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GENETIC BASIS AND THE CURRENT BREEDING EFFORTS FOR QUALITY PROTEIN MAIZE IN SOUTHERN AFRICA

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ABSTRACT

Maize (*Zea mays* L.) is deficient in essential amino acids, lysine and tryptophan. Opaque-2 maize mutant discovery that is high in lysine and tryptophan, offers an avenue for maize protein quality improvement. Quality protein maize (QPM), a product of the extensive development of the Opaque-2 mutant, is an affordable and viable option for overcoming the scourge of protein malnutrition in humans and monogastric livestock especially in sub-Saharan Africa. The objective of this review was to scrutinise the genetic basis of quality protein maize (QPM), and current breeding efforts, and propose potential uptake pathways for QPM products in southern Africa. The conventional QPM breeding methods are based on phenotypic selection to identify genotypes carrying the recessive Opaque-2 alleles. However, phenotypic selection is negatively influenced by the environment and has huge drain on resources such as time, money and labour, with low genetic gains. From this, marker assisted breeding methods are clearly the most efficient way of QPM breeding. Institutions such as the International Maize and Wheat Improvement Centre (CIMMYT) are currently employing molecular breeding in QPM breeding programmes so as to quicken and ease the process of QPM breeding. To date, a number of QPM varieties have been released and are being promoted using various pathways and policies.

Key Words: Opaque-2 gene, phenotypic selection, QPM, *Zea mays* L.

RÉSUMÉ

Le Maïs (*Zea mays* L.) est déficient en acides aminés essentiels, lysine et tryptophane. La découverte du maïs mutant Opaque-2 qui a un taux élevé en lysine et tryptophane, offre une voie pour une amélioration de la qualité de protéine dans le maïs. Le maïs à haute teneur protéique (QPM), un produit du développement extensif du mutant Opaque-2, est une option économique et viable pour réduire le taux de malnutrition protéique chez les humains et les animaux monogastriques spécialement en Afrique sub-saharienne. L'objectif de cette revue était d'examiner la base génétique du maïs à haute teneur protéique (QPM), et les efforts récents d'amélioration génétique, et de proposer un moyen d'adoption des produits QPM en Afrique du Sud. Les méthodes conventionnelles d'amélioration pour QPM sont basées sur la sélection phénotypique pour identifier les génotypes portant les allèles récessifs d'Opaque-2. Cependant, la sélection phénotypique est négativement influencée par l'environnement et nécessite assez de ressources telles que le temps, argent et la main d'œuvre, avec moins de gain génétique. De là, les méthodes de sélection assistée par les marqueurs sont clairement les moyens les plus efficaces pour la sélection pour QPM. Les institutions telles que le Centre International l'Amélioration du Maïs et du Blé (CIMMYT) sont actuellement entrain d'employer la sélection moléculaire dans les programmes

d'amélioration pour QPM de façon à accélérer et faciliter le processus d'amélioration pour QPM. Au jour d'aujourd'hui, un certain nombre de variétés QPM ont été livrées et sont en cours d'être promues en utilisant différents chemins et politiques.

Mots Clés: Gène Opaque-2, sélection phénotypique, QPM, *Zea mays* L.

INTRODUCTION

Maize (*Zea mays* L.) is ranked third after wheat and rice, as a crucial food and feed source in numerous developing countries (Babu *et al.*, 2005; Olakojo *et al.*, 2007). It is the most consumed cereal in Africa, Latin America and Asia (Sofi *et al.*, 2009). Shiferaw *et al.* (2011) described maize as a crop whose levels of consumption is over 130 kg per *capita* per year in southern Africa, especially Lesotho, Malawi, Zambia and Zimbabwe. The highest quantities of maize are consumed in southern Africa, at 85-130 kg per *capita* per year as compared to 27 kg per *capita* per year in East Africa and 25 kg per *capita* per year in West and Central Africa (Babu *et al.*, 2013). Prasanna *et al.* (2001) reported that 15-26% of total daily calories are contributed by maize in about 25 developing countries. Of the daily human protein consumed in these developing countries, maize is reported to provide up to 60% (Musila *et al.*, 2010; Sofi *et al.*, 2009). In this regard, maize has gained a reputation of being the world's nutritia-cereal (Pixley, 2001). Normal maize however lacks essential amino acids namely lysine and tryptophan (Krivanek *et al.*, 2007).

According to Babu *et al.* (2013), a typical maize kernel comprises 65-70% starch, 10-15% water, 1.4-2% soluble sugars, 1.5-2.1% crude fiber, 1.5-2% ash, 3.5-4.5% oil and 8-10% protein. The endosperm harbors all the starch and about 70% of the protein (Babu *et al.*, 2013). The remaining protein and high levels of oils are found in the germ. Sofi *et al.* (2009) explained that both the endosperm and the germ contain protein, but the germ proteins are superior in quality. The endosperm comprises of about 80% of the mature dry kernel weight; while the germ comprises about

10% of the mature kernel weight (Vivek *et al.*, 2008).

In the normal maize endosperm, Hallauer (2001) described the average proportions of the distinct fractions of protein as albumins 3% (water soluble), globulins 3% (salt soluble), zeins or prolamine 60% (alcohol soluble), and glutelins 34% (dilute alkali soluble). Since the zein or prolamine fraction of the endosperm protein is the largest, and it comprises alpha (α), beta (β), gamma (γ) and delta (δ) zeins, it makes them most abundant proteins and comprise 60-80% of total proteins (Ignjatovic'-Mic'ic *et al.*, 2008). The zeins are the most abundant proteins and comprise 80% of total proteins (Ignjatovic'-Mic'ic *et al.*, 2008). Generally, the zeins contain high levels of glutamine, proline, and leucine; but are highly deficient in lysine and tryptophan (Balconi *et al.*, 2007). It was therefore deduced that the poor nutritional quality of maize protein is due to the zein fraction, which is highly unbalanced in amino acid composition and deficient in lysine and tryptophan (Gupta *et al.*, 2013). In agreement with this assertion, Balconi *et al.* (2007) reported that the economic and nutritional value of the maize kernel is mostly derived from the endosperm.

Conventional or normal endosperm maize is deficient in essential amino acids, namely lysine and tryptophan (Sofi *et al.*, 2009; Azevedo and Arruda, 2010; Mbuya *et al.*, 2011). These amino acids are essential because they cannot be synthesized in the body; but are rather obtained solely from the diet (Tome, 2012). The deficiency of these amino acids results in poor net protein utilisation and its low biological value causing malnutrition that leads to kwashiorkor (Upadhyay *et al.*, 2009; Prakash *et al.*, 2017). Rolfes *et al.* (2009) explained that kwashiorkor is a health problem

that emanates from chronic protein and energy imbalance and heightens susceptibility to life threatening diseases such as gastroenteritis and tuberculosis. The known symptoms of kwashiorkor include an abdomen that is swollen, listlessness as well as changes in hair colour (Nuss *et al.*, 2011). Lee *et al.* (2008) in agreement with Rolfes *et al.* (2009), described kwashiorkor as a weaning disease because the onset of the symptoms in many young children occurs at the time of the shift in diet from breast milk to soft cereals, mainly maize in southern Africa.

It was reported that maize based diets in developing countries, with no complementary sources of these essential amino acids lead to protein energy undernutrition, especially to infants and lactating or pregnant mothers (Babu *et al.*, 2014). Pre-school children in developing countries are reported to be stunted (32%) and underweight (20%) due to protein energy undernutrition (Black *et al.*, 2008). Schonfeldt and Hall (2012) also aluded that dietary protein sources are mainly limited to cereals, and to a less extent, animal sources such as eggs, milk and meat in the developing countries. This is due to the scarcity and expensive nature of animal sources of protein. Therefore, the objective of this review was to scrutinise the genetic basis of quality protein maize (QPM), and current breeding efforts, and propose potential uptake pathways of QPM products in southern Africa.

THE QPM DISCOVERY AND BASIS

Opaque-2 mutant discovery. A spontaneous mutant of maize was discovered in a maize field in the 1920's in Connecticut, USA (Krivanek *et al.*, 2007). It had soft and opaque grains and other pleiotropic effects such as susceptibility to pests and diseases. The mutant was later named *Opaque-2* (o_2) In 1994, Dr. Oliver Nelson and his team from Purdue University (USA) found out that the homozygous recessive o_2 allele had elevated levels of lysine (at least 69%) in the grain

endosperm, compared to normal endosperm maize (non-QPM) in 1964 (Vasal, 2001).

Atlin *et al.* (2011) reported that the *opaque-2* gene remarkably reduces the zein fraction by roughly 50%, with concomitant increase in the relative amounts of fractions that are nutritionally superior such as albumins, globulins and glutelins. The amount of lysine in *opaque-2* maize is 3.3 to 4.0g per 100 g of protein. The quality of the protein is 43% higher than that of non-QPM maize and 95% more than that of the milk protein, casein. The decreased level of zein (5-27%) in the o_2 maize, along with reduced leucine content, leads to more tryptophan from niacin synthesis, and thus helps to combat pellagra and significantly improves nutritional quality (Vivek *et al.*, 2008; Babu *et al.*, 2013; Gupta *et al.*, 2013).

At a molecular level, the *opaque-2* gene is located on the short arm of chromosome 7 in the maize genome near the defective endosperm gene 'DEB 30' (Holding and Larkins, 2008; Holding *et al.*, 2008; Sofi *et al.*, 2009; Tripathy *et al.*, 2017). The gene encodes a leucine zipper (bZIP) class transcription factor that is responsible for down-regulating the expression of zein genes as well as a 32-kDa albumin gene *b-32*, which is necessary for the expression of the zein genes (Schimdt *et al.*, 1990; Lohmer *et al.*, 1991; Bass *et al.*, 1992; Prassana *et al.*, 2001). The locus encodes for the leucine binding motif and the protein can bind to the 5' flanking sequence of the gene encoding the 22kDa α zeins, thus reducing their production. Therefore, the *opaque-2* gene reduces but does not completely eliminate transcription of multiple α zein genes (Atlin *et al.*, 2011). According to Sofi *et al.* (2009), the zein proteins are encoded by a large gene family but in contrast, β , δ and γ are encoded by one or two genes.

The negative pleiotropic effects of the *opaque-2* mutation and the birth of quality protein maize. Though the *opaque-2* gene imparted high nutritional quality, it exhibited

negative pleiotropic effects on the agronomic and kernel characteristics. Tripathy *et al.* (2017) highlighted that in addition to amino acid composition alteration, the mutation also affects starch organisation, making the kernel softer, opaque in appearance and have an unpleasant taste. Babu *et al.* (2004) reported that the mutation adversely affected, dry matter accumulation, which results in low grain yield. The maize kernels succumb to slow drying, following physiological maturity as well as a higher incidence of ear rots.

Despite its highly favourable nutritional merits, the *opaque-2* maize did not gain popularity and acceptance by farmers and consumers due to its reduced grain yield, chalky and dull appearance of kernels and susceptibility to ear rots and stored grain pests (Vasal, 2001; Vivek *et al.*, 2008; Gupta *et al.*, 2013). The challenges of the *opaque-2* maize were, however, short-lived. Researchers at the International Maize and Wheat Improvement Center (CIMMYT) developed hard endosperm *opaque-2* genotypes by introgressing endosperm modifier loci (Ignjatovic'-Mic'ic *et al.*, 2008). These transformed the soft and starchy endosperm to a vitreous one that is favoured by farmers, with the retention of elevated levels of lysine and tryptophan. The high lysine and tryptophan maize was named 'Quality Protein Maize' (QPM) (Nurit *et al.*, 2009).

Potential benefits of quality protein maize.

Quality protein maize has many benefits to human beings and monogastric animals, such as the provision of high quality protein and energy. Several researchers (Kiria *et al.*, 2010; Celestino *et al.*, 2012) demonstrated the nutritional superiority of QPM genotypes when used as food and feed. One such example is when children with kwashiorkor responded positively to QPM based diets, compared to non-QPM based diets. Moreover, chickens fed with QPM based diets had more weights and increased breast muscles compared to those fed with non-QPM (Tiwari *et al.*, 2013). In both cases, QPM proved to be nutritionally

superior to non-QPM. Furthermore, the utilisation of QPM as food and, particularly feed can improve the disposable income of maize dependent communities. This is because QPM based feeds reduce production costs and the animals tend to reach maximum weight potential, thereby fetching favourable market prices. Therefore, there is need to intensify QPM breeding so as to develop QPM genotypes, which are high yielding and stable across different environments so as to help alleviate protein-malnutrition related disorders.

QPM BREEDING APPROACHES: PRESENT AND FUTURE PROSPECTS

Genetic systems involved in QPM breeding. According to Atlin *et al.* (2011), breeding of QPM involves the management of three different genetic systems to acquire elevated quantities of tryptophan and lysine as, well as hard and vitreous endosperm. The three genetic systems are (i) recessive mutant allele of the *opaque-2* gene, (ii) endosperm hardness modifier gene and (iii) amino acid modifiers/ genes which have an influence on the free and/ or protein bound amino acid content in the endosperm. Breeders use a suite of phenotypic and genotypic screening methods to combine desirable genotypes for these distinct systems. Conventional breeding involves phenotypic selection of individuals with the *o₂* gene in homozygous recessive state with a modified endosperm.

Phenotypic selection in conventional QPM breeding. The homozygotes are visually selected in QPM generations that are segregating in breeding programmes. Both the availability *opaque-2* recessive mutation and that of the endosperm modifier genes, which are responsible for changing the soft, opaque endosperm to a hard, vitreous endosperm without much loss of protein quality are selected using a simple and cheap method of light based screening (Vivek *et al.*, 2008).

The seeds under selection are placed on a Plexiglass surface above a light; while the light

is projected through the vitreous grains or blocked by the opaque grains (Atlin *et al.*, 2011). Individuals with the opaque endosperm are considered to be homozygous recessive for the *opaque-2* gene (Sofi *et al.*, 2007). This visual assay has the merit of being able to carry out single kernel selection however it is subject to human error. It should be noted however that the light box screening results in some mis-classification of putative o_2 homozygotes. The light box screening therefore appears to have fairly high error rates that differ among crosses and evaluators and may be affected by the dryness of the seed or by the stresses in the seed production environment (Pixley and Bjarnason, 2002).

Despite the presence of o_2 and endosperm hardness modifier genes, the lysine and tryptophan levels among lines from the same cross vary widely necessitating systematic biochemical evaluation and selection for tryptophan or lysine levels during breeding (Vasal, 2001). The confirmation of genotypes carrying the *opaque-2* gene derived from the light box screened kernels either by molecular markers or amino acid content profiling.

Marker assisted foreground selection in QPM breeding. Jonah *et al.* (2011) described marker assisted selection (MAS) as a form of biotechnology which utilises techniques of DNA finger-printing to help breeders of QPM in matching molecular profile to physical properties of a QPM variety. According to Danson *et al.* (2006), three simple sequence repeat markers (*phi112*, *phi057* and *umc1066*) situated as internal repetitive sequences within the o_2 gene on the short arm of chromosome 7 (Tripathy *et al.*, 2017) are being used as foreground selection markers for the *opaque-2* gene.

The analysis using these molecular markers can be accomplished with use of samples of DNA extracted from the leaf tissue of very young plants, thereby enabling the identification of QPM plants early in breeding cycle (Gupta *et al.*, 2013). This therefore allows breeders to discard plants without the o_2 allele prior to

pollinations thus reducing the size of the breeding population and saving both time and money.

Breeders can identify heterozygous plants in order to get homozygous recessive plants after selfing, given that the presence of o_2 in the homozygous recessive state is the aim of the selection of QPM genotype (Vivek *et al.*, 2008). Marker assisted selection enables selection of plants according to their genotypes independent of environment and effects of epistasis. It is approved as a tool that is very applicable that complements and facilitates substantially, the conventional breeding and selection techniques (Ribaut and Hoisington, 1998; Balding *et al.*, 2003).

Presently simple sequence repeat markers are the most widely utilised markers by maize breeders due to their availability in large numbers in the public domain, their simplicity and effectiveness (Maize CrDB: <http://www.maizegdb.org>). These polymerase chain reaction based co-dominant markers are robust, reproducible, hyper variable, abundant and are uniformly distributed in plant genomes, thus offering a significant value in the purposes of breeding (Powell *et al.*, 1996).

Molecular markers increase the reliability; while reducing cost, labour and time taken to obtain QPM varieties (Babu *et al.*, 2004; 2005). Babu *et al.* (2005) reported the development and particular release of MAS derived QPM hybrid, the 'Vivek QPM 9' in Almora, India. The parental lines of Vivek Hybrid 9 (CML145 and CML212) were converted to QPM versions through MAS transfer of the o_2 gene and phenotypic selection of endosperm modifiers in parental lines. The QPM hybrid Vivek QPM 9 exhibited 41% increase in tryptophan, 30% in lysine, 23% in histidine and 3.4% in methionine coupled with a 12% reduction in leucine (Babu *et al.*, 2013).

Current breeding efforts and future directions. Much effort has been invested in the research and breeding of QPM genotypes, and a number of QPM hybrids is on the market (Table 1), thus resulting in improved QPM

TABLE 1. QPM genotypes available in Zimbabwe

Genotype	Source	Coverage in SSA	Attributes
SC643	Seed Co. Pvt. Ltd.	East, central and southern Africa	White kernel QPM hybrid, excellent drought tolerance, exhibits good nitrogen use efficiency
SC527	Seed Co. Pvt. Ltd.	East, central and southern Africa	White kernel, dent ear QPM hybrid, high yield potential
SC535	Seed Co. Pvt. Ltd.	East, central and southern Africa	White kernel, dent ear QPM hybrid, high yield potential, wide adaptation and good heat and drought stress tolerance
PHB3253	Du Pont Pioneer- Pannar Zimbabwe Pvt. Ltd.	East, central and southern Africa	White kernel, dent ear non-QPM hybrid, wide adaptation and good standability
SC513	Seed Co. Pvt. Ltd.	East, central and southern Africa	White kernel, dent ear non-QPM hybrid, high yield potential, relatively high ear placement, wide adaptation and good heat and drought stress tolerance
PAN413	Du Pont Pioneer- Pannar Zimbabwe Pvt. Ltd.	East, central and southern Africa	White kernel, dent ear drought tolerant non-QPM hybrid, prolific (multi-cobbing) characteristics
SC403	Seed Co. Pvt. Ltd.	East, central and southern Africa	White kernel, drought and heat tolerant non-QPM hybrid, flint ear, excellent yield stability, relatively slow drying rate
MQ623	Mukushi Seeds Pvt. Ltd.	Southern Africa	White kernel, dent ear drought tolerant QPM hybrid, prolific (multi-cobbing) characteristics
MH1416	Mukushi Seeds Pvt. Ltd.	Experimental in southern Africa	Yellow kernel, flint ear drought tolerant QPM hybrid
MH1429	Mukushi Seeds Pvt. Ltd.	Experimental in southern Africa	White kernel, flint ear QPM hybrid
MH1410	Mukushi Seeds Pvt. Ltd.	Experimental in southern Africa	White kernel, flint ear QPM hybrid
OPV5195	Mukushi Seeds Pvt. Ltd.	Experimental in southern Africa	White kernel, flint ear QPM open pollinated variety

Adopted from Nyakurwa *et al.* (2018)

production across Africa (Table 2). However, limited information available on how QPM genotypes respond to various stress conditions that include drought, heat stress, low nitrogen, low phosphorus, and high disease pressure (Table 3). Furthermore, the effects of low nitrogen on maize protein quality especially the levels of lysine and tryptophan are largely unknown. This has formed the basis of the studies by Setimela *et al.* (2017) to investigate the effects of different environments on the grain yield of QPM hybrids (Tables 4 and 5). In this study, diverse environments were used during screening on-station (Table 3); followed by on-farm trials to validate the performance results. The results were interesting as they showed genetic gains in yield of quality protein maize under random and mild stress conditions (Tables 3 and 4). Setimela *et al.* (2017) reported that the improved QPM hybrids yielded additional 230 - 300kg per ha and 490 - 600 kg per ha under random and mild stress conditions (Tables 4 and 5).

In addition to the above studies, the issue of yield-drag has not been well documented with regards to QPM genotypes. Generally,

anecdotal evidence shows that bio-fortified crops such as QPM and pro-vitamin A maize (PVAM) produce yields lower than those of their normal maize counterparts suggesting possible existence of some correlation between crop bio-fortification and yield. Furthermore, there is need to evaluate the compatibility of combining both QPM and PVAM into one “highly nutritious” maize crop for maximum nutritional benefit of maize dependent communities in SSA.

Uptake pathways of quality protein maize.

QPM production is mainly promoted in maize dependent communities such as these in sub-Saharan Africa (SSA) (Nyakurwa *et al.*, 2018). Different models are being used for the uptake of QPM and these include; awareness campaigns, food festivals and feeding demonstrations/trials. In countries such as Ghana and Kenya, QPM-based products such as bread, mealie meal and biscuits are available on the market and are clearly labelled for customers to make informed choices (Bett *et al.*, 2014; Abiose *et al.*, 2015). Different seed companies are also producing QPM seed in

TABLE 2. Area under QPM production in sub-Saharan Africa

Country	Hectarages under QPM production	Production description
Ghana	71 250	High
Uganda	46 717	High
Burkina Faso	20 600	High
South Africa	12 500	High
Mozambique	11 250	High
Mali	9000	Medium
Ethiopia	7283	Medium
Nigeria	4500	Low
Benin	4325	Low
Tanzania	4300	Low
Guinea	3875	Low
Malawi	1125	Low
Togo	750	Very low
Cote d'Ivoire	565	Very low
Senegal	500	Very low
Cameroon	305	Very low
Kenya	12	Very low
Zimbabwe	-	New adopters

Modified from Krivanek *et al.* (2007) and Nyakurwa *et al.* (2017)

fortification of every major staple and nutritional education to promote diet diversification so as to reduce malnutrition. With such policies, bio-fortified crops such QPM can get easily promoted and thereby increasing production.

Most importantly, some nations such as Ghana, Uganda and South Africa have crafted and implemented nutrition policies that are inclined to the promotion of QPM varieties and QPM based products (Semakula *et al.*, 2015). Therefore, other maize dependent countries in SSA should emulate the Ghanaian and Ugandan policies on how to best formulate nutrition policies which bring about the desired results in relation to QPM uptake. Such policies include the promotion of crop bio-fortification in order to reduce nutrition supplements. The Government of Zimbabwe recently adopted the compulsory approach on the bio-fortification of common cereals (Nyakurwa *et al.*, 2017).

Non-governmental organisations are also playing pivotal roles in the uptake of QPM genotypes. For instance, in Zimbabwe, a Germany organisation known as Welthungerhilfe has been making tremendous efforts to promote QPM genotypes in the rural communities in Gokwe South District, which is one district with high prevalence rates of poverty and malnutrition in Zimbabwe (Nyakurwa *et al.*, 2017, 2018). Furthermore, in Malawi and Zambia, there is Harvest Plus organisation which is also promoting QPM and pro-vitamin A maize adoption through on-farm participatory trials.

CONCLUSION

Quality protein maize has nutritional can alleviate protein malnutrition in maize dependent countries. Though conventional methods of QPM breeding are still in use, marker assisted breeding with SSR markers for foreground selection can expedite the QPM breeding process. This thereby saves time, money and labour resources with high genetic gains.

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