Nutritionally Enhanced Maize and its Importance in the Developing Countries

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ABSTRACT. Improving nutritional quality of cereals is a noble goal as it benefits hundreds of millions of producers and consumers directly without changing their food habit. Research experience on most crops so far has been disappointing and the success stories limited.In maize, the situation is different as CIMMYT researchers were able to exploit high lysine mutants successfully to develop nutritionally enhanced maize now referred to as Quality Protein Maize (QPM). Maize being a leading crop, contributes significantly to the world's food basket of roughly 2000 million tons every year. Unfortunately, maize has high prolamine content, and is deficient in essential amino acids lysine and tryptophan, thus making it poor in nutritional quality. Biochemists had demonstrated this fact almost hundred years ago.The discovery of high lysine mutants, opaque-2 and floury -2 by Purdue group in mid-sixties spurred enthusiasm and hope to elevate levels of lysine and tryptophan to almost double over what is encountered in normal maize.Conversion programs were initiated worldwide to obtain original soft opaque-2 versions of normal counterpart varieties and hybrids. Testing of such materials exposed a series of problems including poor agronomic performance in yield, unacceptable kernel texture, slower drying following physiological maturity and more vulnerable to ear rots and stored grain pests. As a result pessimism started growing, funding kept on declining and many institutions either completely abandoned their research work or reduced it substantially. Only in a few institutions, momentum continued as usual. Exploratory research on many different approaches was tried at CIMMYT to find a single approach or strategy that will overcome negative association of Opaque-2 gene with other undesirable traits. A combined approach of two genetic systems involving opaque-2 gene and genetic modifiers of opaque-2 locus appeared to be a viable approach. Chance events and a few other factors further enhanced confidence in this approach. A huge volume of QPM germplasm were developed which had a competitive yield performance, acceptable kernel phenotype and free from other undesirable effects.Over the past two decades or so many countries have released QPM varieties and synthetics and the trend now is to release hybrids. Some countries in recent years have released QPM single cross hybrids. Efforts are underway to develop super QPM combining high –oil and some micronutrients such as Fe, Zinc and pro-vitamin A. It is important to mention that maize is a gifted crop and has many competitive technologies which can as well be deployed in QPM research. QPM will certainly play an important role in human nutrition and to correct prevailing problems of Kwashiorkor and pellagra in some developing counties where maize is a staple food. Projected future needs for livestock and use of maize as feed holds a bright future for QPM from the standpoint of better feed ratio and reduced use of high priced supplements. These developments pose numerous challenges for future QPM research.

Introduction

Maize (*Zea mays* L.) is an important and unique crop of worldwide economic importance. It contributes significantly to global grain pool of 2200 million metric tons annually in achieving food and nutritional security. Considering area sown and annual production, it occupies an important position in world economy and trade as food, feed and industrial grain crop. According to the latest statistics, maize is grown on 160 million hectares with a production volume almost touching 800 million metric tons valued at 80 billion US dollars. Projecting a population growth of 80 million people every year and the demand for meat and other animal products to double by 2020, it is expected that demand for maize will increase dramatically in the next decade.

Grain quality of maize is an important and desirable attribute for all types of maize used as grain. It must meet standards and preference of producers, consumers and industrial grain processors with respect to purity, uniformity, grain color, texture, size , freedom from ear rot pathogens and pests, and in some instances possessing specific starch, protein and oil characteristics. Maize grain is indeed fascinating as it offers ample opportunities for altering grain quality characteristics of endosperm, germ and even other parts as aleurone tissue. From my own perspective, improving nutritional quality of maize grain is a noble goal. This becomes particularly important as benefits can be spread to hundreds of millions of people in a most rapid and effective manner without changing the traditional food habits of the people.

Improving protein content and quality of protein has been a concern of nutritionists and breeders for a long time. We are all aware that cereal proteins including maize vary in protein content but in general are of poor protein quality because of lack of balance in amino acid composition. The poor quality of proteins is attributed to high concentration of prolamine storage protein fraction in cereals. This particular fraction is practically negligible or devoid of lysine. The high level of this fraction is the sole cause of poor protein quality in cereals. Unfortunately maize falls in high prolamin group consisting 50-60 % of this protein fraction. It may be of interest to point out that prolamin is one the four protein fractions which make up the grain protein. The other fractions are albumins, globulins and glutelins and are soluble in water, saline solution and alkali solution, respectively. Breeding for improved amino acid composition has thus been attempted in some crops but to date commercially exploitable success of high lysine varieties, commonly referred to Quality Protein Maize (QPM), has been obtained only in maize. In the past 5-6 years, we have witnessed lots of QPM enthusiasm and excitement. At least 22 countries in recent years have released QPM varieties or hybrids based solely on CIMMYT developed QPM germplasm. The enthusiasm and hope could lead to more area coverage but would need tackling remaining future QPM challenges. The QPM germplasm needs to be diversified and more resources devoted to developing QPM germplasm tolerant to a whole spectrum of abiotic stresses. The paper is intended to discuss breeding options, strategies and changing tactics to achieve this objective in a resource effective and efficient manner. The paper provides background information on QPM as well as on germplasm available at CIMMYT and else ware. It also provides a detailed discussion on options, breeding methodologies, and adjustments / modification in strategies to build-up tolerance to some of the prevailing abiotic stresses. The final section discusses importance of QPM in human and animal nutrition in the developing countries

Background Information on QPM, Sequence of Events and Germplasm Available

To start with germplasm accessions were screened for genetic variability for lysine content. Variation was observed in maize but differences were rather small. It would have needed many years to elevate levels sufficiently high to make protein profile reasonably balanced in manifesting superior biological value. Despite increasing realization, the protein quality thus remained more of a concern but with no immediate solutions in sight as no good breeding options were available that could be deployed at that time to affect improvements. A beginning in genetic manipulation of protein quality began with the discovery of high lysine mutant opaque-2 (o2) (Mertz *et al*., 1964) and a year later another mutant floury-2 (Nelson et al. 1965) was discovered by Purdue University researchers. These exciting discoveries generated a lot of enthusiasm and hopes, and paved the way for improving protein quality in maize. It may be of interest to mention that these mutant alleles changed protein quality of endosperm and not that of germ.

These mutants were able to alter amino acid profile of maize endosperm protein resulting in two fold increase in the levels of lysine and tryptophan compared to normal genotypes. The phenotype of the mutants was easily recognizable from their soft chalky appearance. Alterations were noticed in other amino acids as well. An increase was observed for amino acids such as histidine, arginine, aspartic acid and glycine and a decrease in glutamic acid, alanine and leucine. Leucine, isoleucine ratio was improved and became better balanced, which in turn is considered beneficial as it helps to liberate more tryptophan for more niacin biosynthesis and thus helping to combat pellagra. These mutants bring about improvements in lysine and tryptophan by suppressing lysine-deficient zein fraction without altering contribution of other fractions. A reduction in zein fraction causes proportional elevation of other fractions rich in lysine thus resulting in elevation of these two amino acids in protein, but not on absolute basis of per unit of endosperm in the grain. Search was continued for new and better mutants, however, ones found (o7, o6, fl3) were in no way better than opaque-2.

Breeding efforts were thus initially concentrated on opaque-2 and floury-2. Since floury-2 did not hold its promise and thus was also dropped in early seventy's. High quality protein materials developed using o2 did not show competitive performance to their normal counterparts. They suffered from a number of problems including lower grain yield, unacceptable soft chalky endosperm, slower drying, more vulnerable to ear rot pathogens and to stored grain pests. These agronomic deficiencies were serious enough to cause decline in interest and even completely abandoning these efforts in many programs. Only a few institutions such as CIMMYT, Purdue University, Crows Hybrid Seed Company in Milford, Illinois, and University of Natal in South Africa continued sustained efforts choosing different options to develop normal looking agronomically acceptable varieties and hybrids. Success of approach deployed at CIMMYT and germplasm developed will be described in detail in later section.

CIMMYT scientists used opaque-2 gene to enhance levels of lysine and tryptophan in maize endosperm protein as opposed to other genes which did not offer any advantage over the opaque-2 gene. In the beginning emphasis was on developing soft endosperm cultivars. As agronomic problems of reduced grain yield, unacceptable kernel appearance, slower drying following physiological maturity of grain, more vulnerable to ear rots and stored grain pests became obvious, several different options were tried to correct aforementioned problems that could result in acceptable quality protein maize germplasm. These approaches are described in several CIMMYT publications and journal articles (Bjarnason and Vasal, 1992; Vasal *et al.* 1984, Vasal *et al.* 1980; Vasal 1994, Vasal 2000, 2002). Only one approach appeared promising which could resolve all problems confronting soft opaques and result in high-quality protein materials with acceptable yield performance, kernel phenotype and least vulnerable to ear rots and stored grain pests.

The approach involved use of two genetic systems involving the opaque-2 gene and the genetic modifiers of opaque-2 locus. Using this approach, the initial emphasis was on developing hard endosperm opaque-2 donor stocks. Subsequently these donors stocks were used to convert normal maize materials to hard endosperm opaque-2. In addition several broad based gene pools were formed. By late 1978, a huge volume of quality protein maize germplasm was developed with normal looking kernel phenotype. Merging and reorganization was attempted at this point to form a fixed number of pools and populations for systematic handling and improvement (Vasal 1994, 2000). In all 10 populations and 13 QPM pools resulted from this effort. In mid-1980s QPM hybrid effort was initiated. Also two high oil QPM populations were developed. International testing of QPM varieties and hybrids was extensively done and the results were extremely encouraging.

Several countries identified varieties or hybrids which were competitive and either equal or better than the best normal checks included in the trials. Also during mid-1990s, 55 QPM inbreds were announced and made available to public and private sector. Additional QPM lines have been released recently thus increasing the total further. In the past several years, at least 22 countries have released QPM materials including China, India, Indonesia, Mexico and Vietnam. Successful field days were conducted in most of the countries releasing the hybrids. In many instances, high ranking politicians attended the ceremonies. There is enthusiasm and hope of covering more area under QPM in the coming years.

Development and Improvement of Abiotic Stress Tolerant QPM Germplasm

Considering importance of climate change and drought tolerance, below I have attempted to describe some options and strategies to develop abiotic stress tolerant QPM germplasm.

Adaptation vs Development

This is perhaps the simple and most cost effective and efficient approach. It will require selection for a particular stress in a specified stress environment for a few cycles. The QPM related traits affecting kernel phenotype and protein quality due to high frequency of genetic modifier will remain unaffected and may show even slight progress if pressure is applied for modifiers during the selection process. This type of approach has been successfully used in China to improve photoperiod sensitivity of two CIMMYT QPM pools 33 and 34. Three cycles of biparental mass selection brought noticeable change in photoperiod insensitivity.

Improvement of Source of QPM Populations forStress Traits

This approach has been tried in normal materials to improve drought, low-N and acid soil tolerance at CIMMYT headquarter and in CIMMYT's regional programs in south America and Africa. It should also work for QPM materials. Family based selection schemes of full-sibs and selfed progenies were quite effective and resulted in measurable gains equal to or better than non- stress conditions. Since selection was based on family performance under stress and non-stress conditions, the improved populations performed well both under stress and non-stress conditions. From my own experience I could further add some important considerations in the choice of selection schemes with QPM materials. The schemes ought to be simple, cost effective, OPV and hybrid oriented, provide spin off products, possibility of early collaboration with partners, reduced time span per cycle and more importantly should have multiplier effects. Carefully chosen schemes or their modifications should work as well for the improvement of source QPM populations. The schemes should also place least burden on laboratory facilities.

Conversion of Normal Stress Tolerant Germplasm to QPM

This approach requires crossing normal stress tolerant germplasm to one or more QPM donors. The F1's will need to be advanced to F2 **.**In this segregating generation, modified opaque-2 kernels are selected with great care taking into consideration that expected frequency of such kernels does not exceed 25%. Selection of right kernels is highly critical to avoid mistakes in ensuing generations. The quality of modified kernels will dictate the next step, to advance further F2 to F3 or to go for the next backcross. For such conversion programs, skilled and experienced QPM researchers are very much required. Decision to proceed with no backcross or additional backcross(es) will depend on breeder judgement. Sometimes to accelerate progress and to accumulate modifiers, one may choose to backcross with non-recurrent parent.

Conversion of QPM to Stress Tolerant Using QPM Stress Tolerant Donors

This approach appears appropriate and desirable provided QPM stress tolerant donors have been developed and are available. Using this approach the possibilities of making mistakes is reduced, does not dilute the frequency of modifiers and conversion time span is considerably reduced. As more QPM donors are available, one may choose the donor from the same heterotic grouping. Care should also be taken to choose donors that are adapted and are of the same maturity.

Inbred –hybrid Approach

From my own experience I have found this as an efficient and practical approach. It can be done as part of inbred- hybrid program. It requires strong QPM inbred line development program and conducting systematic inbred line evaluation nurseries under specific abiotic stress conditions on regular basis. Results have been encouraging in detecting tolerant lines for various stresses in normal maize inbred lines. Also as tolerant lines are detected, recycling among lines can be initiated to develop more diverse QPM lines. Stress manipulations can also be exercised during inbred line development stages. Use of high density is also recommended asa useful tool in stress breeding work as it creates competition for water and nutrients. Inbred-hybrid approach also has added advantage as being used as a single multi-pronged strategy for selecting simultaneously more than one stress.

Food and Feed Use of Cereals

Cereals are consumed principally as food for humans and feed for livestock. Total production of cereal grains in 2000 was 1870 million tons compared 1581 million tons in 1978. Current estimates now fluctuate around 2200 million metric tons annually. It is estimated that 34% of the world's grain crop is used to feed livestock raised for meat. For humans, cereal grains provide a major portion of calories and protein needed in the diet. Today the world obtains about 50% of its dietary protein from cereals, about 20 % from legumes and 30% from animal products (Oram and Brock 1972). In developing countries, people obtain about 26% of their protein from animal products and the remaining two-thirds from the cereal grain crops. In contrast, the people from the developed world meet 56% of their protein requirement from animal products.

Feed use of cereals has been steadily increasing. On worldwide basis, roughly one-third of grain crops are used for feeding livestock. The feed use of cereals in Asia totaled 158.1 million tons. China was the largest user (103. millions tons) followed by other countries in order of their use, Japan (15.9 millions tons), South Korea (7.6 million tons), Taiwan (5.0 million tons) and India (8.0 million tons). Maize use as feed is quite large in Asia and perhaps exceeds 50% of total production.

The consumption of meat and milk has grown many folds in the developing countries at least in the past 3 decades. The total meat consumption in the world has risen from 139 million tons in 1983 to 184 million tons in 1993. The projected increase for 2020 is 303 million tons. The meat consumption in developing countries increased from 50 in 1983 to 88 million tons in 1993 and the projected figure for 2020 is 188 million tons. Between 1970s to mid -1990s, the consumption of meat in the developing countries grew almost three times as much as it did in the developed world (Pinstrup-Andersen et al. 1999). Consumption grew even at faster rate in the second half of this period with Asia in the lead (Delgado et al. 1999). The future projections are that meat and milk in the developing countries will grow 2.8 and 3.3 percent per year between early 1990s and 2020. The corresponding figures for the developed world growth rates will be 0.6 and 0.2 percent per year.

High Lysine Cereals in Human Nutrition

Most cereals have lysine as the first limiting amino acid. Naturally occurring high lysine cereals are rice and oats. The lysine values range from 3.5 to 4% in protein. Despite high lysine values, the first limiting amino acid in both cereals is lysine. As discussed earlier, conscious effects in further increasing the levels of lysine have not yielded positive results. In respect of protein, rice is quite low (7%) but oat protein content is reasonably high. Here again breeding efforts aimed at increasing protein content in rice have not been very successful but the prospects of developing high protein oats without sacrificing lysine are quite good. Because of high lysine values both rice and oat have demonstrated higher biological value relative to other cereals (Coffman and Juliano 1979; Khush and Juliano 1984; Tanaka 1983; Frey 1977).

Rice will continue to be staple diet of at least half of the world's population. Compared to all other cereals, oat grain combines advantage of both protein content and quality and will have a great use as human food even though its major use is as feed grain. Rice will continue to be an important cereal for food and has the advantage of being high in protein quality despite low concentration of protein.

In the remaining crops, maize, sorghum, barley and millets, the protein quality is not good while the protein quantity is in the range of 9-10% in the whole grain. Except maize, the nutritional improvements for improved amino acid composition through breeding efforts have not been successful so the benefits of nutritionally enhanced characteristics in three crops cannot be harnessed by people and tribes consuming such cereals. The use of high lysine sorghum could be advocated as weaning food as is the case in Ethiopia. The high lysine types are easily recognizable because they are somewhat dented. Farmers could produce high lysine sorghum grain as a protein source for weaning children, pregnant and nursing mothers. Sorghum flour is quite indigestible by the infants and so more studies are needed before recommending as weaning food.

Quality Protein Maize for Human Nutrition

In maize the development of quality protein maize (QPM) has turned out to be a success story. It has similar agronomic performance, appearance and tastes as the normal maize. It has reduced prolamin fraction (25–30%) but elevated levels of other fractions such as glutelins, albumins and globulins. There is two-fold increase in the levels of lysine and tryptophan with high digestibility and biological value. The QPM has balanced leucine-soleucine ratio and thus enhanced production of niacin to help overcome pellagra. The QPM is like eggs and milk, both low in niacin, but offer protection from pellagra because their proteins contain high levels of tryptophan. In comparison to skim milk, the nutritional value of QPM is about 90%. It meets requirements of pre-school children for their protein needs. In countries or communities, where low protein and tuber crops make infant's diet, QPM offers better prospects. There is a tendency for increased nitrogen retention when switch over from normal to QPM is made. It should in turn translate into body weight, stature and protection from protein deficient illnesses. Clinical studies conducted in hospitals have demonstrated that QPM could help prevention and cure of severe protein deficiency disease, Kwashiorkor, in young children by simply using QPM as the only source of protein (Pradilla *et al*. 1975). QPM could be a great weaning food when used alone in maize diets. Substitution of normal by QPM will produce more benefits. QPM could be really helpful in catch-up growth particularly in malnourished and those who are sick especially after diarrhea.

QPM could have a role in improving birth rates. In addressing problems of infant mortality due to low birth weight, QPM fed pregnant women could raise the chances of child survival. Poorer sections of the society lacking resources to buy milk could rely on low cost quality protein maize with almost similar benefits (Singh and Jain 1977). QPM could also be better alternative for those groups who are unable to eat bulk food even if it is available as is the case in infants and children. A diet solely based on QPM is regarded adequate in meeting the energy and protein needs of infants and children (Graham et al. 1980, 1990). It is believed that QPM should be good preventive measure for infants and young children ranging from 3 months to 3 years in age in reducing mortality and growth rates. Studies on adults using QPM are limited, however, indications are that QPM is more efficient than normal corn in supplying the protein requirements of adults (Clark 1966; Clark *et al.* 1977). QPM can also provide a high amount of usable protein as energy 8.3 – 9.6% when a value of 8% is considered adequate for one-year old child. Carotenoids, plant pigments that are precursors and give rise to vitamin A in the body, are better utilized in QPM than in the normal maize. QPM should be considered as special purpose food like weaning food for infants. QPM could also be better alternative for those groups who are unable to eat bulk food even if it is available as is the case in infants and children. From limited studies on humans and animals, it is well demonstrated that it has high biological value (BV), high digestibility and better food efficiency (g food intake/ g weight gain). In defining exact and further role of QPM in human nutrition additional studies are needed to make nutritional and economic assessment.

Value of High-lysine Maize in Animal Nutrition

A variety of animals have been used in demonstrating superior performance of QPM compared to normal alone or in combination with different food rations. It is fair to say that QPM has great potential in monogastric animals such as rats, chickens and swine. In experiments carried out in the last three decades or so, there is a clear evidence of QPM as better feed than normal maize because proteins are well balanced. Other advantages and role of QPM could be seen in substituting high protein costly supplements like soybean or fish meal by QPM to reduce feeding cost.

Feeding trials on rats show that rats fed on opaque-2 maize showed 3-6 fold increase in body weight compared to normal. Bressani has obtained similar results on rats in Guatemala. Results on rats also exhibited greater food intake (162 for QPM and 130.5 for N) and better-feed efficiency (7.0 in normal and 9.4 in QPM).

In chicken feeding, QPM could play a much greater role because of increasing demand of poultry in several countries of Asia. In poultry feeding some special considerations ought to be kept in mind. The growing chicks need high protein and high methionine content diets. With only methionine supplementation, the opaque-2 fed chicken grew faster than normal maize and produced better gains and feed conversion than did normal maize at below optimal protein levels. Feed efficiency results obtained from Guatemala trials were quite striking. The feed efficiency ratio for QPM and normal was 3.5:1 and 8.2:1 respectively. From limited studies that are available in Guatemala, one may conclude that QPM has great promise for chicken if supplemented adequately with methionine.

Field demonstrations of QPM on swine have produced striking and convincing results. Thus pigs can be used as model animal in demonstrating the value of this special maize. For swine, QPM can be fed as the only source of protein during finishing, gestation and pre-gestation periods in the life cycle without reducing growth (Maner, 1975). In Colombian trials, pigs fed QPM grew 3.5 times faster than on normal maize when maize was the sole protein source. Since protein in QPM is not concentrated, it is advisable to add or mix with some supplement. Animals gain weight faster than human especially during early growing period. Piglets and rats, for example, put on 10% of their body weight per day. In contrasts, infant puts on only 1.0 % of its body weight. It is therefore recommended that for baby pigs, growing pigs or lactating sows, opaque-2 corn must be supplemented with extra protein supplement to produce optimum and maximum performance. The dramatic effects of QPM have been demonstrated in other countries such as Guatemala, China, Vietnam and Kenya. In Guizou province of China, QPM intervention through pig raising transformed the livelihoods of the poorest people in the poorest province. From the foregoing it may be concluded that rearing and production of pigs and chicken can be done more efficiently on QPM and this will indirectly improve diets by providing more meat and eggs.

The expanded demand for meat and other animal products has witnessed unprecedented growth. In the next two decades the growth is likely to continue at the rate of 3.3 percent per year. The demand for feed will thus rise rapidly and will have to be met by cereal (s) which have potential for increased productivity and possible improved nutritional value for better feed efficiency. Maize will certainly play a dominant role and QPM will have the added advantage being superior in protein quality and higher in feed efficiency.

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