Review Article

Application of agricultural biotechnology to improve food nutrition and healthcare products

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Crop plants provide essential food nutrients to humans and livestock, including carbohydrates, lipids, proteins, minerals and vitamins, directly or indirectly. The level and composition of food nutrients vary significantly in different food crops. As a result, plant foods are often deficient in certain nutrient components. Relying on a single food crop as source of nutrients thus will not achieve a balanced diet and results in malnutrition and deficiency diseases, especially in the developing countries, due mainly to poverty. The development and application of biotechnology offers opportunities and novel possibilities to enhance the nutritional quality of crops, particularly when the necessary genetic variability is not available. While initial emphasis of agricultural biotechnology has been placed on input traits of crops such as herbicide tolerance, insect resistance and virus resistance, increasing effort and promising proof-of-concept products have been made in output traits including enhancing the nutritional quality of crops since 1990s. Advancements in plant transformation and transgene expression also allow the use of plants as bioreactors to produce a variety of bio-products at large scale and low cost. Many proof-of-concept plant-derived healthcare products have been generated and several commercialized.

Key Words: agricultural biotechnology, food nutrition, plant bioreactors, healthcare products, transgenic plants

INTRODUCTION

Plants are the primary source of food for humans and feed for livestock. Through domestication and agricultural activities of breeding and selection, plants were developed into food crops that serve as the major source of dietary carbohydrates, lipids, proteins, vitamins and minerals for humans and livestock. The level and composition of food nutrients vary significantly in different food crops. As a result, individual plant foods are often deficient in certain nutrient components. For example, while root and tuber crops are rich in carbohydrates, they are low in protein; legumes are usually high in protein, but deficient in essential amino acids methionine; and milled rice is rich in starch but contain little essential amino acid lysine, iron, and no provitamin A (ß-carotene). Relying on a single food crop such as cassava or rice as major staple source of nutrients thus will not attain a nutritionally complete diet and result in malnutrition and deficiency diseases, which often occur in populations of developing countries, due mainly to poverty. Effort to improve the nutritional quality of crops by conventional breeding and selection method, in general, has not met with desired success, and even in promising cases, the improvements often associate with undesirable agronomic traits. Recent advancements in plant sciences and agricultural biotechnology offer new opportunities and possibilities to improve the yield, quality, and production economics of food crops. Although the first generation biotech crops have been dominated by input traits since their commercialization in 1996, such as herbicide tolerance, insect and virus resistance soybeans, corn and canola, interest and effort in research and development of crops with output traits including enhancement of food nutrition is on the rise and many proof-of-concept products have been generated, demonstrating that it is feasible to improve food nutrition. With the advancements in plant transformation and transgene expression, using plants as bioreactors to produce high value enzymes and therapeutic proteins at large scale and low cost has been an area of active research and development. Several of the plant-derived enzymes and therapeutic proteins have been commercialized while many more proof-of-concept prototype products are under active development. This mini-review will summarized with examples the progress of applying agricultural biotechnology to improve food nutrition and the effort in developing plants as bioreactors for production of high value therapeutic products.

IMPROVEMENT OF FOOD NUTRITION

Advancement in agricultural biotechnology has allowed the exploration and development of technologies to correct the deficiency and improve the nutritional quality of food crops. These studies were reviewed with examples given.

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Proteins and essential amino acids

Plant proteins contribute about 65% of the per capita supply of protein on a worldwide basis, with cereal grains and food legumes as the most important suppliers. However, as source of dietary protein, plant proteins are often deficient in certain essential amino acids (EAAs) which are nutritionally indispensable under all conditions. For example, cereal proteins are generally low in lysine (Lys) (1.5-4.5 vs. 5.5% of WHO recommendation) while legume, root, tuber and most vegetable proteins are deficient in the sulphur-containing amino acids [methionine (Met) and cysteine (Cys), 1.0-2.0% vs. 3.5% of the WHO reference protein].

Several molecular approaches have been attempted and developed to correct such deficiency, either by increasing the content of protein bound EAAs in crops, through: a) modifying the protein sequence of a major crop protein to contain higher content of a desired EAA; b) producing a synthetic protein rich in a target EAA; c) expressing a heterologous protein with high content of the desired EAA; d) manipulating the expression of a homologous protein for desired EAA; or by increasing the pool of a specific free EAA through metabolic engineering.[1]

Significant proof-of-concept enhancement, especially for the content of methionine and lysine, has been demonstrated through these approaches. For example, using the protein sequence modification approach, the soybean glycinin was modified by insertion of 4 contiguous methionine residues into the variable regions corresponding to the C-terminal regions of the acidic and basic polypeptides and this modified Met-rich soybean glycinin gene under the control of *GluB-1* promoter was transformed and expressed in rice, amounting to 5% as total protein. Interestingly, the modified soybean Met-rich glycinin not only could assemble into trimeric and hexameric structures in rice as those observed in developing soybean seeds, but also rice glutelin could assemble with the modified soybean to form soluble hetero-trimers and –hexamers and insoluble complexes in the rice seeds.[2]

As an example of synthetic gene approach, based on the structurally well-studied maize zein proteins, Kim *et al.* designed and synthesized an artificial storage protein (ASP1) composed of 78.9% EAAs and transformed the 284bp *asp1* gene under the control of CaMV35S promoter into tobacco. Results showed that the synthetic *asp1* was expressed normally in the transgenic tobacco leaves, resulting in relatively high level accumulation of ASP1 protein, and surprisingly, with remarkable increase in the overall levels of total amino acids and protein in some of the transgenic plants.[3] More recently, the *asp1* gene was transformed and expressed in the leaves and primary roots of cassava plants, resulting in elevated content of some amino acids while no significant difference in the protein content of leaves.[4]

In an attempt to enhance the sulphur-containing amino acids content in plant seeds, Altenbach *et al.* cloned a gene encoding a 2S albumin exceptionally rich in Met (18%) and Cys (8%) from Brazil nut (*Bertholletia excelsa* H.K.B.) and introduced this (heterologous) sulphur-rich protein gene under the control of the seed-specific phaseolin promoter into tobacco plant. Accumulation of the sulphur-rich protein in the seeds of tobacco amounted to 3-8% of the total seed protein, resulting in up to 30% increase in the content of Met in the seeds.[5] This enhancement represents the first successful molecular approach to significantly increase the essential amino acid Met content of seeds.[10] Sunflower seed albumin 8 is rich in the sulphur containing amino acids, with 23% Met and Cys. Expression of this gene in lupine led to 40% increase in the content of sulphur–containing amino acids.[6]

Efforts to identify Lys-rich proteins for enhancing the nutritional value of crops, especially cereals, have met with limited success. Legume seed proteins generally contain higher content of Lys than cereal proteins. Attempts to express a pea seed vicilin, French bean phaseolin and soybean glycinin in plants to enhance the Lys content, however, did not make significant increase in the content of Lys or other EAAs, although these proteins were accumulated to high levels. When a cDNA encoding an 18-kDa protein with 10.8mol% Lys was cloned from edible winged bean and introduced into rice under the regulation of *Gt1* promoter, high level expression and accumulation of the Lys-rich protein in rice seeds resulted in 20% increase in the level of protein-bound Lys.[7]

Likewise, when two soybean vegetative storage proteins (S-VSPα and S-VSPβ), both containing 7% Lys residues, were expressed in tobacco, up to 40% increase in leaf soluble Lys content could be observed.[8] A sightly different approach was used to demonstrate that most of the EAAs in potato could be increased by expressing the gene encoding the seed albumin AmA1 of amaranths (*Amaranthus hypochondriacus*) which contains nutritionally balanced amino acid composition.[9] Using the approach of manipulating a homologous protein for desired EAA, a transgenic hybrid maize line expressing the gene encoding a chimeric 10-kDa zein storage protein rich in Met, with enhanced mRNA stability through overcoming post-transcriptional regulation, was generated. The transgenic maize was shown to contain increased seed Met.[10]

A different approach to enhance the EAA content in crops is by increasing the level of a desired EAA in free form through manipulating its synthetic and/or catalytic pathway. For example, the two key enzymes in Lys biosynthetic pathway are aspartokinase (AK) and dihydrodipicolinate synthase (DHPS), which are feedback inhibited by Lys. Galili and associates were the first to constitutively express the Lys-insensitive form of *Escherichia coli* DHPS in the plastid of transgenic tobacco and found that more than 100-fold increase in the content of frees Lys over the wild-type plants. To further enhance the content of free Lys, the strategy of coupling increasing Lys synthesis with a knockout of its catabolism was tested. Results showed a synergistic boost of free Lys content in the seeds of transgenic *Arabidopsis*.[11]

Vitamins and Minerals

Vitamins and minerals are essential food components for human health. Deficiency in dietary micronutrients such as vitamin A, iron, iodine, or zinc will result in micronutrient malnutrition and various deficiency diseases. Biofortification through transgenic technology is an innovative and promising approach. A good example is the fortification of rice with provitamin A through metabolic engineering.

The first generation of provitamin enriched rice, *Golden Rice 1* (*GR1*) was generated by expressing the *psy* gene from daffodil and the *crtI* gene from bacteria in rice to achieve a yield of 1.6μg provitamin A/g in the endosperm.[12] Further increase in the content of provitamin A was achieved by replacing the *psy* gene of daffodil with that of maize, resulting in second generation *Golden Rice 2* (*GR2*), containing 31μg provitamin A/g rice endosperm.[13] With this improvement, 72 g of *GR2* could provide the daily vitamin A allowance for a 1-3 year-old child, which is within the range of 100-200 g of rice consumed per child per day in the target countries (www.goldenrice.org). Efforts to enhance the synthesis and bioavailability of other vitamins and minerals through biotechnological approaches are also active, including vitamin C, vitamin E, vitamin A, folates, pantothenate (vitamin B_5), iron and zinc.[14-19]

Carbohydrates, lipids and other compounds

Food components such as starch, oils, fatty acids, and secondary compounds have also been demonstrated as feasible to manufacture and modify in transgenic plants. Our understanding of starch, lipid and secondary compound metabolisms in plants allows application of biotechnology to manipulate the genes participating in the pathways for altered structure, property, level and function of these bio-products, so as to achieve desired quality, for food and healthcare utilization. For example, transgenic potatoes that contain only amylopectin and devoid of amylose was first demonstrated in 1991; high-lauric acid (40-50% in) canola oil produced by transgenic technology was first commercialized in 1995; and transgenic tobacco plants containing protoalkaloid tryptamine as high as 260-fold over the control plants was obtained in 1990.[20-22]

PRODUCTION OF HEALTHCARE PRODUCTS

Transgenic plants have emerged as an attractive bioreactor platform for large-scale production of industrial enzymes, pharmaceutical proteins, and other biomolecules. When compared to bioreactors based on other systems, such as bacteria, yeast, transfected animal cell lines, or transgenic animals, the bioreactors based on plants hold several advantages, including: low capital and operating costs, easy to scale up, eukaryote post-translational modifications, low risk of human and animal pathogen contaminations, and a relatively high protein yield. Proof-of-concept production of diverse biomolecules including industrial enzymes and therapeutic proteins has been demonstrated.[23] The first commercialized plant-derived recombinant protein was egg white avidin from maize.[24]

Another example is hirudin, an anticoagulant used to treat thrombosis, produced in transgenic oilseed rape.[25] More recently, bovine trypsin was produced at commercial levels in transgenic maize, with functional equivalence to native bovine pancreatic trypsin and a recombinant antibody against hepatitis B was commercially produced in tobacco plants in Cuba.[26-27] Thus there are convincing progresses that plants, through application of biotechnology, not only can produce nutritious foods but also healthcare products for human consumption and utilization. To further develop plant bioreactors into an efficient production platform, product yield, downstream purification and biosafety are areas that require continuous efforts.

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AUTHOR DISCLOSURES

Samuel SM Sun, no conflicts of interest.

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