A review on quality protein maize

Abenezer Abebe Tefera

Holeta Agricultural Research Center, Holeta, Ethiopia

Abstract

Maize is an important food, feed, as well as raw material for producing high quality protein and carbohydrates products. However, maize is deficient in certain essential amino acids, such as lysine and tryptophan and to correct these deficient scientists have been discovered opaque-2 genes in 1920s. Similarly, researchers at International Maize and Wheat Improvement Center have been developed quality protein maize varieties with acceptable agronomic traits using various breeding methods. More than 167 quality protein maize varieties have so far been released worldwide and out of those eight varieties were released in Ethiopia. Though several quality protein maize varieties have been released, adoption rate is too low. Several scholars have been studied the nutritional benefit of quality protein maize food in health, growth and development of both human and animal. Generally, understanding the effort to develop quality protein maize varieties, monitoring modifier genes in breeding process, selecting best perform quality protein maize variety and controlling seed quality could be indispensible in potential use.

Keywords: Quality, Protein, Maize, opaque 2, Zein

INTRODUCTION

Maize has recognized as one of the most important crops for food, feed and industrial purpose (Prasanna *et al*., 2001; Marcos, 2005; TAAS, 2015) in most parts of the world. Maize is also the leading world cereal in both total yield (1,104.88 million metric tons) and yield in per unit area (FAS, 2019). With such a significant yield potential, it is known as the "Queen of Cereals". It is C4 plant and more efficient in using solar radiation than other cereals. It is cultivated in latitudes up to 58^0 N to 40^0 S from below sea level to altitudes of 3900m in the Peruvian Andes (Russell and Hallauer, 1980). The top maize producing country is the United States of America (USA), and then followed China, Mexico, Brazil and Argentina (USDA, ERS, 2019).

Maize alone contributes over 20% of the total calories in human diets in 21 countries, and over 30% in 12 countries that are home to a total of more than 310 million people (Sentayehu, 2008 ; Aman *et al*., 2016). However, maize, alike other cereals, is deficient in certain essential amino acids, such as lysine and tryptophan (Wubu, 2011; Abate *et al*., 2015). The efforts to improve maize protein quality began in mid-1960s with the discovery of mutants (*opaque* 2 genes) that produce enhanced levels of lysine and tryptophan (FAO, 2002; Mpofu, 2012). Mertz *et al*. (1964) reported that the *opaque -2* (*o2*) maize mutant with an *opaque –2* (*o2*) gene increased the content of lysine and tryptophan and decreased leucine. Luong *et al*. (2017) pointed that the International Maize and Wheat Improvement Centre (CIMMYT) discovered mutant maize with an *opaque-2* gene, which codes for a double increase in levels of the two most limiting essential amino acids(lysine and tryptophan) for growth and development of humans and animals. Lysine is critical in protein synthesis for the growth of tissues and found to be important in the absorption of calcium from the intestinal mucosa (Onimisi *et al.*, 2009). Likewise, Tryptophan is the biological precursor of the B vitamin, niacin. The maize variety with these essential protein and good agronomic traits termed as quality protein (QPM) (Pandey *et al*., 2016; Messing and Rutgers, 2017). Quality Protein Maize (QPM) has hard endosperm and nutritionally enhanced, but gives comparable yield to normal maize (Nuss and Tanumihardjo, 2011). Bressani (1992) reported that the biological value of QPM is about 80%, whereas that of regular maize is 40 to 57% due to the increased digestibility and nitrogen uptake of QPM. This indicates how it is important to deal previous works on quality protein maize and pointing future directions.

Hence, this study is initiated to review the history, genetics and breeding effort, nutritional composition, adaptation and adoption, seed production and market aspect, draw backs of QOM and come up with some sort of conclusions and recommendations to exploit quality protein maize potentials as food, feed and industrial raw materials.

OBJECTIVES

1. Review previous research works on different aspects of quality protein maize

2. To recommend strategic interventions areas for its potential use

History and Genetic basis

Maize mutant with soft and opaque grain was found in maize field in USA during the 1920's, which was later named as *opaque2* maize (Gupta et.al. 2009). The efforts to improve protein quality of maize began in mid-1960s with the discovery of (FAO, 2002; Mpofu, 2012). Prasanna *et al*. (2001) and Vasal, (2000) reported that substantially higher levels of lysine and tryptophan for maize homozygous of opaque2 (o2o2) recessive mutant allele than dominant O2 allele (O2O2 or O2o2) of conventional maize has been discovered by researchers at Purdue University, USA in 1961.

Maize endosperm is rich in Zein protein, which is alcohol soluble (Prasanna *et al*., 2001). Zein (a prolamin group-alcohol soluble) is rich in glutamine (21–26%), leucine (20%), proline (10%) and alanine (10%), but deficient in important essential amino acids e.g., lysine (0.16–0.26%) and tryptophan (0.02–0.06%) which is less than half of the recommended dose specified for human nutrition (Vivek, 2008). The Zein protein polypeptides are products of differential structural gene (Zp) and these genes are simply inherited and are members of a large group of genes (Tripathy *et al*., 2017). Dissecting of Zein fraction by separations based on apparent size (SDS-PAGE) resolves polypeptides with apparent molecular weight of 27, 22, 19, 16, 14 and 10-kD (Larkins *et al*., 1989). These polypeptides are usually classified into four groups: α-(22- and 19-kd polypeptides), β-(14-kd polypeptide), γ-(16- and 27-kd polypeptides) and δ-Zein (10-kd polypeptides) based on their structure (Kirihara *et al*., 1988; Thompson and Larkins, 1989). α- Zeins and γ-Zeins are the two major proteins, 60 to 70% and 20 to 25% of the total Zein fraction, respectively, depending on the genetic background (Thompson and Larkins, 1994). Three clusters of genes which found on chromosomes 4, 7 and 10 are encoding for α-Zeins, while for the rest one or two genes encoding. Non-mutants Opaque (O2) has the greatest impact on the expression of all α-Zein genes and Prolamin-box binding factor (PBF) and O2 heterodimerizing proteins (OHPs) have additive and synergistic effects on their expression (Zhang *et al*., 2015). Defective expression of O2 in maize kernels can result in a 50 to 70% reduction in Zein content (Tsai *et al*., 1978). The reduction of Zein protein would be increase the proportion of lysine and tryptophan.

Mutants such as opaque-2 (o2), floury-2 (fl-2), Mucronate (Mc) and Defective endosperm B30 (DEB30) able to alter the amino acid profile of maize endosperm protein. The opaque mutants are recessive (o1, o2, o5, o9-11, o13, 016, o17), the floury mutations are semi-dominant (fl-1, fl-2 and fl-3) where as Mucronate and Defective endosperm are dominant mutations. Nelson *et al*. (1965) and Krivanek *et al*. (2007) stated that opaque mutations affect the regulatory network, while floury, mucronate and defective endosperm affects the amino acid profile of storage proteins (Gibbon and Larkin 2005). The *opaque-2* gene is located on the short arm of chromosome 7 in the maize genome (Tripathy *et al*., 2017). Transposable element 'rbg' is reported to induce differential expression of opaque-2 mutant gene (Chen *et al*. 2014). The O2 genes activate the expression of Zein protein and down regulate the non-Zein protein; in contrast the mutant o2 recessive genes down regulate Zein gene expression by altering enzyme involved in amino acid and carbon metabolism. In QPM, this recessive allele has to present in a homozygous state (o2o2), while conventional maize has dominant homozygous alleles (O2O2) (Adefris *et al*., 2015). Many other transcriptional factors could be involved in down regulating of Zein and leads to the improvement of protein quality of maize which could need further study. Adefris *et al*, (2015) the presence of the opque2 allele in the recessive condition (o2o2), modifier genes and proper selection during breeding work could ensure high lysine and tryptophan levels or agronomically acceptability.

Breeding efforts

The mutants alter amino acid profile and composition of maize endosperm protein and doubling the levels of lysine and tryptophan compared to normal maize (Tripathy *et al*., 2017). However, soft endosperm of the *opaque 2* maize variety was susceptible to insect pests and fungal diseases and reduced grain yield which gave the researchers further assignments (Gupta *et al*., 2009; Aman *et al*., 20016). As a result, researchers in CIMMYT, identified modifier genes that restored the desirable hard endosperm in materials containing the recessive *opeque-2* which are agronomically acceptable, yields as much or more of normal maize, and nutritionally enhanced materials (Hugo and Mike, 2001; Nuss and Tanumihardjo, 2011).

The breeding program worldwide started converting conventional maize into o2 versions through direct backcross approach and screened of hard kernels in some of the backcross-derived population leads the development of *opeque-2* varieties with hard kernels (Prasanna *et al*., 2001). Conversion of conventional maize genotypes to o2 versions through backcross recurrent selection, regaining original hard endosperm and maintaining protein quality were the focuses of CIMMYT's QPM breeding efforts (Adefris *et al*., 2015). Accordingly, QPM germplasmes are characterized by having higher lysine and tryptophan, normal endosperm, reduced susceptibility post harvest insect pests and disease, comparable to or higher yield than that of conventional maize. The QPM genotype development took over three decades of painstaking research. Prasanna *et al*. (2001) stated that a number of QPM populations and pools with

different ecological adaptation, maturity, grain color, and texture were developed. Several advanced maize populations in CIMMYT's maize program were successfully converted to QPM populations. Pedigree breeding, backcross, molecular marker assisted selection, single seed-based DNA extraction are some of the methods used in QPM breeding program of CIMMYT (Babu and Prasanna, 2014).

More than 167 QPM varieties have so far been released worldwide (Twumasi-Afriye *et al*., 2016). Of the total QPM varieties released, more than half (53% or 89 number) released from Africa, 25% (42) from Latin America and 22% (36) from Asia till 2015. As to the number of QPM varieties released globally, South Africa leads with 26 varieties released (Twumasi-Afriye *et al*., 2016).

QPM genotype development program was launched in Ethiopia by 1994 with the evaluation of open pollinated varieties (OPVs) and pools introduced from CIMMYT for fast-tracking the release of best-bet QPM varieties developed in different CIMMYT maize breeding region. In the country, Ethiopian Institute of Agricultural Research, Nation Maize breeding program with partner (CIMMYT) has been developed and released eight QPM varieties until 2016 for highland, mid-altitude, and moisture stressed maize agro-ecologies of Ethiopia (Getachew *et al*., 2016; Adefris *et al*., 2017). QPM varieties released for commercial use include AMH852Q (Huluka), AMH760Q (Webi), BHQPY545 (Kello), BHQPY42 (Gabissa), BHQP548, Melkasa-1Q and Melkassa-6Q.

Nutritional benefit

Though significant improvements have been made in agricultural research and technological developments, malnutrition remains a widespread problem (Neeraja *et al*. 2017). Black *et al*. (2013) reported that about 45% deaths of children under the age 5 years are associated with malnutrition. The malnutrition could result stunting, wasting, underweight and different disease to children and adults. Adefris *et al.* (2017) noted that in Ethiopia 28% of child mortality is associated with under-nutrition and 67% of adult population suffered from stunting as children. Thus a supplement of balanced nutrition is required for healthy growth and development of humans, particularly essential amino acids, vitamins, and minerals (Bouis *et al*., 2011).

The QPM nutritive value is 90% of milk protein, while conventional maize is 40% in young children (Prasanna *et al*., 2001). Traditional food from QPM are more acceptable in palatability and cooking quality due to softness, perceived sweetness and longer shelf life in eastern African countries (Akalu *et al*., 2010). Quality protein maize has better leucine/isoleucine ratio and higher niacin. The low leucine in QPM helps in liberating more tryptophan for niacin biosynthesis which reduces pellagra significantly (Vasal, 2001). Moreover, sufficient protein enables to alleviate 'kwashiorkor', a potentially fatal syndrome characterized by initial growth failure, irritability, skin lesions, edema, and fatty liver (Dong, 2015). Gunaratna *et al*. (2010) reported that studies in different countries found 12% weight gain in children consuming QPM over the conventional maize. In Ethiopia, specifically, at Sibu Sire area, 7 to 56 children consuming QPM showed on average 20% increase in weight than those who consume conventional maize (Adefris *et al*., 2017).

The nutritional benefits and biological superiority of QPM for animal feed also has been assessed via several trials. Burgoon *et al*. (1992) noted the weight gain doubled in pigs raised on QPM as compared to the ones fed on only normal maize. In poultry too, QPM improves the growth performance of broilers resulting higher gain in weight (Onimisi *et al*., 2008).

Adoption and Adaptation

Though several QPM varieties released in various countries and diverse reports showing the importance of producing QPM grain, its adoption has been limited worldwide. By 2015, it is estimated that about 1 million ha of land were covered with QPM in Sub-Saharan Africa, with Ghana and Uganda together accounting for ~50% of this, and 18% for the rest. It is estimated about 150,000 ha and 250,000 ha of land covered by QPM in Latin America and Asia (Twumasi-

Table 1. Nutritional value of QPM and CM compared to milk.

Food	Nitrogen balance	Quality protein (% of milk)
CM	0.31	39
QPM	0.72	90
Milk	0.8	100

*CM=conventional maize, QPM=quality protein maize, Source: Adefris *et al*. (2017).

Table 2. Lysine and tryptophan levels as percentage of total protein in whole grain flour of conventional and quality protein.

Items	CM	QPM
Protein	>8	>8
Lysine in protein	1.6 – 2.6 (mean= 2.0)	2.7 – 4.5 (mean=4.0)
Tryptophan in protein	0.2 – 0.6 (mean =0.4)	0.5 – 1.1 (mean = 0.8)

*CM=conventional maize, QPM=quality protein maize, Source: Adefris *et al*. (2017).

Table 3. Released QPM varieties with their agro-ecological adaptations in Ethiopia.

SN	QPM varieties	Year released	Variety type	Adaptation
1	AMH852Q (Huluka)	2016	Hybrid	Highland
2	AMH760Q (Webi)	2011	Hybrid	Highland
3	BHQPY542 (Gabissa)	2001	Hybrid	Moist mid-altitude
4	BHQPY545 (Kello)	2008	Hybrid	Moist mid-altitude
5	BHQP548	2015	Hybrid	Moist mid-altitude
6	Melkassa-6Q	2008	OPV	Low moisture stress area
7	Melkassa-1Q	2013	OPV	Low moisture stress area
8	MHQ138	2012	Hybrid	Low moisture stress area and Moist mid-altitude

Source: Adefris Teklewold, Kaleb Kelemu, Abraham Tadesse and Dagne Wegary.2017. A Quality Protein Maize (QPM) Manual for Agricultural Extension Workers in Ethiopia. CIMMYT, Addis Ababa, Ethiopia.

Afriye *et al*., 2016). The author suggested that the wide adoption of QPM varieties in Ghana and Uganda would be because of superior agronomic performance of QPM, nutritional benefit, consumer acceptance and involvement of governmental entities in promoting, popularizing and disseminating QPM. Atlin *et al.* (2010) suggested that the reason for poor adoption of QPM varieties in most country could be the invisibility of the QPM trait which makes QPM grain visually indistinguishable from conventional maize grain and added cost of breeding QPM varieties. Hossain *et al*. (2019) also noted that mistaken perception of low-yielding potential, phenotypically invisibility of nutritional traits, dilution of nutritional quality by contamination, lack of awareness on health benefits, lack of profitable markets for commercial producers and the absence of government incentive to encourage adoption by subsidizing the price of QPM seed are the challenges for dissemination of the QPM technology.

Gupta *et al*. (2015) suggested that minimum support price and/or premium price for biofortified maize grains in the market will encourage the farmers to grow more biofortified maize. Kisembo (2009) reported that the participator plant breeding and participatory variety selection were significantly boosted the adoption of improved varieties in Uganda. Generally, the challenges in adoption of QPM indicate the need of collaborative works among stakeholders at different levels or sectors.

As to adaptation, development of diverse high yielding QPM hybrids via broadening of germplasm base would provide wider opportunities for adaptation to different agro-ecologies (Hossain *et al*., 2016). In Ethiopia, different QPM varieties with different maturity have been released for three agro-ecologies (low moisture stress, mid-altitude and highland) (Adefris *et al*., 2015). Under each agro-ecology the varieties are different in their yield potential, maturity and other related traits.

Seed maintenance and production

The production and maintenance of QPM require performing tryptophan and protein analyses to ensure that values are above the required minimum standard the rest procedures are similar with that of conventional maize seed maintenance and production (Adefris *et al*., 2017). Pollination contamination of QPM cultivar by non-QPM (dominant O2 gene) pollen makes the harvested grain non-QPM because the op2 allele that confers the QPM trait is homozygous recessive. Contamination of a white QPM open pollinated variety by a conventional yellow maize variety planted 0.4 ha QPM plots has been studied at several sites in Ghana (Twumasi-Afriyie *et al.*, 1996). They suggested that contamination declined to insignificant levels approximately 12m from the nearest conventional maize plant. Machida *et al*. (2012) conducted a similar study in Zimbabwe using 50 m x 42 m plots and they found that the percent out-crossing of QPM with conventional yellow maize fell to <20% within 5m and <10% within 10m of the windward plot border. Machida *et al.* (2012) suggested that farmers will not lose the benefits of QPM under normal farming conditions if there are non-QPM plots in the surrounding.

QPM maintenance and production work should follow proper land preparation, isolation distance/time, rouging, field management and inspection, detasseling, post-harvest activities and seed certification standard. Particularly, QPM seed production requires an isolation distance range of 200–400m depending on the environment (e.g wind direction). Generally, strengthening the seed chain to produce and supply good quality seeds is one of the important steps for the popularization QPM.

LIMITATIONS

1. QPM field contamination from conventional maize pollen grain leads the grower to harvest seed of non-QPM due to the opaque-2 genes are homozygous recessive.

2. If breeders fail to maintain both opaque-2 and modifier genes the level of lysine and tryptophan will not improved by opaque 2 genes alone.

3. The invisibility of the QPM trait which makes QPM grain visually indistinguishable from conventional maize grain this leads poor adoption of QPM varieties in most country

CONCLUSION AND RECOMMENDATION

Maize is an important crop worldwide as food, feed and industrial raw materials. However, the conventional maize is rich in Zein protein which lack essential amino acid namely lysine and tryptophan. Accordingly, begging from 1920s researchers have been made effort in discovering opaque 2 and developing maize varieties of improved protein quality. As a result, large number of quality protein maize (QPM) varieties has been developed and released worldwide with large share in Africa (53% of varieties). The varieties were released for different agro-ecologies and breeding for biofortified crop is non stopped work. Currently, the adoption rate of QPM varieties is low and needs collaborative works between researchers and other stakeholders from various sectors. It has been confirmed that the nutrition from QPM varieties has great positive impact in human and animal health, growth and development. The grower to harvest non-contaminated seed of QPM should follow seed production procedures. Generally, awareness of QPM in every aspect enables users to exploit its potential use. Following the review here below the recommendations have been made;

1. For the successful development of QPM variety with appropriate level of lysine and tryptophan the presence of modifier/enhancer genes with opaque 2 genes during breeding processes need to be ensured

2. Enhancement of the collaboration between researchers and farmers through participatory approaches in the breeding process could be crucial in elevating adoption rate for QPM varieties. The knowledge and information shared among researchers and farmers are important in the adoption.

3. It is better to grow the right QPM variety to the right agro-ecology for harvesting quality seed and high yield. The seed production of QPM also needs proper isolation in planting time and distance to ensure true-to-type and high quality seed.

REFERENCES

Abate, T., Shiferaw, B., Menkir, A., Wegary, D., Kebede, Y., Tesfaye, K. and Keno, T. (2015). Factors that transformed maize productivity in Ethiopia. Food security, 7:965-981.

Adefris T, Gissa, D. W., Tadesse, A., Tadesse, B., Bantte, K., Friesen, D. and Prasanna, B. M. (2015). Quality protein maize (QPM): a guide to the technology and its promotion in Ethiopia.

Kelemu, K., Tadesse, A. and Gissa, D. W. (2017). A quality protein maize (QPM) manual for agricultural extension in Ethiopia.

Akalu, G., Taffesse, S., Gunaratna, N. S. and De Groote, H. (2010). The effectiveness of quality protein maize in improving the nutritional status of young children in the Ethiopian highlands. Food and Nutrition Bulletin, 31:418-430.

Aman, J., Bantte, K., Alamerew, S. and Tolera, B. (2016). Evaluation of Quality Protein Maize hybrids at Jimma, WesternEthiopia. Journal of Forensic Anthropology 1:2- 6

Atlin, G. N., Palacios, N., Babu, R., Das, B., Twumasi-Afriyie, S., Friesen, D. K. and Pixley, K. V. (2011). Quality Protein Maize: Progress and Prospects. Plant breeding reviews, 34: 83.

Babu, R. and Prasanna, B. M. (2014). Molecular breeding for quality protein maize (QPM). In Genomics of plant genetic resources Springer, Dordrecht.489-505.

Black, RE., Victora, C.G., Walker, SP., Bhutta, AZ., Christian, P., Onis de, M., Ezzati, M., Grantham-McGregor, S., Katz, J., Martorell, R. and Uauy R (2013). Maternal and child under nutrition and overweight in low-income and middle-income countries. Lancet 382:427–451

Bouis, H. E., Hotz, C., McClafferty, B., Meenakshi, J. V. and Pfeiffer, W. H. (2011). Biofortification: a new tool to reduc emicronutrient malnutrition. Food and nutrition bulletin, 32:S31-S40.

Bressani, R. (1992). Nutritional value of high-lysine maize in humans. Quality protein maize, 205-224.

Burgoon, K. G., Hansen, J. A., Knabe, D. A. and Bockholt, A. J. (1992). Nutritional value of quality protein maize for starter and finisher swine. Journal of Animal science, 70: 811-817.

Dong, N. (2015). Adaptation of Quality Protein Maize (Zea Mays L.) to Northern US Corn Belt (Doctoral dissertation, North Dakota State University).

FAO (United Nation Food and Agriculture Organization). (2002). Protein Sources for the Animal Feed Industry. Expert Consultation and workshop Bangkok, 29 April-3 May 2002.

Getachew, A., Abdulesemed, A., Yeshitila, M., Tafa, J., Agidew, B., Daniel, M., Firew, M., Getachew, T., Mizan, A. and Million E. (2016). Plant Variety Release, Protection and Seed Quality Control Directorate.

Gibbon, B. C. and Larkins, B. A. (2005). Molecular genetic approaches to developing quality protein maize. TRENDS in Genetics, 21:227-233.

Gupta, HS., Hossain, F., Muthusamy, V. (2015). Biofortification of maize: an Indian perspective. Indian J Genet 75:1–22

Gupta, H.S., Agrawal, P.K., Mahajan, V., Bisht, G.S., Kumar, A., Verma, P., Srivastava, A., Saha, S., Babu, R., Pant, M.C. and Mani, V.P. (2009). Quality Protein Mazie for nutritional security: rapid development of short duration hybrids through molecular marker assisted breeding. Current Science, 96:230-237.

Hossain, F., Muthusamy, V., Bhat, J. S., Jha, S. K., Zunjare, R., Das, A. and Kumar, R. (2016). Maize. In Broadening the Genetic Base of Grain Cereals, Springer, New Delhi, 67-88.

Hossain, F., Muthusamy, V., Zunjare, R. U. and Gupta, H. S. (2019). Biofortification of Maize for Protein Quality and Provitamin-A Content. In Nutritional Quality Improvement in Plants . Springer, Cham. 115-136.

Hugo, C. and Mike, L. (2001). Quality Protein Maize: Improved Nutrition and Livelihoods for the poor. In: Sustainable Maize Production Systems for Nepal. Proceedings of Maize Symposium, Held December 3-5, 2001, Kathmandu, Nepal.

Kirihara, J.A., Hunsperger, J.P., Mahony, W.C., and Messing, J.W. 1988. Differential expression of a gene for a methionine-rich storage protein in maize. Mol. Gen. Genet. 211:477-484.

Kisembo, L. G. (2009). The impact of participatory plant breeding and selection on adoption of improved sweet potato varieties in Uganda (Doctoral dissertation, Makerere University).

Krivanek, A. F., De Groote, H., Gunaratna, N. S., Diallo, A. O. and Friesen, D. K. (2007). Breeding and disseminating quality protein maize (QPM) for Africa. African Journal of Biotechnology, 6:312-324.

Machida, L., Derera, J., Tongoona, P., Mutanga, O. and MacRobert, J. 2012. Geostatistical analysis of quality protein maize out crossing with pollen from adjacent normal endosperm maize varieties. Crop Science 52: 1235-124

Marcos, L. (2005). Combining ability for grain yield of quality protein maize under low soil nitrogen (Doctoral dissertation, University of Zambia).

Messing, J. and Wu, Y. (2017). Compositions and methods for rapid and efficient production of quality protein maize, U.S. Patent No. 9,603,317. Washington, DC: U.S. Patent and Trademark Office.

Mpofu, I. D. T., Sibanda, S., Shonihwa, A., and Pixley, K. (2012). The nutritional value of quality protein maize for weaner pigs. J. Pet. Environ. Biotechnol, 3:129.

Neeraja, C. N., Babu, V. R., Ram, S., Hossain, F., Hariprasanna, K., Rajpurohit, B. S. and Datta, S. K. (2017). Biofortification in cereals: progress and prospects. Curr Sci, 113:1050-1057.

Nelson, O. E., Mertz, E. T. and Bates, L. S. (1965). Second mutant gene affecting the amino acid pattern of maize endosperm proteins. Science, 150:1469-1470.

Nuss, E. T. and Tanumihardjo, S. A. (2011). Quality protein maize for Africa: closing the protein inadequacy gap in vulnerable populations. Advances in Nutrition, 2:217-224.

Onimisi, P. A., Dafwang, I. I., Omage, J. and Onyibe, J. E. (2008). Apparent digestibility of feed nutrients, total tract and ileal amino acids of broiler chicken fed quality protein maize (Obatampa) and normal maize. Int. J. Poult. Sci, 7:959-963.

Onimisi, P. A., Omage, J. J., Dafwang, I. I. and Bawa, G. S. (2009). Replacement value of normal maize with quality protein maize (Obatampa) in broiler diets. Pakistan Journal of Nutrition, 8:112-115.

Pandey, N., Hossain, F., Kumar, K., Vishwakarma, A. K., Muthusamy, V., Saha, S. and Gupta, H. S(2016). Molecular characterization of endosperm and amino acids modifications among quality protein maize inbreds. Plant Breeding, 135:47-54.

Prasanna, B.M., Vasal, S.K., Kassahun, B. and Singh, N.N., (2001). Quality protein maize. *Current science*, 1308-1319.

Russell, W.A. and Hallauer, H.H, (1980). Hybridization of crop plants (No. BOOK). American Society of Agronomy and Crop Science Society of America.

Sentayehu, A. (2008). Protein, Tryptophan and Lysine contents in Quality Protein Maize, North India. Ethiop J Health Sci, 18:10-15.

TAAS (Trust for Advancement of Agricultural Sciences). (2015). Up scaling Quality Protein Maize for Nutritional Security Indian Agricultural Research Institute, Pusa Campus, New Delhi-110 012, India.

Thompson, G.A. and Larkins, B.A. (1989). Structural elements regulating zein gene expression. BioEssays 10: 108-113.

Tripathy, S.K., Ithape, D.M., Maharana, M. and Prusty, A.M. (2017). Quality Protein Maize (QPM): Genetic basis and breeding perspective. Trop. Plant Res, 4:145-152.

Tsai, C.Y., Larkins, B.A. and Glover, D.V. (1978). Interaction of the opaque-2 gene with starch-forming mutant genes on the synthesis of zein in maize endosperm. Biochemical genetics, 16:883-896.

Twumasi-Afriye, S., Palacios Rojas, N., Friesen, D., Teklewold, A., Gissa, D.W., De Groote, H. and Prasanna, B.M. (2016). Guidelines for the quality control of Quality Protein Maize (QPM) seed and grain.

Vasal SK. (2001). High quality protein corn. In: Hallauer A (ed) Speciality corn, 2nd edn. CRC Press, Boca Raton, FL, pp 85–129

Vasal, S. K. (2000). The quality protein maize story. *Food and Nutrition Bulletin*, *21*(4), 445-450.

Vivek, B. S. (2008). *Breeding quality protein maize (QPM): Protocols for developing QPM cultivars*. CIMMYT.

Wubu, T. Z. 92011). Effect of graded level of Quality Protein Maize and normal maize on egg production, egg quality and hatchability of white leghorn hens. MSc Thesis submitted to the school of graduate studies, Haramaya University, Ethiopia.

Zhang, Z., Yang, J., & Wu, Y. (2015). Transcriptional regulation of zein gene expression in maize through the additive and synergistic action of opaque2, prolamine-box binding factor, and O2 heterodimerizingproteins.*ThePlantCell*, *27*(4)1162-1172.