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Strategies for improving the quality of maize protein

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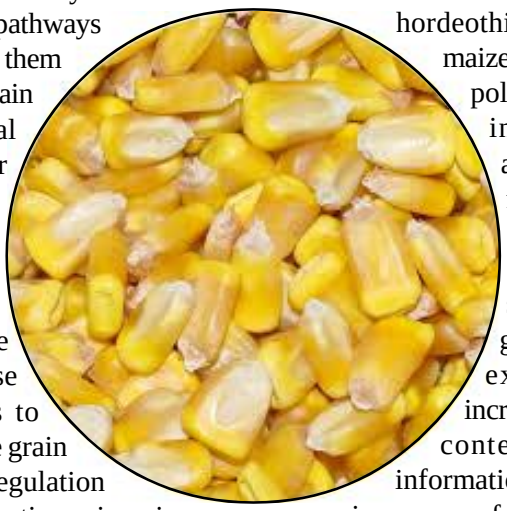
Maize is an important food and feed crop worldwide. It occupies 144.4 m ha globally with a production of 695.2 m tones. Maize (*Zea mays* L.) is the third most important cereal in India after wheat and rice. The area under maize in India is 7.6 m ha with a production of 14.7 m tones. Although maize is widely used as both food and feed, normal maize kernels do not provide sufficient quantities of two essential amino acids, lysine and tryptophan. The nutritional well-being and health of all people are vital prerequisites for the development of societies. Significant advances have been made in genetic enhancement of crop plants for nutritional value.

Grain protein quality is governed by various biochemical pathways and once those pathways are elucidated, it is easier to manipulate them for bringing about desired changes in grain protein profile. Various biochemical pathways are potential targets for manipulation using genetic engineering. Modification of genes encoding zein and genetic engineering of key enzymes involved in the lysine biosynthetic pathway, namely aspartate kinase and dihydropicolinate synthase are some of the other alternatives to enhance the nutritional value of maize grain by increasing the lysine content. Deregulation of lysine biosynthetic pathway via genetic engineering may prove to be effective, provided there is no impairment of normal metabolic functions in the vegetative tissues and the increased lysine is confined to the kernel.



Genetic engineering in improving the protein quality of maize : Genetic engineering has targeted all the traits amenable to manipulation and quality is not exception to it. The use of genetic engineering in improving protein quality of maize is a very potential area of application of biotechnology since conventional breeding suffers from various draw backs. Genetic and genetic engineering strategies to increase both total protein content and the composition of essential amino acids have been employed. These include the exploitation of mutant high lysine genes and the use of transformation to either express additional proteins which are rich in lysine and/or methionine or to increase the free pools of these amino acids.

Increasing lysine rich proteins : Introduction of proteins that carry higher proportion of lysine residues is a unique approach to improve grain protein. This approach offers advantages as it is not associated with any adverse effect on grain texture. Furthermore, since a number of such proteins have been identified among cereals it will not attract too many regulatory controversies. A number of proteins have been found to have higher lysine content including β- amylase (5%), protein Z(7.1%), chrymotrypsin inhibitors CI-I (9.5%) and CI-2 (11.5%) and hordeothionin of barley. Transformation of maize with a lysine rich protein from potato pollen resulting in 50 per cent increase in grain protein and lysine is an alternative strategy. The sb401 gene from potato (*Solanum berthaultii*) encoding a pollen-specific protein with high lysine content was successfully integrated into the genome of maize plants and its expression was correlated with increased levels of lysine and total protein content in maize seeds. But lack of information about its biological functions is a major concern for using it on large scale.

Regulating zein synthesis : The predominant proteins in maize grain are a family of alcohol-soluble prolamin storage proteins called zeins. They account for >50 per cent of total seed proteins but are deficient in several essential amino acids. As a result, the corn grain is considered to be nutritionally poor. Thus, corn mutants with reduced levels of zeins, such as *opaque-2* (*o2*), have been demonstrated to possess grain with improved nutritional quality characteristics. The *o2* mutant has a superior amino acid composition and has been used through conventional breeding to develop Quality Protein Maize (QPM) for human and animal consumption in developing countries. With the understanding of molecular genetics of zeins and progress in biotechnology, an alternative

approach to zein reduction is explored. Through the targeted reduction of the 19-kDa α-zeins, increased levels of lysine, tryptophan, and methionine have been engineered in grain of transgenic hybrids.

In maize, RNA interference induced α-zeins resulted in higher lysine content (upto 16-20% of more lysine) much below the O_2 mutants. Double stranded RNA had been used as a refined approach to simultaneously down regulate α-zeins resulting in increase in lysine from 2.83 to 5.62 per cent and tryptophan from 0.69 to 1.22 per cent. The advantage of this approach is based on the fact that the dominant nature of transgene ensures maintenance of quality in farmers fields under varying degrees of contamination from pollen of normal maize, which is a major problem in case of recessive O_2 mutants.

Lower ~-zein content in *o2* endosperm results in protein bodies that are about one-fifth to one tenth the normal size which alter packing of starch grains during seed desiccation, thereby conferring a characteristic soft texture to the kernel. With the reduction of *a*-zeins in the endosperm due to *o2* mutation, there is an usually concomitant increase in the level of γ-zein. γ-Zeins are essential for endosperm modification in quality protein maize.

Increasing the level of free amino acids : Even though free amino acids account for small proportion of grain proteins, they are also a potential target for genetic manipulation. The essential aminoacids such as lysine, threonine and methionine, in all higher plants are synthesised from aspartic acid via a pathway that is highly branched and under complex feed back inhibition. The key enzymes of pathway are aspartate kinase (AK) and dihydropicolinate synthase (DHPS). Aspartate kinase is important at early steps of pathway and is inhibited by both lysine and threonine while DHPS is inhibited by lysine only. *Corynebacterium* DHPS gene driven by globulin-1 promoter in aleurone and embryo of maize increase 50-100 per cent of free lysine. The expression of DHPS in maize increases the free lysine content in grain from 2 to 30 per cent of total amino acid pool. Monsanto in 2006 has also released transgenic maize with high lysine by expressing feed back insensitive DHPS gene from *Corynebacterium* driven by globulin-1 gene promoter. The free lysine in grain increased from 2500-2800 ppm to 3500-5300 ppm.

MAS, a promising approach to conventional plant breeding : Indian Council of Agricultural Research (ICAR) have developed a marker-assisted technique that enables a simple, rapid and efficient way to breed QPM maize. Marker assisted selection is an appropriate technology for traits such as high lysine in maize and can be a cost effective procedure for selecting *o2* locus in breeding populations. The conventional breeding procedures have been used successfully to convert commercial lines to QPM forms, however, the procedure is highly cumbersome and time-consuming utilizing the opaque-2 gene, conventional breeding procedures have been successfully used to convert commercial maize lines into so called Quality Protein Maize (QPM). A natural mutant, *opaque 2* (*o2*) causes reduction of zeins, an increase of nonzein proteins, and as a consequence, doubling of lysine levels. However, *o2*'s soft inferior kernels precluded its commercial use. Breeders subsequently overcame kernel softness, selecting several quantitative loci (QTLs), called *o2* modifiers, without losing the high-lysine trait. These maize lines are known as "quality protein maize" (QPM).

With sequencing of maize genome being finished, a large number of market system are now available that are associated with *o2* and endosperm modification phenotype. An appropriate application of such markers will greatly enhance the efficiency of selection for improvement of grain protein in maize besides cutting down the cost and time. Both foreground MAS and background MAS can be effectively employed for selecting *o2* phenotype besides ensuring maximum recovery of recurrent parent. MAS have been successfully used for development of QPM parental lines of Vivek-9 hybrid and could develop QPM hybrid in less than half the time required through conventional breeding. They found that using marker for QPM and endosperm modification in tandem can greatly enhance the selection efficiency for isolating fully modified kernels in QPM background. MAS programmes to convert locally-adapted maize germplasm to QPM have also been initiated.

In this context, the story of quality protein maize (QPM) assumes significance. The protein profile of QPM maize is 90 per cent of the milk protein. CIMMYT, Mexico played a significant role in the development of QPM maize. This achievement at CIMMYT paved the way for the transfer of QPM traits to other local/regional inbreds, composites and the synthetic cultivars. Many Asian, African and South American countries including India, China, Mexico, Ghana, Peru and Brazil have commercialized QPM cultivars. Globally QPM occupies 9.0 million acres. India released many CIMMYT QPM hybrids *viz.*, Shaktiman 1, Shaktiman 2, Shaktiman 3, Shaktiman 4, HQPM 1 and HQPM 5, besides few composites.....

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