Protein Quality in Transgenic Plants: Improvements

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INTRODUCTION

Past expansions of the world's food supply have relied primarily on plant breeding directed toward improving yields, increases in available cultivable lands, and augmentation of irrigation techniques. Because we are now encountering further constraints in all of these areas, future emphasis must include enhancing the ''nutritional content'' of the world's basic food and feed crops, especially those that are indigenous to developing nations. Such nutritional enhancement can result in lowering per capita intake of plant-based food crops, ultimately making more food available for expanding populations. These developments are made possible through advances in the fields of biochemistry and molecular biology, which has caused the ''biotechnology revolution.''

The composition of plant storage proteins, a major food reservoir for developing seeds, roots, and tubers, determines the nutritional value of plants and grains when they are used as foods and feed for humans and domestic animals.[1] The amount of protein varies with genotype or cultivar, but in general, cereals contain 10% of the dry weight of the seed as protein, while in legumes, the protein content varies between 20% and 30% of the dry weight. Roots and tubers retain far less, generally around 2–3%. In many seeds, storage proteins account for 50% or more of the total protein and thus determine the protein quality of seeds. Each year, the total world cereal harvest amounts to some 1700 million tons of grain.[2] This yields about 85 million tons of cereal storage proteins harvested each year and contributes about 55% of the total protein intake of humans. It has been difficult to produce significant increases in the level of protein and essential amino acids of crop plants utilizing classical plant breeding approaches. This is primarily because of the fact that the genetics of plant breeding is complex and that an increase in either trait may be offset by a loss in other agronomically important characters. In addition, it is probable that the storage proteins are very conserved in their structure and their essential amino acid composition would be little modified by these conventional techniques.

With respect to human and animal nutrition, most seeds do not provide a balanced source of protein because of deficiencies in one or more of the essential amino acids in the storage proteins. Consumption of proteins of unbalanced composition of amino acids can lead to a malnourished state which is most often found in people inhabiting developing countries where plants are the major source of protein intake. Thus the development of a more nutritionally balanced protein for introduction into plants takes on extreme importance.

PAST WORK

Over the last two decades, much work has been performed in an attempt to improve the nutritional quality of plant storage proteins by transferring heterologous storage protein genes from other plants.[3] The development of genetic engineering and the various gene transfer systems have made this approach possible. Genes encoding storage proteins containing a more favorable amino acid balance, by and large, do not exist in the genomes of major crop plants. Furthermore, modification of native storage proteins has met with difficulty because of their instability, low level of expression, and limited host range. However, there has been some success in recent years in improving the content of single amino acids using this approach. For example, 2S methionine-rich Brazil nut albumin (18% methionine) has been used to enhance levels of seed protein methionine in canola. A chimeric gene regulated by a phaseolin promoter was fused to the 17-kDa Brazil nut albumin and expressed in transgenic canola plant seeds. The methionine-rich protein exhibited temporal regulation with significant accumulation of the protein late in development, thereby correlating with that of wild-type 11S-canola seed protein. There was a 33% increase in the methionine level, as well as a 4% increase in the total protein level.[4] In the case of Brazil nut 2S albumin, the highly allergenic nature of the protein, however, renders it unsuitable for use in food plants. A possible alternative to the chimeric gene approach would be to design de novo a more nutritionally balanced protein that retains certain characteristics of the natural storage proteins of plants, yet contains all of the essential amino acids at their proper ratio for the feeding of humans and animals.

The biosynthesis of amino acids from simpler precursors is a process vital to all forms of life as these amino acids are the building blocks of proteins. Organisms differ markedly with respect to their ability to synthesize amino

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Table 1

Food stuff	Requirement in grams/day[a]
Cassava	4400
Corn	1800
Plantain	6100
Potato	2100
Rice	3100
Sweet potato	5760
Wheat	2300
Beef	170
Egg	180

[a] The values are what are necessary to consume in grams/day to achieve minimum daily requirement for all essential amino acids for a 10-year-old child. This assumes that the protein, in each foodstuff, is 100% bioavailable, and we know it is not, so these numbers should be increased to an even higher level.

acids. In fact, virtually all members of the animal kingdom are incapable of manufacturing some amino acids. There are 20 common amino acids that are utilized in the fabrication of proteins and essential amino acids are those protein building blocks that cannot be synthesized by the animal. It is generally agreed that humans require 8 of the 20 common amino acids in their diet: isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine, to maintain good health.[5] Protein malnutrition can usually be ascribed to a diet that is deficient in one or more of the essential amino acids. Therefore a nutritionally adequate diet must include a minimum daily consumption of these amino acids.

When diets are high in carbohydrates and low in protein, over a protracted period, essential amino acid deficiencies result. The name given to this undernourished condition is ‘‘kwashiorkor’’ which is an African word meaning ‘‘deposed child’’ (deposed from the mother’s breast by a newborn sibling). This debilitating and malnourished state, characterized by a bloated stomach and reddish-orange discolored hair, is more often found in children than in adults because of their greater need for essential amino acids during growth and development. In order for normal physical and mental maturation to occur, a daily source of essential amino acids is a requisite. Essential amino acid content, or protein quality, is as important a feature of the diet as total protein quantity or total calorie intake.

Some foods, such as milk, eggs, and meat, have very high nutritional values because they contain a disproportionately high level of essential amino acids. As mentioned previously, many plants are notoriously deficient in essential amino acids. The amino acid composition of most plants is insufficient to sustain proper human growth and development. To rely solely on plants as a source of food (as so many people in developing countries must do) requires large intakes and mixtures of plant material to obtain all of the essential amino acids required to sustain life. To satisfy the minimum daily requirement of essential amino acids of a human child, a very unbalanced amount of plant foodstuffs are required as compared with the amounts necessary to consume from egg and beef (Table 1). As we know from experience, obtaining such essential amino acids from animal products creates an increasing demand on basic food crops such as corn, soybeans, and wheat. At this time, increases in animal production to meet future food needs are not viable options, at least through traditional methods.

PRODUCTION OF NOVEL PROTEIN

De novo artificial plant storage proteins have been designed to accomplish this nutritional goal.[6,7] These proteins can be adjusted to accommodate any composition of essential amino acids desired for the consumption by animals and humans, based on any parent crop. Moreover, unlike many storage proteins found naturally in plants—that are only ‘‘partially’’ bioavailable to those consuming them—the proteins produced as a result of these designs are near 100% bioavailable. In collaboration with Dr. Marceline Egnin and Dr. C.S. Prakash, of Tuskegee University, we have introduced one of these artificial plant storage protein genes into sweet potatoes. Several years of field trials have been completed and small animal feeding studies have been conducted. The results of this work have been most promising. The roots of this transgenic plant contain a more balanced amino acid composition provided by the new gene, as well as

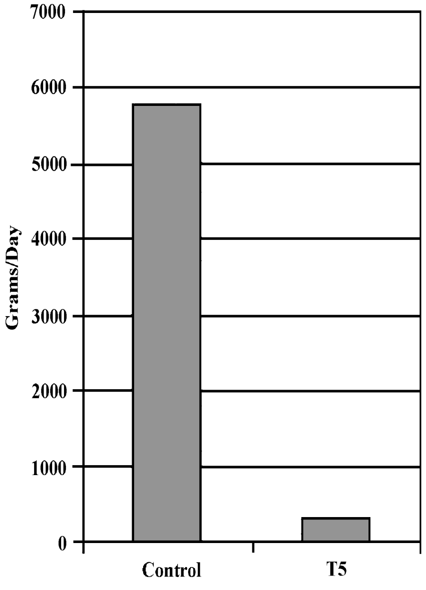

Fig. 1

substantially higher levels of overall protein content[8] (Fig. 1). Thus we have within our grasp the capability of producing indigenous, edible plant foodstuffs and feedstuffs for humans and domesticated animals that would be efficient, cost-effective, and provide complete protein and essential amino acid sources; no supplementation with animal protein sources would be necessary. It is estimated that the total food or feed intake necessary to meet these daily needs could be reduced by more than 75% after this technology is implemented.

F1/AQ3

Improving the essential amino acid composition of basic food and feed crops, as well as increasing their overall protein content, can make a major contribution toward helping to meet the world's future food needs. That advancement combined with conference of disease and stress resistance could result in a better-fed world in the future.

AQ4
AQ5

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