#### **ISB NEWS REPORT**

## PLANT RESEARCH

## Can a Foreign Protein Improve the Amino Acid Balance of Corn?

#### Paul Scott

Corn grain is an important component of feed for non-ruminant animals and food for humans. It is an excellent source of starch, but is a poor source of protein nutrition. Protein content is low, usually less than 10% of the kernel mass, and moreover, the quality of maize protein is not ideal. Maize protein is deficient in certain amino acids—lysine, tryptophan, and methionine—that are required by non-ruminant animals (including humans). To remedy these deficiencies, protein supplements are provided to create a well-balanced diet, which adds to the cost of feed and food. Genetic improvements that increase the levels of lysine, tryptophan, and methionine have been actively sought by researchers for the past fifty years. Recently, several approaches involving one or more transgenes have been successful<sup>1,2,3</sup>.

A recently published approach<sup>3</sup> tests the hypothesis that amino acid balance in maize kernels can be improved by production of a foreign protein to serve as a sink for amino acids. The foreign protein used in this case is the porcine milk protein alpha-lactalbumin, which is introduced transgenically. The transgene contains a maize seed storage protein gene promoter and a synthetic coding sequence optimized for expression in maize. The coding sequence is modified by the addition of a maize signal sequence and an endoplasmic reticulum retention sequence to direct accumulation of the transgene product only to targeted regions in the cell. This transgene design was predicted to result in accumulation of alpha-lactalbumin only in the endoplasmic reticulum of the kernel endosperm, mimicking the pattern of accumulation of the most abundant natural seed proteins in the kernel, the zeins. The authors of this study found that transgenic kernels accumulate a protein that cross-reacts with antiserum raised to alpha-lactalbumin and is consistent with the size predicted for the transgene product.

The key experiment in this work is a comparison between transgenic and non-transgenic endosperm tissue produced on the same ear. In two events evaluated, transgenic and nontransgenic endosperm differ in their amino acid balance, and the difference generally reflects the amino acid balance of alpha-lactalbumin. Lysine content is 29 - 47% higher in transgenic endosperm than in the non-transgenic sibling kernels. In most cases, total protein content is not changed. Kernel characteristics such as density, mass, and seed storage protein content are not altered. Thus it appears that this modification results in a specific alteration to the endosperm amino acid balance that does not detectably disrupt kernel development.

The change in amino acid content, together with the accumulation of alpha-lactalbumin in the kernels, suggests that alpha-lactalbumin may be acting as an amino acid sink. Closer inspection reveals, however, that the level of alpha-lactalbumin in the kernels is not sufficient to explain the change in amino acid composition. Thus it seems that the introduction of alpha-lactalbumin to the kernels creates a sink for amino acids but the nature of the sink remains unclear.

The choice of a milk protein is interesting because it could result in nutritional benefits in addition to improved amino acid balance. Several studies have shown that derivatives of alphalactalbumin have benefits such as antimicrobial and anti-tumor activities. It is hypothesized that some of the health benefits enjoyed by nursing infants are conferred by proteins in milk such as alpha-lactalbumin. The nutritional benefits of alpha-lactalbumin have led several researchers to conclude that human alpha-lactalbumin would be a beneficial component of infant formulas. A logical extension of this idea is to include milk proteins from specific farm animals as supplements to their diets, especially when young animals are weaned. By illustrating the feasibility of producing porcine alpha-lactalbumin in corn, the Bicar study enabled the researchers to test the hypothesis that corn containing porcine alpha-lactalbumin is beneficial in swine diets.

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One drawback to using a milk protein is its potential for allergenicity in humans. Porcine alpha-lactalbumin has not been evaluated for its allergenic potential. Most milk allergies involve a reaction to bovine beta-lactoglobin protein; however some individuals react to other milk proteins, and some individuals with bovine milk allergies also react to goat milk proteins. A thorough evaluation of the allergenicity of porcine alpha-lactalbumin is therefore a prerequisite for application of this technology. Regardless of the outcome of this evaluation, this work illustrates the important point that it is possible to alter amino acid balance in a predictable way by adding a foreign protein to seeds using genetic engineering.

The challenge of increasing grain amino acids can be thought of in terms of increasing the strengths of either the source or the sink of the amino acids of interest. As shown in the Bicar et al. report, adding a foreign protein to grain may increase the strength of the amino acid sink. Some transgenic approaches that successfully alter lysine content involve manipulation of lysine metabolism enzymes, essentially increasing the strength of the source of lysine. As described by Kirihara et al.<sup>4</sup>, an effective method to improve amino acid balance could involve alterations that increase both the source and the sink strengths for the amino acids of interest. Thus, the pathways for amino acid metabolism would be altered to increase the level of amino acids available for incorporation into proteins, and a sink for these amino acids would be created by manipulation of the seed protein content and/or composition. This approach has been successfully put into practice<sup>1</sup>. In this case, the sink for amino acids is created by reducing the levels of the native, low lysine proteins of corn, which presumably results in an increase in high lysine native proteins. The work of Bicar et al. suggests that a foreign protein can create an effective sink for amino acids and would therefore be compatible with this approach as well. A carefully selected protein could not only create an amino acid sink, but could also confer nutritional or other types of benefit on the seed.

#### References

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# **Transgenic Wheat Has Increased Polyamines**

Teresa Capell

#### Importance of arginine decarboxylase in plants

Crop productivity in modern agriculture relies heavily on abundant supplies of water for irrigation. Although drought and salt tolerance genes are present in wheat, breeding for stress tolerance is time- and labour-intensive and complicated by the multigenic nature of stress tolerance and the complexity of wheat genetics. The polyamine biosynthetic pathway in higher plants is a useful model in which to examine the components that affect the levels of intermediates and end products in the pathway. By introducing appropriate transgenes into plants and measuring the effects of transgene products on end product accumulation, we may begin to understand how individual components of the pathway contribute towards their concerted regulation<sup>1</sup>.

The polyamines spermidine and spermine, and their precursor putrescine, are ubiquitous in all living organisms and are involved in many diverse physiological, developmental, and biochemical processes. Pyridoxal phosphate (PLP)-dependent ornithine decarboxylase (ODC) is the initial enzyme in the pathway committed to polyamine synthesis. In plants and some

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