Nutritional Improvement of Corn Proteins Through Biotechnology II.1/2

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Executive Summary

The primary objective of these projects is to improve the protein quality of corn by increasing the quantity of lysine in seeds, without affecting yield or other desirable traits. Specific aims are to: 1) Screen transgenic corn events containing genes for altered tRNAs and lysyl tRNA synthetase and select those with the highest levels of lysine in their seeds; 2.) Introgress DNA sequences conferring enhanced lysine trait into elite germplasm and select lines with the highest levels of lysine and protein and kernel quality; 3) Characterize high lysine corn for introduced DNAs, for feed quality and for properties that might affect safety and public acceptance.

Significant progress has been achieved for aims 1+2. Several independent transgenic events have been generated that have significant increases in lysine content. The trangenes of these events have been introgressed into elite germplasm selected for high protein content and partially characterized. Aim 3 will be implemented with the events having the highest protein and lysine content.

Research Activities

The grain from individual plants randomly selected from the hybrid HiII AxB parental (nontransgenic) population had lysine contents ranging from 2.43% to 2.81%. The lysine contents of the grain from the independent transgenic events expressing AtKRS-1 without or with the $tRNA^{Lys}$(CUG) transgene were significantly higher, despite only 50% of the harvested kernels from the T_0 transgenic plants being predicted to being transgenic, due to out-crossing with untransformed plants No significant difference was observed between events with or without the $tRNA^{Lys}$(CUG) gene.

T_1 plants from transgenic events served as donors in a backcrossing (BC) scheme using improved maize inbreds as the recurrent parents. The parental inbreds (AR16035:S02-

670)-03-01) and (CH05015:N12-7-1-B-B) represent each side of the two dominant heterotic patterns used commercially. During backcrossing, segregants containing the transgenes in plant tissues were identified using polymerase chain reaction (PCR), and sub-events with high lysine content were carried forward. In the BC2 generation, selected plants expressing AtKRS-1 were self-pollinated and also crossed to the recurrent parent. The grain of the self-pollinated transgenic plants, expected to be enriched for the *AtKRS-1* transgenes contained up to 26% elevated lysine content indicating that expression of *AtKRS-1* has a significant positive impact on lysine content in diverse genetic backgrounds.

Lysine is incorporated into zeins. As the maize zeins contain no lysine recoding of lysine at internal sites should be detectable by susceptibility to Lysine C, a serine endoproteinase highly specific for peptide bonds at the carboxyl side of lysine. α-zeins from transgenic plants expressing At-KRS were reproducibly reduced by 12-15% following incubation with Lysine C indicating they contained lysine. These data agree with the increased lysine levels observed in grain of transgenic lines further supporting the occurrence of translational recoding of lysine.

Bibilography

Xing Rong Wu, Alexander Kenzior, David Wilmot, Sarah Scanlon, Zhihong Chen , Andrey Topin, Shao Hua He, Ashley Acevedo and William R. Folk . 2007. Altered expression of plant lysyl tRNA synthetase promotes tRNA misacylation and translational recoding of lysine The Plant Journal 50: 627-636.

Relevance

Plants provide the majority of the food and fiber for mankind, and through intensive selection and breeding, highly desirable traits and increased yields have been realized in many crops. Biotechnology offers continued advances in the development of improved traits and yields, particularly in areas where more traditional methods have been only moderately successful.

One area in which plant breeders have been struggling has been the improvement of protein quality in our important crops. Human food and animal feed derived from corn, soybeans and most cereals are deficient in several of the essential amino acids required in an animal diet. Corn is a preferred animal feed because it is a low cost energy source, but it is deficient in lysine, methionine and tryptophan. Approximately 200,000 tons of lysine are produced annually by microbial fermentation to supplement animal feed, at a cost of $500 million dollars. A smaller amount of tryptophan is produced annually for supplementation of animal feeds. Were the quantities of these amino acids in crops to be increased sufficiently to obviate the need for supplementation, the added value is estimated to exceed one billion dollars/yr.

Although varieties of corn have been found which produce kernels with higher lysine and tryptophan content, they have not been adopted by producers because of reduced yield or fertility, greater sensitivity to pests and increased kernel fragility. The objective of the research is to improve the protein quality of corn by increasing the quantity of lysine in seeds, without affecting yield or other desirable traits. Completion of these efforts can then lead to similar approaches for improving the content of methionine and tryptophan.