines by using MAS, introgressed VTE4 allele (Das *et al.* 2018) [10]. Enriching iron and zinc inside the QPM cultivars is also been reported in different parts of the country over that time (Mallikarjuna *et al.* 2015; Gupta *et al.* 2015; Pandey *et al.* 2015; Chakraborti *et al.* 2011) [30, 16, 43, 7].

Table 2: Events and developments of QPM varieties on year basis

Year	Work or achievements	References
1970	Three *opaque 2* composite varieties released by India- Rattan, Shakti and Protina. But those have poor agronomic performances and kernel type.	Dhillon and Prasanna, 2001 [44]
1960-1970s	The *opaque 2* hybrids are experimented and grown in in some countries like USA, Brazil, South Africa, Colombia, India and Hungary.	Prasanna *et al.*, 2001 [44]
1980-1990s	Many QPM were developed from the normal germplasm by CIMMYT which are as good as the non QPM varieties in terms of yield and different agronomic performances.	Vasal, 2001 [44]
1990s	Two CIMMYT scientists Dr. S.K. Vasal and Dr. E. Villegas used opaque 2 with genetic modifiers which results the desirable hard kernel along with good tryptophan and lysine content in QPM. This is the result of their 35 years of hard work.	Vasal, 2001; Prasanna *et al.* 2001 [44],
1997	Two QPM cultivars were commercialized, BR451 and BR473.	Vasal 2000 [55].
1998	Variety Shakti 1 was released by India, it contains desirable agronomic traits and performance.	Dhillon and Prasanna, 2001 [44].
2000	QPM hybrids Shaktiman 1 and Shaktiman 2 was released by CIMMYT.	Prasanna *et al.* 2001 [44].
2000	HL 1, HL 2, and HL 8 was released by South Africa which shows good agronomic performances and tolerance to some diseases.	Vasal 2000 [55]
2000	In Ghana more than 50% of maize cultivated area was covered by QPM variety (OBTANPA).	Vasal 2001 [44]
2003	Across the world, 23 countries released QPM varieties.	Nedi *et al.* 2016 [39]
2003	In total about 3.5 million hectares and in Mexico alone about 2.5 million hectares were under QPM cultivation.	Nedi *et al.* 2016 [39]
2008	Vivek QPM 9 was released by India, this is the which have 30% more lysine and 41% more tryptophan than Vivek hybrid 9.	Gupta *et al.* 2013 [17]
2015	Some QPM inbred lines were registered, those are BQPM 9, BQPM 12, BQPM 15, BQPM 10, BQPM 14, BQPM 17, BQPM 16, BQPM 11, BQPM 13	Worral *et al.* 2015[61]
2015	16 QPM hybrids and 4 OPVs were released from South Africa	https://www.nda.agric.za/docs/statsinfo/Preliminary. htm
2017	Hybrid named, Pusa HM 4 Improved, Pusa HM 8 Improved and Pusa HM 9 Improved was released which are developed by back cross breeding.	Hossain *et al.* 2018 [10]
2015-2019	CIMMYT released 39 QPM varieties in the span of those 5 years.	CIMMYT's Annual CRP Maize Report, 2015, 2016, 2017, 2018 and 2019.
2019	In Asia and China alone about 250000 ha area is under the cultivation of QPM varieties.	Maqbool *et al.* 2021 [32]
2020	QPM hybrid ZPQPM13 showed promising result in temperate environment in terms of yield and protein content.	Ignjatovic- Micic *et al.* 2020 [22]
2020	By using the conventional breeding methods more than 40 varieties of QPM developed and released.	Prasanna *et al.* 2020 [45]

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