Improving the Content of Essential Amino Acids in Crop Plants: Goals and Opportunities[1]

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The inability of humans and many farm animals to synthesize certain amino acids has long triggered tremendous interest in increasing the levels of these so-called essential amino acids in crop plants. Knowledge obtained from basic genetic and genetic engineering research has also been successfully used to enrich the content of some of these essential amino acids in crop plants. Among the essential amino acids, Lys, Trp, and Met have received the most attention because they are most limiting in cereals (particularly Lys and Trp) and legumes crops (particularly Met), which represent the major sources of human food and animal feed worldwide. Enriching crop plants in essential amino acids has both economical and humanitarian interest. In developed countries, the interest is mostly for the livestock feeding industry because farm animals generally provide sufficient amount of essential amino acids for human diets. In developing countries, where plants directly account for the majority of the food, the interest is both humanitarian and economical.

So far, the success of genetic approaches has been mostly restricted to maize (*Zea mays*) by generating quality protein maize (QPM) cultivars, which are enriched in Lys and to some extent Trp in their seeds. However, genetic approaches have resulted in relatively limited success in other crop species. This is mostly due to limited availability of genetic resources for plant breeding, and the fact that genetic traits for high contents of Lys, Trp, or Met are generally associated with abnormal plant growth because these traits do not operate in a seed-specific manner. In contrast, results from genetic engineering research appear to be more promising, particularly because this approach allows seed-specific expression of specific traits of interest, using seed-specific promoters. In fact, one high-Lys maize cultivar, LY038, developed by genetic engineering, represents the first genetically modified (GM) crop with high nutritional value to be approved for commercial use in a number of countries. The potential to increase the contents of Trp and Met in a seed-specific manner have already been proven successful in basic research studies. Another advantage of genetically engineered traits is that they can be transformed into multiple plant species and genotypes and function synergistically with many other agronomically important traits. These genetic engineering approaches were generally aimed at tailor-made improvements of essential amino acid metabolic pathways and expressing native and genetically engineered proteins enriched in essential amino acid contents. However, improvements of metabolic pathways by genetic engineering also requires a detailed understanding of how these pathways interact with regulatory networks that fine tune plant development. These are now beginning to be elucidated by modern systems biology approaches, including transcriptomics, proteomics, and metabolomics. Due to space limitation, we focus this review only on approaches that have been extensively studied and proven suitable to improve the nutritional quality of food and feed. We also only cover research associated with nutritional improvements for monogastric mammals, namely, human and certain farm animals, particularly poultry and swine. These approaches are not as important for ruminant livestock, such as beef, because these animals require the presence of the essential amino acids in proteins that are resistant to rumen proteolysis. Approaches suitable for ruminant animals are discussed in the following reviews (Galili et al., 2002; Amir and Tabe, 2006).

INCREASING SEED LYS AND TRP CONTENTS IN MAIZE BY GENETIC APPROACHES: THE STORY OF QPM

Maize is one of the most important cereal crops, providing between 50% and 70% of the dietary protein for humans, depending on geographical distribution. It is also one of the major crops used for feeding farm animals, particularly poultry and swine. Since maize seeds are very low in Lys, a major effort was initiated at the mid 20th century to identify high-Lys corn varieties by genetic approaches. These efforts resulted in the discovery of the high-Lys *opaque2* mutant (Mertz et al., 1964; Mertz, 1997), which contains low levels of the Lys-poor seed storage proteins (called zeins) and a compensatory increase in Lys- and Trp-rich, non-zein, seed proteins as well as free Lys and Trp, compared to normal maize. The *opaque2* mutant seeds, together with

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minerals and vitamins, were proven to significantly increase growth in rat feeding trials (Mertz et al., 1964), and they were found to have 90% the value of milk protein when fed to Guatemalan children (Bressani, 1966). A diet based solely on *opaque2* flour was further shown to cure children who suffered from the protein deficiency disease kwashiorkor (Harpstead, 1971). Success with *opaque2* maize stimulated extensive research to identify similar mutants in other cereals, including barley (*Hordeum vulgare*; Munck et al., 1970; Doll et al., 1974), and sorghum (*Sorghum bicolor*; Singh and Axtell, 1973).

Despite the initial optimism, field analyses showed that opaque mutations were associated with inferior agronomic traits that could not be easily overcome. The undesirable traits included reduced yield and protein content as well as a soft endosperm that increased disease and insect susceptibility, kernel breakage, and poor food processing (Glover, 1992; Munck, 1992). Commercial utilization of *opaque2* mutants seemed unlikely until 1992, when researchers at the Maize and Wheat Improvement Center in Mexico and the University of Natal in South Africa (Geevers and Lake, 1992; Glover, 1992) generated *opaque2*-derived QPM lines with normal kernel properties and yields comparable to other maize cultivars. The QPM genotypes are generally associated with an increased level of the 27-kD γ-zein storage protein, which somehow compensates for the reduced level of the Lys-poor α- and β-zein storage proteins, but the molecular nature of this compensation is not understood. Progress with developing commercial QPM lines has been slow, particularly because of the complexity and multiple loci of the *opaque2* modifier genes. Research led to the discovery of the molecular basis of *opaque2* and other *opaque* mutant genotypes in maize (Gibbon and Larkins, 2005), and this knowledge has accelerated the development and commercialization of QPM maize varieties. Further details associated with the molecular basis of different opaque mutants in maize can be found in the following references (Or et al., 1993; Coleman et al., 1995; Lopes et al., 1995; Burkhardt et al., 1997; Gillikin et al., 1997; Sun et al., 1997; Kim et al., 2004, 2006).

Since its discovery, QPM maize has been used worldwide (see, for example, Bockholt and Rooney, 1992; Magnavaca et al., 1993; Zarkadas et al., 2000). Based on adaptation of QPM maize in many developing countries, the World Food Prize was awarded to the Maize and Wheat Improvement Center in Mexico researchers who contributed significantly to their development. The performance and nutritional value of the QPM varieties appears promising as exemplified by analysis of a number of QPM cultivars adapted to conditions in Canada (Zarkadas et al., 2000).

The high Lys and Trp contents of *opaque2* and QPM maize genotypes is associated with a major reduction in the Lys-poor α- and β-zein storage proteins. Attempts have also been made to reduce the content of these storage proteins by genetic engineering, using constructs designed to reduce their expression in seeds (Segal et al., 2003; Huang et al., 2005, 2006). Interestingly, specific reduction of zeins by this approach also causes an opaque phenotype (Segal et al., 2003), implying that this phenotype is directly associated with the reduction of zein storage proteins. Since many of the natural opaque mutations are associated with regulatory components of maize seed development (Kim et al., 2004; Gibbon and Larkins, 2005), it will be interesting to test the agronomical potential of zein reduction traits when combined into QPM genotypes.

IMPROVING LYS CONTENT BY GENETIC ENGINEERING

Lys is considered the most important essential amino acid, because it is most limiting in the cereals grains, which are major crops worldwide. Hence, much genetic engineering research was devoted to: (1) understanding the regulation of Lys metabolism and its exploitation for increasing free Lys level in seeds; and (2) using proteins that are enriched in Lys content. Research on increasing free Lys content provides an excellent example of how utilizing model plants can speed up research and later efficiently utilize it to improve crops, particularly maize, which is a staple crop for the livestock feeding industry.

Lys is synthesized by a branch of the Asp family pathway that also leads to the synthesis of two additional essential amino acids, namely, Met and Thr (Galili, 2002). Flux through the Lys biosynthetic branch is strongly regulated by a feedback inhibition loop in which Lys inhibits the activity of dihydrodipicolinate synthase (DHDPS), the first enzyme specifically committed to Lys biosynthesis (Galili, 2002). Genetic mutations in the tobacco (*Nicotiana tabacum*) *DHDPS* gene, rendering the enzyme Lys insensitive, or constitutive expression of a bacterial Lys-insensitive DHDPS in transgenic tobacco or Arabidopsis (*Arabidopsis thaliana*) plants caused Lys overproduction in all plant organs, including the seeds (Negrutiu et al., 1984; Frankard et al., 1992; Shaul and Galili, 1992a, 1992b; Ben Tzvi-Tzchori et al., 1996). However, high levels of Lys in all plant tissues can cause abnormal vegetative growth and flower development that, in turn, reduce seed yield (Negrutiu et al., 1984; Frankard et al., 1992; Shaul and Galili, 1992a, 1992b). Targeting the expression of bacterial Lys-insensitive DHDPS to seeds of tobacco, using a seed-specific promoter, resulted in plants with normal growth characteristics that also accumulate higher amounts of Lys in their seeds, but not yet nutritionally desirable levels (Karchi et al., 1994).

The increased accumulation of Lys in tobacco seeds was correlated with enhanced activity of a bifunctional LKR/SDH enzyme that controls the first two reactions of the α-amino adipic acid pathway of Lys catabolism (Karchi et al., 1994, 1995). To elucidate the role of Lys catabolism in balancing the Lys level in seeds, a bacterial Lys-insensitive DHDPS was expressed in a seed-specific manner either in wild-type Arabidopsis

or in an Arabidopsis *LKR/SDH* knockout mutant (Zhu and Galili, 2003). While seeds of transgenic plants expressing the bacterial DHDPS or the knockout mutant contained approximately 12- or 5-fold higher levels of seed free Lys, respectively, than wild-type plants, the combination of these two traits caused a synergistic approximately 80-fold increase in the seed free Lys level (Zhu and Galili, 2003). This showed that Lys catabolism can become very important when Lys overaccumulates.

The excessive Lys accumulated in mature seeds of Arabidopsis plants expressing bacterial DHDPS in the LKR/SDH knockout mutant background also severely reduced seed germination (Zhu and Galili, 2003). Since the *LKR/SDH* knockout eliminates Lys catabolism in all tissues, it could not be determined whether the inhibition of seedling growth was due to a negative physiological effect of excess Lys on seed maturation or defective postgermination catabolism of the high Lys level. This question was addressed by coexpressing the bacterial *DHDPS* gene with an RNAi construct of the Arabidopsis *LKR/SDH* gene, both under control of the same seed-specific promoter (Zhu and Galili, 2004). In this genetic background, both enhanced Lys synthesis and its suppressed catabolism are restricted to developing seeds. Coexpression of the two seed-specific constructs significantly boosted seed Lys content, while seed germination was significantly improved (Zhu and Galili, 2004).

Following the studies with model plants, the bacterial DHDPS was expressed in a seed-specific manner in the embryos of soybean (*Glycine max*), rapeseed (*Brassica napus*), and maize. Unlike tobacco and Arabidopsis, the transgenic soybean and rapeseed showed a significant elevation of free Lys in the mature seeds, which in some cases nearly doubled total seed Lys content (Falco et al., 1995; Mazur et al., 1999; Frizzi et al., 2008). The differences in Lys content between plant species may be due to the use of different DHDPS enzymes from different bacterial sources. Lys overproduction in these plants was also associated with increased levels of various Lys catabolites (Falco et al., 1995; Mazur et al., 1999; Huang et al., 2005; Frizzi et al., 2008). Interestingly, the bacterial DHDPS caused Lys overproduction only when expressed in the embryo, but not in the endosperm (Mazur et al., 1999; Frizzi et al., 2008). This is likely because Lys is efficiently catabolized in the endosperm, as it was also shown that endosperm-specific reduction of Lys catabolism by an RNAi approach significantly increased Lys content in maize (Houmard et al., 2007). The observation that Lys catabolism is highly active in the maize endosperm (Kemper et al., 1999; Arruda et al., 2000) provided additional support for this supposition. To overcome Lys catabolism in the endosperm, maize plants were transformed with a single endosperm-specific bifunctional expression/silencing transgene, which encodes a bacterial feedback-insensitive DHDPS with a LKR/SDH RNAi sequence in an intron (Frizzi et al., 2008). This construct resulted in a significant elevation in the seed Lys level, proving that maize endosperm possesses enzymatic activity responsible for both Lys biosynthesis and catabolism.

Substantial increases in free Lys in the embryos of transgenic plants expressing a bacterial feedback-insensitive DHDPS were in some cases associated with abnormal seed germination (Falco et al., 1995; Mazur et al., 1999). Yet, shifting Lys overproduction into the endosperm enabled free Lys accumulation in maize grains to over 4,000 ppm, compared to less than 100 ppm in control plants, with no detectable negative effect on seed germination (Frizzi et al., 2008). This could be explained by the fact that either Lys is more toxic in the embryo than in the endosperm and/or that the volume of endosperm is much larger than the embryo; hence, Lys can accumulate in the endosperm to higher levels, while maintaining a relatively low cellular concentration of this amino acid.

Most of the free amino acids in sink tissues, such as developing seeds, are incorporated into storage proteins. Thus, another approach to enhance the level of a given amino acid in seeds is to increase the protein sink for this amino acid. This can be done by transforming plants with genes encoding stable proteins that are rich in the desired amino acid(s), and can accumulate these proteins to high levels. Several types of recombinant genes encoding Lys-rich proteins have been tested so far: (1) natural genes encoding Lys-rich proteins derived from different plant or nonplant sources; (2) natural genes that have been mutated to increase the number of Lys codons and make proteins richer in Lys; and (3) synthetic genes encoding Lys-rich proteins. However, most of these attempts did not prove satisfactory because the proteins were unstable and did not accumulate to sufficiently high levels (see Sun and Liu, 2004; Beauregard and Hefford, 2006, and refs. therein for extensive discussion of this issue). Yet, among the different proteins that were tested, some were proven to accumulate to reasonably high steady-state levels. The most significant increases in seed Lys levels were obtained by expressing a genetically engineered gene encoding HORDOTHIONINE12 or the BARLEY HIGH LYSINE8 (BHL8) protein, which contain 28% and 24% Lys, respectively (Jung and Carl, 2000). These proteins accumulated in transgenic maize seeds to 3% to 6% of total grain protein, and when introduced together with a bacterial DHDPS resulted in an elevation of total Lys to over 0.7% of seed dry weight, compared to around 0.2% in wild-type maize (Jung and Carl, 2000). BHL8 is a recombinant protein derived from a barley CHYMOTRYPSIN INHIBITOR-2, which was genetically engineered to substantially increase the number of Lys codons and those of other essential amino acids, based on a three-dimensional structure analyses (Roesler and Rao, 2000). This protein also serves as a model for elucidating mechanisms of protein folding (for review, see Daggett and Fersht, 2003, and refs. therein).

In conclusion, a number of studies in maize have shown that it is possible to increase the Lys content in cereal grains to sufficiently high levels to meet the

requirements for animal feeding, with no need for addition of supplemental Lys. These studies demonstrate that a combination of the traits of *DHDPS* expression, *LKR/SDH* suppression, and expression of genetically engineered high-Lys proteins, all in an endosperm-specific manner, shows the greatest potential for producing cereal crops with an optimal dietary Lys level and minimal penalties of growth performance and yield. Yet, even simpler approaches employing only the expression of a bacterial feedback-insensitive DHDPS in an embryo-specific manner could suffice for commercial use in livestock feed. Indeed, a high-Lys maize line (LY038) expressing a bacterial feedback-insensitive DHDPS in an embryo-specific manner (Dizigan et al., 2007) has recently been approved for commercial use in the livestock feeding industry in a number of countries. The maize LY038 line, as well as a maize LY038 × MON 810 hybrid, proved superior in broiler chicken performance, compared to the same lines that lacked the LY038 trait (Lucas et al., 2007).

Recently, another interesting approach has been adapted to increase the Lys content in cereal seeds, utilizing a recombinant tRNA(lys) species that introduces Lys at alternative codons during protein synthesis (Wu et al., 2003). Expression of a recombinant gene encoding this tRNA in transgenic rice (*Oryza sativa*) caused a meaningful enrichment of the Lys content of seed proteins (Wu et al., 2003). Notably, even stable expression of a wild-type Arabidopsis gene encoding a Lys tRNA synthetase in transgenic maize caused translational recoding of Lys into the Lys-deficient zein storage proteins, apparently using non-Lys codons, and resulted in significantly enriching the Lys content in the grain (Wu et al., 2007). It will be interesting to test the extent to which such transgenes also introduce Lys at nonnatural locations in other seed proteins and what impact this approach has on the performance of the crop plants and on their safety regulations.

IMPROVING MET CONTENT BY GENETIC ENGINEERING

Met is synthesized by another branch of the Asp-family pathway that also synthesizes Lys (Galili et al., 2005). Besides being a building block of proteins, Met, through its catabolic product *S*-adenosyl-Met (SAM), is a precursor for the hormone ethylene, for polyamines, and it is also the primary methyl-group donor for multiple biological processes, such as DNA replication, cell wall development, and secondary metabolite production (Amir et al., 2002). It is likely that under favorable growth conditions, a significant portion of the flux through the Asp-family pathway results in the production of Met and in its further metabolism. A number of attempts were made to improve the Met level in plants, mostly by manipulation of genes encoding various Asp-family and Cys biosynthetic enzymes, which were described in previous reviews (see, for example, the following reviews: Amir et al., 2002; Hesse et al., 2004; Amir and Tabe, 2006; Azevedo et al., 2006). These experiments generally met with limited success, either because they were associated with severe phenotypes or Met did not accumulate, due to extensive catabolism. Indeed, reduced catabolism of Met to SAM, due to suppression of SAM synthase, generally caused increased levels of soluble Met (Boerjan et al., 1994; Goto et al., 2002; Shen et al., 2002). It is also likely that many of the metabolic phenotypes resulting from an increased Met level are indirectly associated with the reduced production of SAM. The major regulatory enzyme of Met biosynthesis is cystathionine γ-synthase (CGS), and this activity in Arabidopsis is also regulated by the level of SAM via a compound posttranscriptional control mechanism involving interactions with a highly regulatory multicomponent domain located in the N terminus of the mature CGS polypeptide (Inaba et al., 1994; Chiba et al., 1999; Hacham et al., 2002; Ominato et al., 2002; Hacham et al., 2006). Interestingly, mutations in this region, or its deletion, result in overproduction of Met, which is likely independent of a reduction of SAM synthesis (Chiba et al., 1999; Hacham et al., 2002, 2006, 2008; Ominato et al., 2002). Moreover, the combination of expression of a mutated form of CGS with a bacterial feedback-insensitive Asp kinase (the first enzyme of the Asp-family pathway) further increases the accumulation of Met (Hacham et al., 2008). Utilization of CGS enzymes with either deleted or mutated N-terminal domains currently appears as a potentially promising approach to increase the production of free Met with minimal negative effects. Yet, it is still questionable whether the regulatory characteristics of the Arabidopsis CGS are conserved in other plant species (Kreft et al., 2003). It also remains to be demonstrated whether utilization of a mutated CGS can improve Met production in seeds.

Another important metabolite regulating Met metabolism in plants is *S*-methyl-Met (SMM), a Met storage and phloem mobile metabolite that can be efficiently transported from leaves to developing seeds (Bourgis et al., 1999). SMM is synthesized from Met by Met *S*-methyltransferase (MMT) and is recycled back to Met by homo-Cys methyltransferase. However, despite the efficient transport capability of SMM, Arabidopsis and maize *mmt* mutants, which are unable to synthesize SMM, grow and reproduce normally, suggesting a minimal regulatory role for SMM in sulfur transport of at least these two species (Kocsis et al., 2003). Notwithstanding, a recent report (Lee et al., 2008) showed that a mutant Arabidopsis plant overaccumulating Met in its seeds is due to a mutation eliminating the activity of HMT2, one of the three Arabidopsis HMT isozymes that recycle SMM into Met (Lee et al., 2008). The *HMT2* gene is expressed in vegetative tissues where it apparently shifts the balance from Met to SMM in these source tissues. The accumulated SMM in this mutant apparently transports more efficiently than Met into the sink tissues, where it is converted back to Met by the two other HMT isozymes (Lee et al., 2008). Why plants

need a delicate balance between Met and SMM is an interesting question, but certainly the increased accumulation of Met in the seeds of the *HMT2* mutant suggests a novel approach to increase the nutritional value of crop plants.

Another approach to increase the Met content in plants involved the expression of sulfur-rich proteins. Although a large array of sulfur-rich proteins have been tested (see the following reviews as examples: Muntz et al., 1998; Tabe and Higgins, 1998; Tabe and Droux, 2002; Hagan et al., 2003; Galili et al., 2005; Amir and Tabe, 2006), the most commonly used and most successful approaches in terms of increased Met content included expressing heterologous, natural Met-rich storage proteins, such as the 2S albumin from Brazil nut (*Bertholletia excelsa*) and sunflower (*Helianthus annuus*). Therefore, we will focus in this review on expression of these two proteins in seeds of transgenic plants.

The Brazil nut and sunflower 2S albumins have been transgenically expressed in seeds of a number of plant species, including tobacco, canola (*B. napus*), narbon bean (*Vicia narbonensis*), and soybean. Significant enhancement of total seed Met was observed in some of these plant species, and in some cases was still below the optimal level required for human food and animal feed (Altenbach et al., 1992; Saalbach et al., 1995; Molvig et al., 1997; Tabe and Higgins, 1998; Tabe and Droux, 2002; Hagan et al., 2003; Lee et al., 2003; Chiaiese et al., 2004; Amir and Tabe, 2006). This was largely because production of the proteins came at the expense of endogenous sulfur-rich compounds, such as free Met, Cys, and glutathione, as well as endogenous Met-rich proteins. These results indicate that the available soluble Cys and Met in seeds of these species limits the accumulation of sulfur-rich proteins. Moreover, studies preformed in lupin (*Lupinus angustifolius*) expressing the sunflower 2S albumin demonstrated that the seed Cys and Met content is not only dependent on their transport from the canopy, but also on their de novo synthesis in seeds (Tabe and Droux, 2002).

Transgenic lupin seeds expressing the sunflower 2S albumin were tested in various nutritional studies. Rat feeding experiments showed not only an increase of Met availability, but also an increase in general dietary value (Molvig et al., 1997). Furthermore, the transgenic lupin seeds were superior to control lupin seeds for poultry feeding, requiring lower amounts of supplemental Met (Ravindran et al., 2002). They also increased the efficiency of wool growth in Merino sheep (White et al., 2001). Unfortunately, despite their nutritional potential, the plant 2S sulfur-rich albumins were found to be allergenic (Pastorello et al., 2001), reducing their usefulness as Met sink proteins, at least for human nutrition. Nevertheless, accumulating data suggest that a combination of specific transgenes that allow increased free Met accumulation and its incorporation into sulfur-rich proteins could considerably enhance Met accumulation in crops, such as the forage alfalfa (*Medicago sativa*; Avraham et al., 2005; Bagga et al., 2005).

IMPROVING TRP CONTENT BY GENETIC ENGINEERING

Trp is the second most limiting essential amino acids in cereal grains. Trp synthesis in plants is strongly feedback regulated by inhibiting its biosynthetic enzyme, anthranilate synthase. The discovery that a mutation rendering the Arabidopsis α-subunit of anthranilate synthase insensitive to feedback inhibition by Trp enhances Trp accumulation (Kreps et al., 1996; Li and Last, 1996) led to a number of studies using this trait to improve the Trp content in crop plants. Expression of a transgene, termed *OASA1D*, encoding an analogous feedback-insensitive α-subunit of the rice anthranilate synthase, under control of the constitutive ubiquitin promoter in transgenic rice, led to a significant increase in free Trp in the seeds (Wakasa et al., 2006). Yet, this caused significant negative effects on a limited number of important agronomical traits, including germination, spikelet fertility, and yield (Wakasa et al., 2006). Expression of the transgene also caused a nearly 2-fold increase in auxin content in the seed, which is produced from Trp (Wakasa et al., 2006). Whether constitutive expression of the transgene caused a similar auxin increase in vegetative tissues (Matsuda et al., 2005), and whether the hormonal imbalance is associated with the deleterious agronomical traits is unknown. It could be interesting to test whether specific expression of this transgene, either in the developing embryo or the developing endosperm of the rice grains, will reduce the deleterious agronomical traits. Embryo-specific and endosperm-specific promoters are currently available, both from rice and other cereals, rendering such experiments highly feasible.

The rice *OASA1D* transgene was also shown to raise the free Trp level when expressed in transgenic potato (*Solanum tuberosum*; Yamada et al., 2004), adzuki bean (*Vigna angularis*; Hanafy et al., 2006), and Arabidopsis (Ishihara et al., 2006) plants. In addition, an analogous increase in free Trp was obtained in transgenic soybean plants upon expression of a different transgene encoding a feedback-insensitive tobacco anthranilate synthase driven by the cauliflower mosaic virus 35S promoter (Inaba et al., 2007). These results, when taken together with those obtained by genetic approaches (Kreps et al., 1996; Li and Last, 1996), support an evolutionarily conserved mechanism for regulation of Trp synthesis in plants by feedback inhibition of anthranilate synthase. It is important to notice that besides being an essential building block of proteins, Trp is a precursor for a variety of secondary metabolites and therefore its overproduction may reduce or stimulate their levels. Since some of the Trp secondary metabolites are harmful either to mammals or to plant pathogens, it is important to elucidate whether increased Trp accumulation alters their production.

FUTURE GOALS AND OPPORTUNITIES

Most if not all of the currently grown commercial GM crops contain traits that are considered to be beneficial

for farmers and not consumers, such as the herbicide resistance Roundup Ready trait. GM crops with enhanced nutritional quality, such as the high-Lys LY038 maize, should be beneficial not only to farmers, but also to consumers, an issue that will hopefully increase their public acceptance. It was estimated that doubling the Lys content in maize without changing the grain protein content could increase the gross value of the U.S. maize in the world feed market by additional $360 million (Johnson et al., 2001). This is important in view of the competition between maize used for ethanol production and maize used for feeding. The progressively increasing prices of foods and feeds resulting from the massive and growing markets for biofuels are expected to further increase the value of GM crops with enhanced nutritional quality. Such crops will be important in developing countries for uses as food and feed. High Lys QPM maize has already penetrated into developing countries, such as those in Africa, and it is likely that GM crops with enhanced nutritional quality will do the same if freely available to developing countries. Individual traits for enhanced nutritional quality, such as those described in this review, could be combined into a single plant, along with other valuable traits, such as high protein and high oil content, to further improve a crop's value and competitive abilities. Moreover, the technology developed for producing high-Lys corn could be transferred to other crops used as foods and feeds, particularly to important cereals such as wheat, rice, and barley. Since cereals have comparable seed characteristics, namely, relatively large endosperms and small embryos, it is likely that the endosperm-specific approaches that have been proven most successful in maize will also succeed in the other cereals. The success with high-Lys maize also provides an optimistic basis for generating GM crops with enhanced levels of other important essential amino acids. A second GM crop that has already been proven beneficial in feeding trials is the transgenic high-Met lupin. In addition, future production of GM crops with higher levels of two other important amino acids, namely, Trp and Thr, is also expected.

Another important aspect of enhancing the nutritional quality of crops is related to the rapidly growing demand for crops for biofuel production. Ethanol production from maize generates large amounts of nonextractable seed material, called dried distiller grain solubles (DDGS; Rausch and Belyea, 2006), the production of which progressively increased in recent years. Historically, most of the DDGS has been fed to cattle, but in the past several years, researchers have been evaluating its nutritional value and feeding recommendations for swine and poultry (Fastinger et al., 2006; Pedersen et al., 2007). Hence, research projects under way could modify the amino acid composition, protein composition, and phosphorous content of DDGS (Singh et al., 2005; Rausch and Belyea, 2006). The feed quality of DDGS can be further improved by expressing recombinant proteins enriched in essential amino acids that will not be lost during ethanol extraction. Nutritional improvement for cattle requires the presence of essential amino acids in proteins that are resistant to rumen proteolysis (Galili et al., 2002; Amir and Tabe, 2006).

Like all other GM crops, the opportunities for and the impacts of GM crops with enhanced nutritional quality depend on public acceptance. Although recent years have shown gradual increases in the acceptance of these crops and GM foods in some countries, there is still public debate about the safety of GM crops. A detailed study based on a number of categories, including molecular studies and comparative safety assessments, concluded that the high-Lys LY038 maize is as safe as conventional maize (Glenn, 2007). Yet, despite the fact that the high-Lys LY038 maize was approved for commercial use in a number of countries, there remains public debate about its safety, which can be viewed on the Internet. The outcome of this debate is central to the development of additional GM crops with a high content of essential amino acids.

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LITERATURE CITED

Altenbach SB, Kuo CC, Staraci LC, Pearson KW, Wainwright C, Georgescu A, Townsend J (1992) Accumulation of a Brazil nut albumin in seeds of transgenic canola results in enhanced levels of seed protein methionine. Plant Mol Biol **18:** 235–245

Amir R, Hacham Y, Galili G (2002) Cystathionine gamma-synthase and threonine synthase operate in concert to regulate carbon flow towards methionine in plants. Trends Plant Sci **7:** 153–156

Amir R, Tabe L (2006) Molecular approaches to improving plant methionine content. *In* KJ Pawan, K Jaiwal, PS Rana, eds, Plant Genetic Engineering: Metabolic Engineering and Molecular Farming II, Vol 8. Studium Press LLC, Houston, pp 1–26

Arruda P, Kemper EL, Papes F, Leite A (2000) Regulation of lysine catabolism in higher plants. Trends Plant Sci **5:** 324–330

Avraham T, Badani H, Galili S, Amir R (2005) Enhanced levels of methionine and cysteine in transgenic alfalfa (*Medicago sativa L.*) plants over-expressing the Arabidopsis cystathionine gamma-synthase gene. Plant Biotechnol J **3:** 71–79

Azevedo RA, Lancien M, Lea PJ (2006) The aspartic acid metabolic pathway, an exciting and essential pathway in plants. Amino Acids **30:** 143–162

Bagga S, Potenza C, Ross J, Martin MN, Leustek T, Sengupta-Gopalan C (2005) A transgene for high methionine protein is posttranscriptionally regulated by methionine. In Vitro Cell Dev Biol Plant **41:** 731–741

Beauregard M, Hefford MA (2006) Enhancement of essential amino acid contents in crops by genetic engineering and protein design. Plant Biotechnol J **4:** 561–574

Ben Tzvi-Tzchori I, Perl A, Galili G (1996) Lysine and threonine metabolism are subject to complex patterns of regulation in *Arabidopsis*. Plant Mol Biol **32:** 727–734

Bockholt AJ, Rooney LW (1992) QPM hybrids in the United States. *In* ET Mertz, ed, Quality Protein Maize. American Association of Cereal Chemists, St. Paul, pp 111–121

Boerjan W, Bauw G, Vanmontagu M, Inze D (1994) Distinct phenotypes generated by overexpression and suppression of S-adenosyl-L-methionine synthetase reveal developmental patterns of gene silencing in tobacco. Plant Cell **6:** 1401–1414

Bourgis F, Roje S, Nuccio ML, Fisher DB, Tarczynski MC, Li CJ, Herschbach C, Rennenberg H, Pimenta MJ, Shen TL, et al (1999) S-methylmethionine plays a major role in phloem sulfur transport and is synthesized by a novel type of methyltransferase. Plant Cell **11:** 1485–1497

Bressani R (1966) Protein quality of opaque2 maize in children. *In* ET Mertz, OE Nelson, eds, Proceedings of the High Lysine Corn Conference. Corn Industries Research Foundation, Lafayette, IN, pp 34–39

Burkhardt PK, Beyer P, Wunn J, Kloti A, Armstrong GA, Schledz M, von Lintig J, Potrykus I (1997) Transgenic rice (*Oryza sativa*) endosperm expressing daffodil (*Narcissus pseudonarcissus*) phytoene synthase accumulates phytoene, a key intermediate of provitamin A biosynthesis. Plant J **11:** 1071–1078

Chiaiese P, Ohkama-Ohtsu N, Molvig L, Godfree R, Dove H, Hocart C, Fujiwara T, Higgins TJV, Tabe LM (2004) Sulphur and nitrogen nutrition influence the response of chickpea seeds to an added, transgenic sink for organic sulphur. J Exp Bot **55:** 1889–1901

Chiba Y, Ishikawa M, Kijima F, Tyson RH, Kim J, Yamamoto A, Nambara E, Leustek T, Wallsgrove RM, Naito S (1999) Evidence for autoregulation of cystathionine gamma-synthase mRNA stability in Arabidopsis. Science **286:** 1371–1374

Coleman CE, Lopes MA, Gillikin JW, Boston RS, Larkins BA (1995) A defective signal peptide in the maize high-lysine mutant floury-2. Proc Natl Acad Sci USA **92:** 6828–6831

Daggett V, Fersht A (2003) The present view of the mechanism of protein folding. Nat Rev Mol Cell Biol **4:** 497–502

Dizigan MA, Kelly RA, Voyles DA, Luethy MH, Malvar TM, Malloy KP, inventors (January 2, 2007) High lysine maize compositions and event LY038 maize plants. United States Patent No. 7157281

Doll H, Koie B, Eggum BO (1974) Induced high lysine mutants in barley. Radiat Bot **14:** 73–80

Falco SC, Guida T, Locke M, Mauvais J, Sandres C, Ward RT, Webber P (1995) Transgenic canola and soybean seeds with increased lysine. Biotechnology (N Y) **13:** 577–582

Fastinger ND, Latshaw JD, Mahan DC (2006) Amino acid availability and true metabolizable energy content of corn distillers dried grains with solubles in adult cecectomized roosters. Poult Sci **85:** 1212–1216

Frankard V, Ghislain M, Jacobs M (1992) Two feedback-insensitive enzymes of the aspartate pathway in *Nicotiana sylvestris.* Plant Physiol **99:** 1285–1293

Frizzi A, Huang S, Gilbertson LA, Armstrong TA, Luethy MH, Malvar TM (2008) Modifying lysine biosynthesis and catabolism in corn with a single bifunctional expression/silencing transgene cassette. Plant Biotechnol J **6:** 13–21

Galili G (2002) New insights into the regulation and functional significance of lysine metabolism in plants. Annu Rev Plant Biol **7:** 153–156

Galili G, Amir R, Hoefgen R, Hesse H (2005) Improving the levels of essential amino acids and sulfur metabolites in plants. Biol Chem **386:** 817–831

Galili G, Galili S, Lewinsohn E, Tadmor Y (2002) Genetic, molecular, and genomic approaches to improve the value of plant foods and feeds. Crit Rev Plant Sci **21:** 167–204

Geevers HO, Lake JK (1992) Development of modified opaque2 maize in South Africa. *In* ET Mertz, ed, Quality Protein Maize. American Association of Cereal Chemists, St. Paul, pp 49–78

Gibbon BC, Larkins BA (2005) Molecular genetic approaches to developing quality protein maize. Trends Genet **21:** 227–233

Gillikin JW, Zhang F, Coleman CE, Bass HW, Larkins BA, Boston RS (1997) A defective signal peptide tethers the floury-2 zein to the endoplasmic reticulum membrane. Plant Physiol **114:** 345–352

Glenn KC (2007) Nutritional and safety assessments of foods and feeds nutritionally improved through biotechnology: lysine maize as a case study. J AOAC Int **90:** 1470–1479

Glover DV (1992) Corn protein-genetics, breeding, and value in foods and feeds. *In* ET Mertz, ed, Quality Protein Maize. American Association of Cereal Chemists, St. Paul, pp 49–78

Goto DB, Ogi M, Kijima F, Kumagai T, van Werven F, Onouchi H, Naito S (2002) A single-nucleotide mutation in a gene encoding S-adenosylmethionine synthetase is associated with methionine over-accumulation phenotype in Arabidopsis thaliana. Genes Genet Syst **77:** 89–95

Hacham Y, Avraham T, Amir R (2002) The N-terminal region of Arabidopsis cystathionine gamma-synthase plays an important regulatory role in methionine metabolism. Plant Physiol **128:** 454–462

Hacham Y, Matityahu I, Schuster G, Amir R (2008) Overexpression of mutated forms of aspartate kinase and cystathionine gamma-synthase in tobacco leaves resulted in the high accumulation of methionine and threonine. Plant J **54:** 260–271

Hacham Y, Schuster G, Amir R (2006) An in vivo internal deletion in the N-terminus region of Arabidopsis cystathionine gamma-synthase results in CGS expression that is insensitive to methionine. Plant J **45:** 955–967

Hagan ND, Upadhyaya N, Tabe LM, Higgins TJV (2003) The redistribution of protein sulfur in transgenic rice expressing a gene for a foreign, sulfur-rich protein. Plant J **34:** 1–11

Hanafy MS, Rahman SM, Khalafalla MM, El-Shemy HA, Nakamoto Y, Ishimoto M, Wakasa K (2006) Accumulation of free tryptophan in azuki bean (*Vigna angularis*) induced by expression of a gene (OASA1D) for a modified α-subunit of rice anthranilate synthase. Plant Sci **171:** 670–676

Harpstead DD (1971) High lysne corn. Sci Am **225:** 32–34

Hesse H, Kreft O, Maimann S, Zeh M, Hoefgen R (2004) Current understanding of the regulation of methionine biosynthesis in plants. J Exp Bot **55:** 1799–1808

Houmard NM, Mainville JL, Bonin CP, Huang S, Luethy MH, Malvar TM (2007) High-lysine corn generated by endosperm-specific suppression of lysine catabolism using RNAi. Plant Biotechnol J **5:** 605–614

Huang S, Frizzi A, Florida CA, Kruger DE, Luethy MH (2006) High lysine and high tryptophan transgenic maize resulting from the reduction of both 19- and 22-kD alpha-zeins. Plant Mol Biol **61:** 525–535

Huang SS, Kruger DE, Frizzi A, D'Ordine RL, Florida CA, Adams WR, Brown WE, Luethy MH (2005) High-lysine corn produced by the combination of enhanced lysine biosynthesis and reduced zein accumulation. Plant Biotechnol J **3:** 555–569

Inaba K, Fujiwara T, Hayashi H, Chino M, Komeda Y, Naito S (1994) Isolation of an Arabidopsis thaliana mutant, Mto1, that overaccumulates soluble methionine: temporal and spatial patterns of soluble methionine accumulation. Plant Physiol **104:** 881–887

Inaba Y, Brotherton JE, Ulanov A, Widholm JM (2007) Expression of a feedback insensitive anthranilate synthase gene from tobacco increases free tryptophan in soybean plants. Plant Cell Rep **26:** 1763–1771

Ishihara A, Asada Y, Takahashi Y, Yabe N, Komeda Y, Nishioka T, Miyagawa H, Wakasa K (2006) Metabolic changes in Arabidopsis thaliana expressing the feedback-resistant anthranilate synthase alpha subunit gene OASA1D. Phytochemistry **67:** 2349–2362

Johnson LA, Hardy CL, Baumel CP, Yu TH, Sell JL (2001) Identifying valuable corn quality traits for livestock feed. Cereal Foods World **46:** 472–481

Jung R, Carl F (2000) Transgenic corn with an improved amino acid composition. *In* K Müntz, W Ulrich, eds, 8th International Symposium on Plant Seeds. Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany

Karchi H, Miron D, Ben-Yaacob S, Galili G (1995) The lysine-dependent stimulation of lysine catabolism in tobacco seeds requires calcium and protein phosphorylation. Plant Cell **7:** 1963–1970

Karchi H, Shaul O, Galili G (1994) Lysine synthesis and catabolism are coordinately regulated during tobacco seed development. Proc Natl Acad Sci USA **91:** 2577–2581

Kemper EL, Neto GC, Papes F, Moraes KCM, Leite A, Arruda P (1999) The role of Opaque2 in the control of lysine-degrading activities in developing maize endosperm. Plant Cell **11:** 1981–1993

Kim CS, Gibbon BC, Gillikin JW, Larkins BA, Boston RS, Jung R (2006) The maize Mucronate mutation is a deletion in the 16-kDa gamma-zein gene that induces the unfolded protein response. Plant J **48:** 440–451

Kim CS, Hunter BG, Kraft J, Boston RS, Yans S, Jung R, Larkins BA (2004) A defective signal peptide in a 19-kD alpha-zein protein causes the unfolded protein response and an opaque endosperm phenotype in the maize De*-B30 mutant. Plant Physiol **134:** 380–387

Kocsis MG, Ranocha P, Gage DA, Simon ES, Rhodes D, Peel GJ, Mellema S, Saito K, Awazuhara M, Li CJ, et al (2003) Insertional inactivation of the methionine S-methyltransferase gene eliminates the S-methylmethionine cycle and increases the methylation ratio. Plant Physiol **131:** 1808–1815

Kreft O, Hoefgen R, Hesse H (2003) Functional analysis of cystathionine gamma-synthase in genetically engineered potato plants. Plant Physiol **131:** 1843–1854

Kreps JA, Ponappa T, Dong WQ, Town CD (1996) Molecular basis of alpha-methyltryptophan resistance in amt-1, a mutant of Arabidopsis

thaliana with altered tryptophan metabolism. Plant Physiol **110:** 1159–1165

Lee M, Huang T, Toro-Ramos T, Fraga M, Last RL, Jander G (2008) Reduced activity of Arabidopsis thaliana HMT2, a methionine biosynthetic enzyme, increases seed methionine content. Plant J **54:** 310–320

Lee TTT, Wang MMC, Hou RCW, Chen LJ, Su RC, Wang CS, Tzen JTC (2003) Enhanced methionine and cysteine levels in transgenic rice seeds by the accumulation of sesame 2S albumin. Biosci Biotechnol Biochem **67:** 1699–1705

Li J, Last RL (1996) The Arabidopsis thaliana trp5 mutant has a feedback-resistant anthranilate synthase and elevated soluble tryptophan. Plant Physiol **110:** 51–59

Lopes MA, Takasaki K, Bostwick DE, Helentjaris T, Larkins BA (1995) Identification of two opaque2 modifier loci in quality protein maize. Mol Gen Genet **247:** 603–613

Lucas DM, Taylor ML, Hartnell GF, Nemeth MA, Glenn KC, Davist SW (2007) Broiler performance and carcass characteristics when fed diets containing lysine maize (LY038 or LY038 x MON 810), control, or conventional reference maize. Poult Sci **86:** 2152–2161

Magnavaca R, Larkins BA, Schaffert RE, Lopes MA (1993) Improving protein quality in maize and sorghum. *In* DR Buxton, R Shibles, RA Forsberg, BL Blad, KH Asay, GM Paulsen, RF Wilson, eds, International Crop Science. Crop Science Society of America, Madison, WI, p 85

Matsuda F, Yamada T, Miyazawa H, Miyagawa H, Wakasa K (2005) Characterization of tryptophan-overproducing potato transgenic for a mutant rice anthranilate synthase alpha-subunit gene (*OASA1D*). Planta **222:** 535–545

Mazur B, Krebbers E, Tingey S (1999) Gene discovery and product development for grain quality traits. Science **285:** 372–375

Mertz ET (1997) Thirty years of opaque2 maize. *In* BA Larkins, ET Mertz, eds, Quality Protein Maize: 1964-1994. Purdue University Press, West Lafayette, IN, pp 25–37

Mertz ET, Bates LS, Nelson OE (1964) Mutant gene that changes protein composition and increases lysine content of maize endosperm. Science **145:** 279–280

Molvig L, Tabe LM, Eggum BO, Moore AE, Craig S, Spencer D, Higgins TJV (1997) Enhanced methionine levels and increased nutritive value of seeds of transgenic lupins (*Lupinus angustifolius L*) expressing a sunflower seed albumin gene. Proc Natl Acad Sci USA **94:** 8393–8398

Munck L (1992) The case of high-lysine barley breeding. *In* PR Shewry, ed, Barley: Genetics, Biochemistry, Molecular Biology and Biotechnology. CAB International, Wallingford, UK, pp 573–601

Munck L, Karlsson KE, Hagberg A, Eggum BO (1970) Gene for improved nutritional value in barley seed protein. Science **168:** 985–987

Muntz K, Christov V, Saalbach G, Saalbach I, Waddell D, Pickardt T, Schieder O, Wustenhagen T (1998) Genetic engineering for high methionine grain legumes. Nahrung **42:** 125–127

Negrutiu I, Cattoir-Reynearts A, Verbruggen I, Jacobs M (1984) Lysine overproducer mutants with an altered dihydrodipicolinate synthase from protoplast culture of *Nicotiana sylvestris* (Spegazzini and Comes). Theor Appl Genet **68:** 11–20

Ominato K, Akita H, Suzuki A, Kijima F, Yoshino T, Yoshino M, Chiba Y, Onouchi H, Naito S (2002) Identification of a short highly conserved amino acid sequence as the functional region required for posttranscriptional autoregulation of the cystathionine gamma-synthase gene in Arabidopsis. J Biol Chem **277:** 36380–36386

Or E, Boyer SK, Larkins BA (1993) opaque2 modifiers act post-transcriptionally and in a polar manner on gamma-zein gene expression in maize endosperm. Plant Cell **5:** 1599–1609

Pastorello EA, Pompei C, Pravettoni V, Brenna O, Farioli L, Trambaioli C, Conti A (2001) Lipid transfer proteins and 2S albumins as allergens. Allergy **56:** 45–47

Pedersen C, Boersma MG, Stein HH (2007) Digestibility of energy and phosphorus in ten samples of distillers dried grains with solubles fed to growing pigs. J Anim Sci **85:** 1168–1176

Rausch KD, Belyea RL (2006) The future of coproducts from corn processing. Appl Biochem Biotechnol **128:** 47–86

Ravindran V, Tabe LM, Molvig L, Higgins TJV, Bryden WL (2002) Nutritional evaluation of transgenic high-methionine lupins (*Lupinus angustifolius L*) with broiler chickens. J Sci Food Agric **82:** 280–285

Roesler KR, Rao AG (2000) A single disulfide bond restores thermodynamic and proteolytic stability to an extensively mutated protein. Protein Sci **9:** 1642–1650

Saalbach I, Waddell D, Pickardt T, Schieder O, Muntz K (1995) Stable expression of the sulfur-rich 2S albumin gene in trangsenic vicianarbonensis increases the methionine content of seeds. J Plant Physiol **145:** 674–681

Segal G, Song RT, Messing J (2003) A new opaque variant of maize by a single dominant RNA-interference-inducing transgene. Genetics **165:** 387–397

Shaul O, Galili G (1992a) Increased lysine synthesis in tobacco plants that express high levels of bacterial dihydrodipicolinate synthase in their chloroplasts. Plant J **2:** 203–209

Shaul O, Galili G (1992b) Threonine overproducing in transgenic tobacco plants expressing a mutant desensitized aspartate kinase from *Escherichia coli*. Plant Physiol **100:** 1157–1163

Shen B, Li CJ, Tarczynski MC (2002) High free-methionine and decreased lignin content result from a mutation in the Arabidopsis S-adenosyl-L-methionine synthetase 3 gene. Plant J **29:** 371–380

Singh R, Axtell JD (1973) High lysine mutant gene (*hl*) that improves protein quality and biological value of grain sorghum. Crop Sci **13:** 535–539

Singh V, Johnston DB, Naidu K, Rausch KD, Belyea RL, Tumbleson ME (2005) Comparison of modified dry-grind corn processes for fermentation characteristics and DDGS composition. Cereal Chemistry **82:** 187–190

Sun SSM, Liu QQ (2004) Transgenic approaches to improve the nutritional quality of plant proteins. In Vitro Cell Dev Biol Plant **40:** 155–162

Sun Y, Carneiro N, Clore AM, Moro GL, Habben JE, Larkins BA (1997) Characterization of maize elongation factor 1A and its relationship to protein quality in the endosperm. Plant Physiol **115:** 1101–1107

Tabe L, Higgins TJV (1998) Engineering plant protein composition for improved nutrition. Trends Plant Sci **3:** 282–286

Tabe LM, Droux M (2002) Limits to sulfur accumulation in transgenic lupin seeds expressing a foreign sulfur-rich protein. Plant Physiol **128:** 1137–1148

Wakasa K, Hasegawa H, Nemoto H, Matsuda F, Miyazawa H, Tozawa Y, Morino K, Komatsu A, Yamada T, Terakawa T, et al (2006) High-level tryptophan accumulation in seeds of transgenic rice and its limited effects on agronomic traits and seed metabolite profile. J Exp Bot **57:** 3069–3078

White CL, Tabe LM, Dove H, Hamblin J, Young P, Phillips N, Taylor R, Gulati S, Ashes J, Higgins TJV (2001) Increased efficiency of wool growth and live weight gain in Merino sheep fed transgenic lupin seed containing sunflower albumin. J Sci Food Agric **81:** 147–154

Wu XR, Chen ZH, Folk MR (2003) Enrichment of cereal protein lysine content by altered tRNA(lys) coding during protein synthesis. Plant Biotechnol J **1:** 187–194

Wu XR, Kenzior A, Willmot D, Scanlon S, Chen Z, Topin A, He SH, Acevedo A, Folk WR (2007) Altered expression of plant lysyl tRNA synthetase promotes tRNA misacylation and translational recoding of lysine. Plant J **50:** 627–636

Yamada T, Tozawa Y, Hasegawa H, Terakawa T, Ohkawa Y, Wakasa K (2004) Use of a feedback-insensitive at subunit of anthranilate synthase as a selectable marker for transformation of rice and potato. Mol Breed **14:** 363–373

Zarkadas CG, Hamilton RI, Yu ZR, Choi VK, Khanizadeh S, Rose NG, Pattison PL (2000) Assessment of the protein quality of 15 new northern adapted cultivars of quality protein maize using amino acid analysis. J Agric Food Chem **48:** 5351–5361

Zhu X, Galili G (2003) Increased lysine synthesis coupled with a knockout of its catabolism synergistically boosts lysine content and also trans regulates the metabolism of other amino acids in *Arabidopsis* seeds. Plant Cell **15:** 845–853

Zhu X, Galili G (2004) Lysine metabolism is concurrently regulated by synthesis and catabolism in both reproductive and vegetative tissues. Plant Physiol **135:** 129–136