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Review

Output traits in crop plants: Nutrients and pharmaceuticals

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Abstract Output traits centered on improved plant-based products will find their way to consumers in such ways as nutritionally enhanced foods, therapeutic proteins for disease treatment and vaccines, bio-industrial products, modified oil quality and biofuels. Significant progress in biotechnology has occurred over the last several decades. The importance of output traits development and production using biotechnology will impact not only agribusiness, but also pharmaceutical and food industries. The objective of this paper is to review briefly the current status of output traits development in crop plants using nutrients and pharmaceuticals as examples.

Keywords Output traits - Nutritional improvement - Fortification - Micronutrients - Edible vaccines - Pharmaceuticals

What are "input" and "output" traits?

In the early years of plant biotechnology, efforts and products focused on traits called "input traits". Input traits enhanced growing crop plants, considered the environmental and biological factors that limit production in a farmer's field such as resistance/tolerance or biotic/abiotic stress. Unlike input traits that are designed to protect and enhance yield, "output traits" promise to enhance the value of crops for the consumer. By using biotechnology, output traits can help consumers by enhancing the quality of food and food derived products Therefore, "output traits" are also called value added traits or traits of the next generation of biotechnology (Wilmitzer 1999). Various output traits are now in development such as nutritionally improved traits (Table 1 and 2), modified plant oils, pharmaceutical products (Table 3), bio-industrial products, biofuel etc.

There is growing interest in enhancing the nutrition of plant products as well as producing pharmaceutical products in plants. One good example is golden rice (Enserink 2008). Golden rice producing provitamin A has the potential to help prevent blindness for many impoverished people who might benefit from eating it. Although application of biotechnology is supported by many people and organizations, there are also some who oppose the technology (Enserink 2008). This paper gives a brief overview of output traits in crops, using examples of nutrients and pharmaceuticals.

Nutrients

Agricultural crops are important sources of food and nutrition for human and animal populations. In addition to the calories that food crops provide, crop plants have an incredible capacity for producing a variety of complex chemical compounds such as proteins, vitamins, minerals, lipids, and fiber. Crops high in protein content are critical for human diets and animal rations. In some crops, even those high in protein content, the nutritional value can be improved by modifying the composition of particular amino acids. For example, in soybean seeds, both methionine and cysteine contents are rather low, which may limit the nutritional value of soybean (Krishnan 2005). Efforts to increase the levels of these two essential amino acids are underway by focusing on the sulfur assimilatory pathway in soybean. Adequate supplies of sulfur amino acids in developing seed may facilitate accumulation of sulfur-rich proteins to a level sufficient to reach the nutritional requirements of livestock and poultry (Krishnan 2005).

Humans require at least 44 known nutrients (more than 22 micronutrients) in adequate and consistent amounts from an appropriate diet, primarily plant foods or supplementation. However, these interventions have not always been suc-

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cessful and mineral imbalances often occur in the human body. One micronutrient example is folates. Humans cannot synthesize folates de novo. However, the lack of folates is the most common vitamin deficiency in the world and has serious health consequences, including increased risk of neural tube defects in infants, cancers, and vascular diseases (Scott et al. 2000). The current approach in terms of cost-effective and efficient methods to enhance folate content in crops is biofortification. Biofortification is the process of increasing the bioavailable concentrations of an element in edible portions of crop plants through agronomic intervention (White and Broadley 2005). It differs from ordinary fortification because it emphasizes making plant foods more nutritious as the plants are growing, rather than having nutrients added to the foods when they are being processed. Table 2 demonstrates current studies of biofortified crops development and their target levels for micronutrient contents.

The plant folate synthesis pathway is now largely elucidated, and the first trials of metabolic engineering are under way in various laboratories around the world. Of 10 enzymes specific to the pathway, eight have been cloned and characterized from plants (reviewed in Gilles et al. 2005). Gilles et al (2005) proposed, three approaches to increase the concentrations of folate in plants: (i) increasing synthesis by over-expression of one or more enzymes (or transporters) that contribute steps that limit overall flux through the pathway; (ii) increasing the transport of folates into the vacuole or other metabolically inert compartment where they can be stored; and (iii) decreasing chemical and enzymatic breakdown, either by downregulation of catabolic enzymes, or by rerouting folate synthesis toward 5-formyl-THF (tetrahydrofolate), which is the most stable natural folate.

In addition to biofortification, we also must consider the amount of the nutrient that can be absorbed by humans or animals after processing (Bouis and Welch 2010; White and Broadley 2005). Crop plants often contain antinutrients that interfere with the absorption or utilization of these nutrients in humans (Welch and Graham 1999). For example, crop seeds and grains contain very low bioavailable levels of Fe and increasing the bioavailable amounts of Fe from 5 to 20% would be equivalent to increasing the total Fe by fourfold (Bouis and Welch 2010). Antinutrients that depress Fe bioavailability (such as phytate) or promoter substances (such as ferritin) have fewer genes involved in its biosynthesis and metabolism compared with the uptake, transport, and deposition of Fe in edible seeds and grains (Bouis and Welch 2010). An integrated genetic, physiological, and biochemical strategy can be used to identify molecular markers for improving Fe bioavailability in cereal crops. In addition, breeders could breed for genotypes that contain lower con-

Table 1 Selected nutritionally improved output traits and crops

Crops	Output Traits
Rice	Provitamin A enriched
Rice	Iron fortification
Corn	Detoxification of mycotoxins
Soybean, Canola	Modified oil
Canola	Vitamin E enriched
Tomato	Modified fruit ripening
Tomato	Beta-carotene, lycopene enriched
Cassava	Detoxification of cyanogens
Coffee beans	Caffeine-free

Table 2 Examples of target levels for micronutrient content of biofortified crops (adapted from Bouis and Welch 2010)

Improved micronutrients	Criteria	Rice	Wheat	Corn	Beans	Sweet potato
Per capita consumption	Adult women (g/d)	400	400	400	200	200
	Children 4-6 yr (g/d)	200	200	200	100	100
Fe (RDA* 8-18 mg)	Final target content (ug/g)	13	52	52	94	28
	Final target content as dry wt (ug/g)	15	59	60	107	85
Zn (RDA* 8-11 mg)	Final target content (ug/g)	24	33	33	49	23
	Final target content as dry wt (ug/g)	28	38	38	56	70
Provitamin A (RDA* 0.7-0.9 mg)	Final target content (ug/g)	15	15	15	30	32
	Final target content as dry wt (ug/g)	17	17	17	34	91

* RDA: recommended daily allowances (or adequate intakes per day)

centrations of antinutrients or molecular biologists could alter plant genes in ways that reduce or even eliminate antinutrients from plants using gene silencing or transgenic strategy (Bouis and Welch 2010).

To find solutions for a range of nutritional deficiencies, the importance of interdisciplinary communication between plant scientists and human nutrition scientists are being increased. For example, human nutritionists need to be informed about the extent to which the vitamin and mineral density of specific foods, as well as compounds that promote and inhibit their bioavailability, can be modified (Bouis and Welch 2010; Nestel et al. 2006). Plant scientists need to be aware of both the major influence that agricultural research may have had on nutrient utilization in the past and the potential of plant research for future improvements in nutrition (Bouis and Welch 2010; Nestel et al. 2006).

Pharmaceuticals

Production of pharmaceuticals using plants has became an attractive output trait approach and one of the most widely discussed applications in plant biotechnology since early 1990 (Daniell et al. 2001; Zimmermann 2004). It is often called "pharming" and it is a portmanteau of farming and pharmaceutical and refers to the use of biotechnology to insert genes that encode pharmaceutical proteins into host plants that would otherwise not express those genes. As a consequence, host plants manufacture the pharmaceutical product in large quantity, which can then be used as a drug product. There are two approaches of pharming to develop pharmaceuticals using plants; i) edible vaccines, customers consume edible parts of plants directly and, ii) plant-made pharmaceuticals (PMPs), customers consume the bio-products purified from host plants.

Edible vaccines hold great promise as a cost-effective, easy-to-administer, easy-to-store, and fail-safe. Creating edible vaccines involves introduction of selected desired genes into plants and then inducing these altered plants (transgenic plants) to manufacture the encoded vaccine proteins (Daniell et al. 2001; Korth 2008). In general, the proteins are produced not by the pathogens themselves, but by expression of the gene encoding the protein in a "surrogate organism / host plant." At this stage the idea of producing food crops genetically engineered to produce vaccines in their edible parts, which could then be eaten when inoculations were needed, has become technically feasible (Zimmermann 2004).

The strategy of producing edible vaccines (and PMPs as well) in plants could have several advantages. Transgenic plants offer the economies of scales to grow and harvest large amounts of biomass expressing the target product on relatively little land. In addition, plants provide an economically superior alternative for scaling-up production for recombinant proteins, simply by planting more acreage of these plants. Genetically modified plants to make vaccines are not only economically effective, but also safer for humans than the current system of vaccine production using animals' cells (Zimmermann 2004). That is because animal cells often carry virus and bacteria that can infect humans as well. Plant cells, on the other hand, do not contain pathogens that can harm humans and, therefore, reduce the risks of contamination. Studies carried out in animals over the past 10 years, and small tests in people, encourage hope that edible vaccines can work (Zimmermann 2004).

The development of plant-based vaccines directed at human and animal diseases has provided a new and promising opportunity for agricultural crops, thus increasing the uses and profitability of these agricultural crops. However, not only are these plants useful for production of recombinant proteins, but for some crops, e.g., fruit, leafy vegetables, or grains, they can also serve as delivery systems of these high-value proteins to human and animal populations (Howard 2005). To date, the vast majority of biopharmaceuticals and bio-industrial products are produced using *E. coli*, yeast, or mammalian cell cultures (Kaiser 2008). However, the tremendous variety and opportunity of chemicals produced in plants has been long recognized, as many have powerful effects on human health and physiology such as salicylic acid, cocaine, morphine, taxol etc (Kaiser 2008). The first plant-made pharmaceuticals were monoclonal antibodies from a tobacco plant could be engineered to crank out an antibody (Hiatt et al. 1989). Monoclonal antibodies were being used to treat a growing number of diseases, from arthritis to cancer, but were expensive to make in mammalian cells; Hiatt et al (1989) envisioned harvesting cheap supplies of therapeutic proteins, antibodies, and vaccines from vast field of crops (Table 3).

Most of the antibodies as PMPs are currently expressed in tobacco and potato. Tobacco especially, has advantages as a host plant in various ways; high yielding, low cost of cultivation, producing lots of seeds, and long seed storage time (Zimmermann 2004). Thus far, this technology has resulted in a $40 billion industry of new therapeutics and industrial enzymes, and promises to grow much larger (Korban 2005; Van Arnum 2003). Table 3 shows selected pharmaceuticals produced using crops as host plants.

Even though there is worry that pharma transgenic crops may escape from their fields and taint the food supply, academic scientists and some companies have persisted,

Table 3 Selected pharmaceuticals related output traits (Daniell et al. 2001, Kaiser 2005, Zimmermann 2004)

Category	Crops	Potential Application	Pharmaceuticals
Edible vaccines	Potato	Hepatitis B virus	Hepatitis B vaccines
	Potato	Cholera	Vibrio cholerae vaccines
	Potato, Corn	Diarrhea	Enterotoxigen *E. coli vaccines*
	Potato	Gastrointestinal	Norwalk virus vaccines
	Rice, Potato	Measles virus	Measles virus vaccines
	Tomato	Respiratory syncytial virus	Respiratory syncytial vaccines
Therapeutics antibodies	Tobacco	Dental caries	Streptococcal antigen
	Alfalfa	Diagnostic	Anti-human IgG
	Wheat	Cancer treatment	carcinoembryonic antigen
	Rice	Cancer treatment	carcinoembryonic antigen
	Tobacco	Cancer treatment	carcinoembryonic antigen
	Tobacco	B-cell lymphoma treatment	Idiotype vaccine
	Tobacco	Colon cancer	Surface antigen
Purified products for human drugs	Tobacco	Collagen	Homotrimeric collagen protein
	Tobacco	Wound repair	Epidermal growth protein
	Tobacco	Liver cirrhosis, burns, surgery	Serum albumin protein
	Tobacco	Blood substitute	Hemoglobin α, β protein
	Potato	Antimicrobial	Lactoferrin protein
	Carrot*	Gaucher disease	Glucocerebrosidase
	Duckweed*	Hepatitis C	Interferon-α protein
	Safflower*	Insulin	Diabetes
	Corn*	Lipase	Cystic fibrosis

* Under clinical trials stages

improving innovative ways to keep research inside the lab, or the greenhouse. One of promising and progressing technique is from industry lab, Large Scale Biology Corp, CA, USA (Kaiser 2008). It does not require generating transgenic plants, it simply sprayed tobacco plants with a tobacco mosaic virus carrying the appropriate gene. The leaves produced useful amounts of the vaccine protein within two weeks. The vaccine product worked in mice, suggesting that vaccine designed for lymphoma patients' tumors could be made in plants in just weeks. It is not a transgenic plant which carried the foreign gene, only the leaves until they were shed; this was potentially more acceptable by public than permanently genetically modified crops. Several plant-made pharmaceuticals (PMPs) are now in patient trials, but not yet in final clinical trials (Kaiser 2008). Notably, antibody generated from tobacco which can be used to purify the vaccine of hepatitis B developed from CIGB, Cuba is on the market (Kaiser 2008).

In conclusion, the review demonstrates the current status of output traits development and productions in various applications using crop plants. It becomes clear that the scientific achievements will led to a tremendous variety of new products in plants with important, economic beneficial and hopefully positive consequences for the farmers, customers and the environment.

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