MOLECULAR GENETIC IMPROVEMENT OF PROTEIN QUALITY IN MAIZE

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Contents

1. Introduction
2. Maize Seed Proteins
3. Genetic manipulation of maize kernel protein fractions
3.1 Molecular Genetic Manipulation of *opaque-2* and Quality Protein Maize (QPM)
3.2 Investigation of the High-Methionine Inbred Line BSSS53
4. Conclusions and Future Prospects
Acknowledgements
Glossary
Bibliography
Biographical Sketches

Summary

Increasing the nutritional value of maize grain may contribute to improved human nutrition and health. Although maize is widely grown both for human consumption and for animal feed, maize is an inadequate source of essential amino acids in many diets. Maize protein is deficient in lysine and tryptophan, and in many poultry diets, maize protein is inadequate in the sulfur amino acids (methionine and cysteine). Many attempts have been made to change the amino acid composition of maize grain by changing the relative abundance of different seed proteins in the kernel. Considerable research has focused on the molecular genetic control of kernel protein accumulation. Genetic investigation of the *opaque-2* mutant and the modifier genes which restore kernel vitreousness in Quality Protein Maize (QPM) has provided new marker-assisted and transgenic approaches to achieving improved protein quality in maize. Genetic studies of the high-methionine inbred line BSSS53 have identified genomic regions influencing whole-kernel methionine levels. The development of more efficient molecular marker systems will enhance traditional breeding programs aimed at improving the protein quality of elite maize varieties. Genetic engineering strategies to modify amino acid biosynthetic pathways and to over-express high-quality proteins will also yield varieties with improved protein quality.

1. Introduction

Maize (*Zea mays* L.) is one of the most productive and widely adapted crop species of

the world. Maize is an important staple food crop in Latin America and much of Sub-Saharan Africa. Additionally, maize grain is an important livestock feed in China, Southeast Asia and North America (see *Improving the Nutritional Quality of Maize and Wheat for Human Consumption*). Maize is also widely harvested as silage for dairy production. As maize is highly productive and widely grown, considerable research has focused on improving its nutritional value. Improvement of the nutritional quality of maize grain will increase its value as a food source and will improve the efficiency of animal production. Both of these outcomes can improve human health and nutrition and may have a broad impact, considering the global importance and widespread acceptance of maize.

Although maize grain is a valuable source of dietary energy, it is typically comprised of only 8-10% protein. Furthermore, maize is an inadequate source of essential amino acids in many diets. For monogastric animals, including humans, maize protein is deficient in lysine and tryptophan; and in many poultry diets, maize protein is inadequate in the sulfur amino acids (methionine and cysteine). The amino acid most often lacking in human diets is lysine. In swine and poultry rations balanced for lysine, tryptophan, and the sulfur amino acids, threonine can become limiting. Increasing the levels of lysine, tryptophan, threonine and methionine in maize grain would improve the nutritional value of maize for human nutrition and for livestock feed (see *Plant Based Sources of Proteins and Amino Acids in Relation to Human Health*).

In addition to improving the nutritional value of maize for poultry feed, increasing the methionine content of maize grain may also prove beneficial for reducing micronutrient deficiency in humans. There is increasing evidence that methionine may improve the bioavailability of zinc. Zinc deficiency is a widespread nutritional disorder that can lead to growth failure, pregnancy complications, low birth weight and impaired immune function (see *Global Importance of Zinc Deficiency in Humans: its Relation to Malnutrition and Strategies for its Prevention*). In a rat nutrition study comparing normal maize with conventionally selected high-methionine maize, high-methionine maize had higher levels of bioavailable zinc resulting in elevated dietary zinc intake. Increasing the methionine level of maize grain may alleviate the problems associated with zinc deficiency by increasing the bioavailability of dietary zinc.

Both traditional plant breeding and molecular genetic approaches have been utilized in order to improve the protein quality of maize. For the purposes of this article, traditional plant breeding will be assumed to include both the utilization of naturally occurring genetic variation through controlled crossing of maize lines or strains followed by artificial selection and the use of mutagenic agents to increase genetic variation prior to selection. Molecular genetic approaches will be assumed to include those methods for which molecular sequence information is required during development or implementation. These methods include the development of transgenic plants with novel phenotypic characteristics and the utilization of molecular markers to augment traditional breeding efforts.

The objective of this article is to highlight molecular genetic attempts to improve maize protein quality. To achieve this objective, the results of traditional breeding programs aimed at maize protein quality improvement will be referred to, since improved

germplasm arising out of these programs has provided a starting point for molecular genetic efforts in many cases. Several attempts have been made to alter the amino acid composition of the maize kernel by changing the relative abundance of different seed proteins. In order to summarize this research, we first will briefly review the nomenclature of maize kernel proteins and the amino acid profiles of these proteins.

Considerable research has been performed to better understand the metabolic pathways involving lysine, threonine and methionine synthesis and degradation. Lysine, methionine and threonine are all produced via the aspartate biosynthetic pathway. Due to the nutritional importance of these amino acids, considerable effort has focused on understanding and manipulating this critical biosynthetic pathway. Elucidation of amino acid metabolic pathways and the key regulatory enzymes of these pathways have led to the design of molecular genetic approaches for improving the amino acid balance of maize grain.

2. Maize Seed Proteins

The relative proportions of different seed proteins in the kernel influence the nutritional value of maize protein. Maize kernel protein is divided into four major classes based on solubility properties (Table 1). The commonly used Osborne seed protein classification system separates seed storage proteins into albumins, globulins, glutelins and prolamins. The albumin proteins are soluble in water or low-salt aqueous solutions, and the globulins are soluble in high-salt aqueous solutions. Although the albumins and globulins are relatively high in lysine (5.0-7.0%), these two protein fractions combined represent only 6-12% of the total kernel protein. Glutelins are seed proteins soluble in dilute alkali solutions and they contain 3.0-3.4% lysine. Glutelins are the second most abundant class of maize seed proteins accounting for 35-45% of total kernel protein.

Protein class	Solubility properties	Relative abundance	Nutritional value
Albumins	Water or low-salt aqueous solutions	Low	High
Globulins	High-salt aqueous solutions	Low	High
Glutelins	Dilute alkali solutions	High	Intermediate
Prolamines (Zeins)	Alcohol	High	Low*

*Specific maize prolamines are high in methionine, however these tend to be lower in abundance

Table 1. Major classes of maize kernel proteins.

The major class of proteins in the maize kernel is the prolamine fraction. Maize prolamines, referred to as zeins, account for approximately 50-60% of the total kernel

protein. Considerable research has been directed at understanding and manipulating the various zein proteins. Zeins compose a large, heterogeneous group of storage proteins (Table 2).

The commonly used nomenclature system distinguishes four classes: α-, β-, γ-, and δ-zeins based on solubility properties and structural similarities. Specific proteins within each class are further characterized by apparent molecular mass following SDS-PAGE. The α-zeins class consists of 19- and 22-kDa proteins and account for the majority of zein protein in common genetic backgrounds. Both the 19- and 22-kDa α-zeins are mixtures of related polypeptides encoded by large gene families. The α-zeins contain no lysine and low levels of methionine. The β-, γ-, and δ-zeins are soluble in alcohol in the presence of a reducing agent. The β-zein is a 14-kDa protein containing no lysine and approximately 11% methionine. The γ-zeins, including 16- and 27-kDa proteins, are also devoid of lysine and are less than 2.0% methionine. The δ-zein class is comprised of a 10-kDa protein encoded by a single structural gene and an 18-kDa protein encoded by the duplicate locus of the 10-kDa zein structural gene. The δ-zeins have a very high methionine content. The 10-kDa δ-zein is 23% methionine and the 18-kDa δ-zein is 27%. Additionally, the 18-kDa δ-zein contains one lysine and two tryptophan codons.

Zein class	Molecular mass	Lysine and methionine content
α	19-kDa 22-kDa	Low levels of methionine; no lysine
β	14-kDa	Intermediate levels of methionine; no lysine
γ	16-kDa 27-kDa	Low levels of methionine; no lysine
δ	10-kDa 18-kDa	High levels of methionine; the 18-kDa zein structural gene also contains one lysine codon

Table 2. Major classes of zein proteins.

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Biographical Sketches

Michael S. Olsen is a corn breeder for Monsanto Company. His current research effort is aimed at developing maize hybrids with enhanced grain quality traits for animal nutrition. He completed a B.A. degree from Bethel College in St. Paul, Minnesota before earning the M.S. and Ph.D. degrees from the University of Minnesota. His dissertation focused on the genetics of whole-kernel methionine in maize. Prior to joining Monsanto, he worked for three years as a specialty traits corn breeder for Wilson Genetics, L.L.C., a joint venture between Syngenta Seeds and Land O' Lakes. His research focus at Wilson Genetics was the development of maize hybrids with enhanced grain quality traits.

Ronald L. Phillips is Regents' Professor, University of Minnesota. He earned the B.S. and M.S. degrees from Purdue University and a Ph.D. from the University of Minnesota; his postdoctoral training was at Cornell University. Throughout his career, Dr. Phillips has coupled the techniques of classical cytogenetics with research advances in tissue culture and molecular biology to enhance our understanding of basic biology of cereal crops and to improve these species by innovative methods. His research program at the University of Minnesota was one of the early programs in modern plant biotechnology related to agriculture. He is a founding member and former Director of the Plant Molecular Genetics Institute of the University of Minnesota. He has served on numerous editorial boards, has edited six books, and has published over 50 chapters, 100 refereed journal articles, and 200 abstracts. Dr. Phillips teaches a world renowned course in cytogenetics and is invited to teach the course or present the results of his research at numerous university, governmental, and industrial institutions in the U.S. and abroad. He served as Chief Scientist of the USDA (1996-1998) in charge of the National Research Initiative Competitive Grants Program. Awards include Fellow of ASA, CSSA, and AAAS, the Purdue University Agriculture Distinguished Alumni Award, an Honorary Degree from Purdue University, and the Dekalb Genetics Crop Science Distinguished Career Award. In 1991, he was elected a member of the National Academy of Sciences. Dr. Phillips has also served as the President of the Crop Science Society of America.