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Vitamin, protein and essential mineral enhancement of cereal crops for food security

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SUMMARY – In the developing world 840 million people are chronically undernourished. It is estimated that more than half of the world's population suffers from diseases caused by dietary deficiencies and inadequate supplies of essential micronutrients. The endosperm tissues of cereal crops (maize, wheat, and rice) serve as major staple foods worldwide. However, they are deficient in essential vitamins (A, E, C and folate), lysine, and minerals (iron, selenium and zinc). The cloning of a near complete set of identified genes required for the biosynthesis of vitamins, lysine and methionine, and for the absorption and bioavailability of Fe, Se and Zn during the past decade, combined with the capabilities to introduce multiple transgenes into cereal crops in recent years, makes it possible to simultaneously enhance all such micronutrients in agriculturally important cereal crops. Our ongoing research towards the simultaneous enhancement of vitamins (A, E, C and folate), essential amino acids (Lys and Met), and minerals such as Fe, Se and Zn, in cereal endosperm tissues by genetic manipulation in order to combat deficiencies in human populations will be presented.

Introduction

Most plants are deficient in some essential amino acids, vitamins and minerals but a balanced diet provides adequate quantities of all. Problems arise when the diet is restricted to a single plant source, which is often the case for both humans and domestic animals in developing countries (Graham and Gregorio, 2001). For example, cereal storage proteins are deficient in lysine and threonine while legumes tend to lack the sulfur-containing amino acids methionine and cysteine. A diet solely comprising one of these protein sources will therefore be deficient for one or more essential amino acids. Milled cereal grains for example are deficient in several vitamins, the most important of which are vitamins A, E and folate.

Vitamin A deficiency is prevalent in the developing world, and is probably responsible for the death of 2 million children every year. In surviving children, vitamin A deficiency is a leading, but avoidable, cause of blindness. Humans can synthesize vitamin A if provided with the precursor molecule beta-carotene (also known as provitamin A, von Lintig and Vogt, 2004). Endosperm tissues of food crops, such as maize and wheat, are low in provitamin A (1-10%) as compared with non-provitamin A carotenoids (Bendich and Olson, 1989; Kurilich and Juvik, 1999). The synthesis of carotenes in plants is a branch of the isoprenoid pathway and the first committed step is the linking of two geranylgeranyl pyrophosphate (GGPP) molecules to form the 40-carbon backbone, phytoene, the first compound of the carotenoid biosynthetic pathway. In order to engineer the conversion of GGPP into beta-carotene in cereal endosperm, expression of at least five enzymes of the carotenoid biosynthetic pathway is required: PSY (phytoene synthase), PDS (phytoene desaturase), ZDS (zeta-carotene desaturase), CRTISO (carotenoid isomerase) and LYCB (lycopene β-cyclase) (Sandmann *et al*., 2006). Alternatively, engineering efforts can be simplified by reducing the number of enzymes required. This can be achieved by using a bacterial phytoene desaturase (CrtI) capable of conversion of phytoene into all-trans lycopene. Two enzymes, BCH (β-carotene hydroxylase) and LYCE (lycopene ε-cyclase), divert the pathway to non-provitamin A xanthophylls that are lower in provitamin A value. High activity of PSY and bacterial CrtI, and higher activity of LYCB, compared to LYCE, in combination with lowering activity of the BCH enzyme, would lead to optimal accumulation of the provitamin A carotenoid, β-carotene in the cereal endosperm.

Vitamin E encompasses a class of lipid antioxidants consisting of four forms each of tocopherol and tocotorienol (DellaPenna and Pogson, 2006). Vitamin E is the common name that describes these eight naturally occurring compounds possessing α-tocopherol activity. Vitamin E is very important for human and animal health. Many human diseases such as certain types of cancer and neurodegenerative and cardiovascular diseases are associated with insufficient intake of vitamin E. Because of its high economical value and importance for human nutrition, much effort has been dedicated to elucidating the tocopherol biosynthetic pathway in plants and cyanobacteria, and to identify rate-limiting steps by over-expression of candidate genes in transgenic plants (Kanwischer *et al*., 2005; DellaPenna and Pogson, 2006). High activity of HPPD and HPT1, VTE3 and γ-TMT enzymes in cereal endosperm, would lead to optimal accumulation of α-tocopherol.

Vitamin C (ascorbic acid, AsA) is required for cardiovascular function, immune cell development, connective tissue, and iron utilization (Chen *et al*., 2003). Humans cannot synthesize AsA which consequently, must be acquired from dietary sources, primarily from plants rich in AsA. Dehydroascorbate reductase (DHAR) allows the plants to recycle dehydroascorbate, therefore recapturing AsA before it is lost. Vitamin C content in plants can be elevated by increasing expression of the DHAR enzyme responsible for recycling AsA (Chen *et al*., 2003).

Tetrahydrofolate and its derivatives (vitamin folates) are essential cofactors for one-carbon transfer reactions in all organisms. Similar to bacteria and yeasts, plants make folates *de novo* from pterin, ρ-aminobenzoic acid (PABA), and glutamate moieties (Hanson and Gregory, 2002). In contrast, humans and other animals lack a complete folate synthesis pathway, thus they depend on plants as the source of dietary folates. Inadequate dietary levels of folate can lead to megaloblastic anemia, birth defects, impaired cognitive development, and increased risk of cardiovascular disease and cancer (Hossain *et al*., 2004 and therein). Because plant foods are major sources of folate, and folate deficiency is a global health problem, enhancing plant folate content is a major target for metabolic engineering (DellaPenna, 1999; Hossain *et al*., 2004). Folates are synthesized *de novo* from pterins and ρ-aminobezoate (PABA) by means of a multi-step pathway, whereas pterins and PABA are synthesized from GTP and chorismate, respectively. The reaction catalysed by plant GTP cyclohydrolase-1 (GCH) is a rate-determining step in *de novo* pterin and folate biosynthesis in plants (Hossain *et al*., 2004).

Minerals such as iron (Fe), zinc (Zn) and selenium (Se) are essential to the body in small amounts because they are either components of enzymes or act as cofactors in controlling chemical reactions. Iron is a carrier of oxygen in red blood cell haemoglobin, transporter of electrons within cells and is an integral part of important enzyme systems in various tissues. Populations with Fe deficiency risk are infants, children, adolescents and pregnant women. Zn deficiency may result in low psychomotor and mental development (children), poor pregnancy, poor immune function, tiredness, and retarded growth. More then 30% people are Zn deficient (White and Broadley, 2005). Selenium is implicated in the protection of body tissues against oxidative stress, in the maintenance of defences against infection and in the modulation of growth and development. Some enzymes contain Se. Se also optimizes the biological functionality of Zn and Fe in humans and has anticarcinogenic properties.

Cloning of genes, transformation vectors, rice and corn transformations

We have cloned all genes shown in Tables 1, 2 and 3 and made appropriate endosperm-specific or constitutive expression vectors for maize and rice transformation, as appropriate. Our strategy calls for the simultaneously enhancement of vitamins (A, E, C and folate), essential amino acids (Lys and Met), and minerals (Fe, Se and Zn), in cereal endosperm tissue. Rice and corn transformation is ongoing.

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Status quo of the project

Table 1. General description of genes for vitamins

Vitamins	Genes	Gene products	Source organisms	Accession number
Pro-vitamin A	*psy*	Phytoene synthase	*Zea mays*	U32636
	crtI	Phytoene desaturase	*Erwinia uredovora*	D90087
	lycb	Lycopene beta cyclase	*Gentiana lutea*	AB017367
	bch	beta-carotene hydroxylase	*Z. mays*	AY844956
Vitamin C	*dhar*	Dehydroascorbate reductase	*Oryza sativa*	AY074786
Vitamin E	*pds1*	HPP dioxygenase (HPPD)	*Arabidopsis thaliana*	AF060481
	sdx1/vte1	Tocopherol cyclase		AF302188
	hpt1/vte2	Homogentisate Phytylprenyltransferase		AY089963
	vte3	MPBQ methyltransferase		AY089963
	vte4	gamma-Tocopherol methyltransferase (γ-TMT)		AF104220
Folate	*folE*	GTP cyclohydrolase-1	*Eschericha coli*	X63910

Table 2. General description of genes for minerals

Mineral	Genes	Gene products	Source organisms	Accession number
Iron	*IRT1*	Iron regulated metal transporter	*O. sativa*	AB070226.1
	NAS1	Nicotianamine synthase 1	*O. sativa*	AB021746.2
	NAAT-A	Nicotianamine aminotransferase	*Hordeum vulgare*	D88273.2
	NAAT-B	Nicotianamine aminotransferase	*H. vulgare*	AB005788.1
	HvYS1	Iron-phytosiderophore transporter	*H. vulgare*	AB214183.1
	Ferritin	Ferritin	*Glycine max*	M64337
	PHYA3	Phytase	*Aspergillus fumigatus*	AJ419776
Zinc	*ZAT*	Zn transporter	*A. thaliana*	NM_130246.2
Selenium	*APS1*	ATP sulfurylase	*A. thaliana*	NM_113189.2

Table 3. General description of genes for essential amino acids

Amino acids	Genes	Gene products	Source organisms	Accession number
Lysine	*AK*	Aspartate kinase	*E. coli*	M11812
	DHPS	Dihydrodipicolinate synthase	*E. coli*	NC_004431
Methionine	*CGS*	Cystathionine γ-synthase	*A. thaliana*	U43709