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Review article

ADVANCES IN TRANSGENIC MAIZE FOR QUALITY IMPROVEMENT

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ABSTRACT: Maize (*Zea mays*) is a major food and animal feed worldwide and occupies a relevant place in the world economy and trade as an industrial grain crop. Currently more than 70% of maize production is used for food and feed; therefore, knowledge of genes involved in grain structure and chemical is important for improving the nutritional and food-making properties of maize. It is a good source of carbohydrates, fats, proteins, vitamins and minerals but deficient in two essential amino acids, Viz., lysine and tryptophan. To overcome this problem and to improve the above quality characters the maize breeders have followed different strategies like opaque 2, QPM and development of transgenic maize with improved quality characters. Finally we can conclude that the conventional breeding techniques and now plant biotechnology are helping meet the growing demand for food production, nutrition security while preserving our environment for future generations.

INTRODUCTION

Maize (*Zea mays*) is a major food and animal feed worldwide and occupies a relevant place in the world economy and trade as an industrial grain crop (White & Johnson, 2003). Currently more than 70% of maize production is used for food and feed; therefore, knowledge of genes involved in grain structure and chemical is important for improving the nutritional and food-making properties of maize. Although, plant breeding has been extremely successful at improving the yield of maize, quality has received less attention. However, important advances were made by breeders in this area as well, resulting in maize with a wide range of compositions. In fact, by exploiting genetic variation, the composition of the kernel was altered for both the quantity and quality (structure and chemical diversity) of starch, protein, and oil throughout kernel development. Furthermore, the ability of plant scientists to use existing genetic variation and to identify and manipulate commercially important genes will open new avenues to design novel variation in grain composition. This will provide the basis for the development of the next generation of specialty in maize and of new products to meet future needs. This chapter focuses on gene discovery, exploitation, and genetic variation known to affect the development and chemical composition of maize kernel. Throughout the chapter we have attempted to summarize the current status in these areas with a particular reference to deposition of storage proteins, starches, lipids, and carotenoids, and research pertinent to enhance kernel quality-related traits. Finally, we provide a brief outlook on future developments in this field and the resultant opportunities and application of conventional and molecular breeding for the development of new maize products better suited to its various end uses.

STRUCTURE OF MAIZE KERNEL

As a typical angiosperm, the maize kernel comprise two zygotic tissues, namely the embryo (germ) and the endosperm, that are embedded in the testa (or seed coat) and the pericarp (or) fruit wall), which fuse into a thin protective envelope. The endosperm is the main storage site of starches and proteins, whereas the embryo reserves mainly lipids. However, the economical and nutritional value of the kernel is mostly derived from the endosperm, a starch-rich tissue, that supports the embryo at germination.

In maize, endosperm makes up the majority of kernel dry matter (70-90%) and is the predominant sink of photosynthates and other assimilates during reproductive growth; therefore, factors that mediate endosperm development to a large extent also determine grain yield. Furthermore, the endosperm of seed can serve as a valuable system to address fundamental questions related to the improvement of seed size in crops.

COMPOSITION OF MAIZE KERNEL

Starch

The major chemical component of the maize kernel is starch, which provides up to 72 to 73 percent of the kernel weight. Other carbohydrates are simple sugars present as glucose, sucrose and fructose in amounts that vary from 1 to 3 percent of the kernel. The starch in maize is made up of two glucose polymers: amylose, an essentially linear molecule, and amylopectin, a branched form. The composition of maize starch is genetically controlled. In common maize, with either the dent or flint type of endosperm, amylose makes up 25 to 30 percent of the starch and amylopectin makes up 70 to 75 percent. Waxy maize contains a starch that is 100 percent amylopectin. An endosperm mutant called amylose-extender (ae) induces an increase in the amylose proportion of the starch to 50 percent and higher. Other genes, alone or in combination, may also modify the amylose-to-amylopectin ratio in maize starch.

Proteins

After starch, the next largest chemical component of the kernel is protein. Protein content varies in common varieties from about 8 to 11 percent of the kernel weight. Most of it is found in the endosperm. The protein in maize kernels has been studied extensively. It is made up of at least five different fractions, according to Landry and Moureaux (1970, 1982). In their scheme, albumins, globulins and non-protein nitrogen amount to about 18 percent of total nitrogen, in a distribution of 7 percent, 5 percent and 6 percent, respectively.

Among different proteins Prolamins occupies 60%. Amounts of the alcohol-soluble proteins (zein proteins) are low in immature maize. They increase as the grain matures. When these fractions were analyzed for their amino acid content, the zein fraction was shown to be very low in lysine content and lacking in tryptophan. Since these zein fractions make up more than 50 percent of the kernel protein, it follows that the protein is also low in these two amino acids. The albumin, globulin and glutelin fractions, on the other hand, contain relatively high levels of lysine and tryptophan. Another important feature of the zein fractions is their very high content of leucine, an amino acid implicated in isoleucine deficiency. Based on their evolutionary relationships, zeins are divided into four protein subfamily of α- (19 and 22-kDa), β- (15 kDa), γ- (16-, 27-, and 50-kDa), and δ-zeins (10- and 18-kDa), that are encoded by distinct classes of structural genes. Quality protein maize differs from common maize in the weight distribution of the five protein fractions. The extent of the change is variable and affected by genotype and cultural conditions. It has been found, however, that the opaque-2 gene reduces the concentration of zein by some 30 percent. As a result, lysine and tryptophan content is higher in QPM varieties than in common maize. The nutritional quality of maize as a food is determined by the amino acid make-up of its protein. In common maize, deficiencies in lysine and tryptophan are evident as compared with QPM. An additional important feature is the high leucine content in common maize and the lower value of this amino acid in QPM.

Oil and fatty acids

The oil content of the maize kernel comes mainly from the germ. Oil content is genetically controlled, with values ranging from 3 to 18 percent.. These values differ to some extent; it may be expected that oils from different varieties have different compositions. Maize oil has a low level of saturated fatty acids, i.e. on average 11 percent palmitic and 2 percent stearic acid. On the other hand, it contains relatively high levels of polyunsaturated fatty acids, mainly linoleic acid with an average value of about 24 percent. Only very small amounts of linoleic and arachidonic acids have been reported. Furthermore, maize oil is relatively stable since it contains only small amounts of linoleic acid (0.7 percent) and high levels of natural antioxidants. Maize oil is highly regarded because of its fatty acid distribution, mainly oleic and linoleic acids. In this respect, populations that consume degermed maize benefit less in terms of oil and fatty acids than populations that consume whole-kernel products.

Dietary fibre

After carbohydrates, proteins and fats, dietary fibre is the chemical component found in the greatest amounts. The complex carbohydrate content of the maize kernel comes from the pericarp and the tip cap, although it is also provided by the endosperm cell walls and to a smaller extent the germ cell walls. Differences in soluble and insoluble dietary fibre are small between samples, even though QPM has higher levels of total dietary fibre than common maize, mainly because of a higher level of insoluble fibre. Maize bran was composed of 75 percent hemicellulose, 25 percent cellulose and 0.1 percent lignin on a dry-weight basis. Dietary fibre content in dehulled kernels would obviously be lower than that of whole kernels.

Other carbohydrates

When mature, the maize kernel contains carbohydrates other than starch in small amounts. Total sugars in the kernel range between I and 3 percent, with sucrose, the major component, found mostly in the germ. Higher levels of monosaccharides, disaccharides and trisaccharides are present in maturing kernels. At 12 days after pollination the sugar content is relatively high, while starch is low. As the kernel matures, the sugars decline and starch increases. For example, sugars were found to have reached a level of 9.4 percent of kernel dry weight in 16-day-old kernels, but the level decreased significantly with age. Sucrose concentration at 15 to 18 days after pollination was between 4 and 8 percent of kernel dry weight. These relatively high levels of reducing sugar and sucrose are possibly the reason why immature common maize and, even more, sweet maize are so well liked by people.

Minerals

The concentration of ash in the maize kernel is about 1.3 percent, only slightly lower than the crude fibre content. Environmental factors probably influence the mineral content. The germ is relatively rich in minerals, with an average value of 11 percent as compared with less than I percent in the endosperm. The germ provides about 78 percent of the whole kernel minerals. The most abundant mineral is phosphorus, found as phytate of potassium and magnesium. All of the phosphorus is found in the embryo, with values in common maize of about 0.90 percent and about 0.92 percent in opaque-2 maize. As with most cereal grains, maize is low in calcium content and also low in trace minerals.

Table 1 - Mineral content of maize (Average of five samples)

Mineral	Concentration (mg/100 g)
P	299.6 ± 57.8
K	324.8 ± 33.9
Ca	48.3 ± 12.3
Mg	107.9 ± 9.4
Na	59.2 ± 4.1
Fe	4.8 ± 1.9
Cu	1.3 ± 0.2
Mn	1.0 ± 0.2
Zn	4.6 ± 1.2

Source: Bressani, Breuner and Ortiz, 1989

The other fat-soluble vitamin, vitamin E, which is subject to some genetic control, is found mainly in the germ. The source of vitamin E is four tocopherols, of which alpha-tocopherol is the most biologically active. Gamma-tocopherol is probably more active as an antioxidant than alpha tocopherol, however.

Water-soluble vitamins

Water-soluble vitamins are found mainly in the aleurone layer of the maize kernel, followed by the germ and endosperm. This distribution is important in processing, which, as will be shown later, induces significant losses of the vitamins. Variable amounts of thiamine and riboflavin have been reported.

The content is affected by the environment and cultural practices rather than by genetic make-up. Variability between varieties has, however, been reported for both vitamins. The water-soluble vitamin nicotinic acid has attracted much research because of its association with niacin deficiency or pellagra, which is prevalent in populations consuming high amounts of maize. As with other vitamins, niacin content varies among varieties, with average values of about 20 µg per gram. A feature peculiar to niacin is that it is bound and therefore not available to the animal organism. Some processing techniques hydrolyze niacin, thereby making it available. The association of maize intake and pellagra is a result of the low levels of niacin in the grain, although experimental evidence has shown that amino acid imbalances, such as the ratio of leucine to isoleucine, and the availability of tryptophan are also important. Maize has no vitamin B12, and the mature kernel contains only small amounts of ascorbic acid, if any. Other vitamins such as choline, folic acid and pantothenic acid are found in very low concentrations.

Changes in chemical composition and nutritive value during grain development

In many countries, immature maize is often used as a food, either cooked whole as corn on the cob or ground to remove the seed-coat, with the pulp used to make thick gruels or foods like tamalitos. The changes in chemical composition that take place upon maturation are important. All relevant studies have shown a decrease in nitrogen, crude fibre and ash on a dry-weight basis and an increase in starch and ether extract. The alcohol-soluble proteins increase rapidly as the kernel matures, while acid- and alkali-soluble proteins decrease. During this biochemical process arginine, isoleucine, leucine and phenylalanine (expressed as mg per g N) increase, while lysine methionine and tryptophan decrease with maturation. Thus, immature maize should be promoted during weaning or for infant nutrition.

Nutritional value of maize

The importance of cereal grains to the nutrition of millions of people around the world is widely recognized. Because they make up such a large part of diets in developing countries, cereal grains cannot be considered only as a source of energy, as they provide significant amounts of protein as well. It is also recognized that cereal grains have a low protein concentration and that protein quality is limited by deficiencies in some essential amino acids, mainly lysine Much less appreciated, however, is the fact that some cereal grains contain an excess of certain essential amino acids that influence the efficiency of protein utilization. The classic example is maize. Other cereal grains have the same constraints but less obviously.

A comparison of the nutritional value of maize protein with the protein quality of eight other cereals is given in Table 2, expressed as percentages of casein. The protein quality of common maize is similar to that of the other cereals except rice. Both opaque-2 maize and the hard-endosperm QPM have a protein quality not only higher than that of common maize, but also significantly higher than that of other cereal grains.

Table 2. - Protein quality of maize and other cereal grains

Cereal	Protein quality (% casein)
Common maize	32.1
Opaque-2 maize	96.8
QPM	82.1
Rice	79.3
Wheat	38.7
Oats	59.0
Sorghum	32.5
Barley	58.0
Pearl millet	46.4
Finger millet	35.7
Teff	56.2
Rye	64.8

The reasons for the low quality of maize proteins have been extensively studied by numerous investigators. Some researchers have reported that tryptophan rather than lysine is the first limiting amino acid in maize, which may be true for some varieties with a high lysine concentration or for maize products modified by some kind of processing. All researchers have agreed that the simultaneous addition of both lysine and tryptophan improves the protein quality of maize significantly; this has been demonstrated in experimental work with animals. The improvement in quality obtained after the addition of lysine and tryptophan has been small in some studies and higher in others when other amino acids have been added. Apparently, the limiting amino acid after lysine and tryptophan is isoleucine, as detected from animal feeding studies Most researchers who reported such findings indicated that the effect of isoleucine addition resulted from an excess of leucine which interfered with the absorption and utilization of isoleucine. It has been reported that high consumption of leucine along with the protein in maize increases niacin requirements, and this amino acid could be partly responsible for pellagra.

Transgenic Approach for Different Characters

Improved Lysine and Tryptophan Content

The use of molecular biology to isolate, characterize and modify individual genes followed by plant transformation and trait analysis will introduce new traits and more diversity into maize database. For example, maize-based diets (animals or humans) require lysine and tryptophan supplementation for adequate protein synthesis. The development of high lysine maize to use in improved animal feeds illustrates the challenges that continually interlace metabolic engineering projects. From a biochemical standpoint, the metabolic pathway for lysine biosynthesis in plants is very similar to that in many bacteria. The key enzymes in the biosynthetic pathway are aspartakinase (AK) and dihydrodipicolinic acid synthase (DHDPS), both of which are feedback inhibited by lysine. Bacterial genes encoding lysine-insensitive forms of aspartakinase (AK) and DHDPS (Falco et al.,1995) were isolated from *Escherichia coli and Corynebacterium,* respectively. Overexpression bacterial DHPS in maize seeds overproduced lysine. Likewise, a gene corresponding to resistant form of the enzyme anthranilate synthase (AS) has been cloned from maize and re-introduced via transformation under the control of seed specific promoters. This altered has reduced sensitivity to inhibition by tryptophan, thus overproduced tryptophan.

Starch Synthesis

Many biochemical and molecular studies on starch synthesis have been also focused on identifying the rate limiting enzymes to control metabolism. In this context, ADP-glucose pyrophosphorylase (AGPase) plays a key role in regulating starch biosynthesis in cereal seeds. Transfer of Bt2 *and Sh2 genes from wild maize,* with an endosperm-specific promoter from 27-kDa zein or an endosperm-specific promoter from 22-kDa zein, into elite inbred lines, solely and in tandem, by *Agrobacterium tumefaciens mediated* transformation, was possible (Li et al., 2008). This would resulted into development of transgenic maize kernels which exhibited higher *Bt2* and *Sh2* gene expression, higher AGPase activity, higher seed weight, and the kernels accumulated more starch compared with non-transgenic plants. The over-expression of either gene enhanced AGPase activity, seed weight (+15%) and starch content compared with the wild type, but the amounts were lower than plants with over-expression of both *Bt2* and *Sh2*. Collectively, these results indicate that over-expression of those genes in transgenic maize plants could improve kernel traits and provide a feasible approach for enhancing starch content and seed weight in maize. Introduction of bacterial *glgC16 gene* into maize which improves ATPase activity and provide a feasible approach for enhancing starch content and seed weight in maize (Wang *et al.* , 2007). *Bacillus amyloliquefaciens SacB gene accumulates* high-molecular weight fructose in mature seed. *This could potentially be* exploited within the high-fructose maize syrup market (Caimi *et al.*1996). Transgenic maize endosperm was developed, via the introduction of a *Streptococcus mutant's gtfD* gene that accumulates novel glucan polymers at levels relevant to commercial production (Zhang *et al*. 2007).

Improved oil Content

Increasing seed oil production is a major goal for global agriculture to meet the strong demand for oil consumption by humans and for biodiesel production. Efforts to increase oil content and composition in maize kernels through breeding have considerable success, but high oil lines have significant reduced yield (Moose et al., 2004). Several and complementary approaches might be considered to try and enhance oil content in maize kernels. This goal may be achieved by increasing the relative proportion of the oil rich embryonic tissue within the grain.

It has been reported that embryo size and oil content could be increased in transgenic maize by ectopic expression of the wheat *Purindoline a and b (PINA and PINB) genes (Zhang, et al., 2010).* While total oil content of the kernel was increased by 25% in these transgenic lines, the molecular mechanism responsible for the increase remains to be clarified. The recent identification of transcriptional regulators of the oil biosynthetic network in maize has opened the way for designing and testing new original biotechnological strategies. A study has shown that seed-specific expression *ZmWRI1*, a WRI1-like gene of maize, enhanced oil accumulation in transgenic maize without detectable abnormalities. However, expression of *ZmLEC1* under similar conditions severely affected growth and development of the resulting transgenic maize plants (Shen, et al., 2010*).* It was also found that *ZmWri1* not only increases the fatty acid content of the mature maize grain but also the content of certain amino acids (Lys, Glu, Phe, Ala, Val) of several compounds involved in amino acid biosynthesis (pyro-Glu, aminoadipic acid, Orn, nor- Leu), and of two intermediates of the TCA cycle. To increase kernel oil content, fungal *diacylglycerol acyltransferase2* (*DGAT2*) genes from *Umbelopsis ramanniana* and *Neurospora crassa* were also introduced into maize using an embryo-enhanced promoter (Oakes et al., 2011). The protein encoded by the *N. crassa* gene was longer than that of *U. ramanniana*. Expression of transgenes in maize resulted in small but statistically significant increases in kernel oil. *S-NcDGAT2* had the biggest impact on kernel oil, with a 26% (relative) increase in oil in kernels of the best inbred.

Carotenoids

Along with their essential role in photosynthesis, carotenoids are of significant economic interest as natural pigments and food additives (reviewed in Botella-Pavía & Rodríguez- Concepción, 2006). Their presence in the human diet provides health benefits as nontoxic precursors of vitamin A and antioxidants, including protection against cancer and other chronic diseases (review by Fraser & Bramley 2004). These motives have promoted scientists to explore ways to improve carotenoid content and composition in staple crops (reviewed in Sandmann et al. 2006; Zhu et al. 2009). Analyses of genotypes with yellow to dark orange kernels exhibits considerable natural variation for kernel carotenoids, with some lines accumulating as much as 66 μg/g (e.g. Harjes et al., 2008), with provitamin A activity (*β*- \cryptoxanthin, *α*- and *β*-carotene is typically small (15% to 18% of the total carotenoids fraction) compared to lutein or zeaxanthin (45% and 35%, respectively; Kurlich & Juvik, 1999; Brenna and Berardo, 2004). Moreover, a moderate to high heritability estimates indicate that breeding for increased levels of both carotenes and xanthophylls should be feasible. Transgenic strategies can also be used as tools to complement breeding techniques in meeting the estimated levels of provitamin A. In this respect, the over expression of the bacterial genes *crtB* (for PS) and *crtI* (for the four desaturation steps of the carotenoid pathway catalyzed by PDS and β-carotene desaturase in plants), resulted in an increase of total carotenoids of up to 34-fold with a preferential accumulation of β-carotene in the maize endosperm (Aluru et al. 2008). The levels attained approach those estimated to have a significant impact on the nutritional status of target populations in developing countries. Furthermore, the same authors, via gene expression analyses, suggested that increased accumulation of β-carotene is due to an up-regulation of the endogenous lycopene β-cylase. These experiments set the stage for the design of transgenic approaches to generate provitamin A-rich maize that will help alleviate vitamin A deficiency in developing countries. Similarly, Naqvi et al. (2009). produced transgenic maize plants with significantly increased contents for *β-carotene, ascorbate, and folate in the endosperm via that* simultaneous modification of 3 separate metabolic pathways .The transgenic kernels contained 169-fold the normal amount of *β-carotene, 6-fold, and 2-fold the normal amount* of ascorbate and folate, respectively

To increase -carotene levels, they introduced corn *phytoene synthase (psy1) cDNA under the control of* the wheat LMW glutenin promoter and the *Pantoea ananatis crtI gene carotene desaturase)* under the control of the barley D-hordein promoter. To increase ascorbate levels they introduced rice dehydroascorbate reductase (*dhar) cDNA, and to increase folate levels introduced* the *E. coli folE gene encoding GTP cyclohydrolase (GCH1), both* under the control of the barley D-hordein promoter.

Reduction in the Phytic Acid Level

Transgenic approaches may help solve an important problem with maize as a feed grain is in the reduction of phytic acid levels. In maize, 80% of the total phosphorous (P) is found as phytic acid, and most of that is in the germ (O'Dell et al., 1972). Phytate P is very poorly digested by non-ruminant animals, therefore inorganic supplementation is necessary. Phytate is also a strong chelator that reduces the bioavailability of several other essential minerals such as Ca, Zn, Cu, Mn, and Fe. In addition, since the phytate in the diet is poorly digested, the excrement of monogastric animals (e.g. poultry and pigs), is rich in P and this contributes significantly to environmental pollution.

Even though, low phytic acid mutants (*lpa-1*) identified, have limited value to breeder because of adverse effects on agronomic traits such as low germination rates, reduced seed weight (*lpa1-1*), stunted vegetative growth and impaired seed development. Shi *et al.* (2007) have identified the gene in maize *lpa1 mutants as a multidrug resistance-associated protein (MRP) ATP binding* cassette (ABC) transporter. Silencing expression of this transporter using the embryo specific globuline promoter produced low-phytic acid, high phosphate transgenic maize seeds.

Iron (Fe)

To increase the amount of bioavailable iron in maize, Drakakaki et al. (2005) have generated transgenic maize plants expressing aspergillus phytase and iron-binding protein ferritin. This strategy has proven effective for increasing iron availability and enhancing its absorption. However, much work is still to be done to transfer this technology to tropical and subtropical maize genotypes normally grown in the areas of greatest need for enhanced iron content maize.

Molecular Farming

A relatively new area in plant biotechnology is the use of genetically-engineered maize to produce high-value end products such as vaccines, therapeutic proteins, industrial enzymes and specialty chemicals. The long-term commercial expectations for this use of “plants as factories”, often also called “molecular farming”, are large. Transgenic maize seed has many attractive features for this purpose, including: i) well-suited for the production and storage of recombinant proteins; ii) ease of scale-up to essentially an infinite capacity; iii) well-established infrastructure for producing, harvesting, transporting, storing, and processing; iv) low cost of production; v) freedom from animal pathogenic contaminants; vi) relative ease of producing transgenic plants which express foreign proteins of interest.

However, there is a need, apart the public issues related with the acceptance of genetically-engineered maize, for continued efforts in increasing expression in order to reduce cost effectiveness for products at protein accumulation levels in transgenic plants to broaden this new uses.

CONCLUSSION

Two prominent features of agriculture in the 20th century have been the use of breeding and genetics to boost crop productivity and the use of agricultural chemicals to protect crops and enhance plant growth. In the 21st century, crops must produce good yields while conserving land, water, and labor resources. At the same time, industries and consumers require plants with an improved and novel variation in grain composition. We expect that genomics will bolster plant biochemistry as researchers seek to understand the metabolic pathways for the synthesis of these compounds. Identifying rate-limiting steps in synthesis could provide targets for genetically engineering biochemical pathways to produce augmented amounts of compounds and new compounds. Targeted expression will be used to channel metabolic flow into new pathways, while gene-silencing tools will reduce or eliminate undesirable compounds or traits. Therefore, developing plants with improved grain quality traits involves overcoming a variety of technical challenges inherent in metabolic engineering programs.

In conclusion, the green revolution and now plant biotechnology are helping meet the growing demand for food production, nutrition security while preserving our environment for future generations.

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