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Vitamin bio-fortification of cereals for food security

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SUMMARY – The objective of the project is to engineer cereal plants with appropriate genes to obtain grains with an enhanced nutritional profile for vitamins for food security targeting specifically developing countries. Our focus is vitamins A, C, E and folate. Target species are rice and maize. The genetic constructs will be co-transformed simultaneously into appropriate target tissue by particle bombardment. We will generate a combinatorial population of plants that express different permutations and numbers of the input transgenes. Past experience indicates that if we generate ca. 100 independent transgenic plants there is a high probability of recovering a number of independent transformants expressing the full complement of transgenes. Transgenic plants will be screened for gene expression at the mRNA, protein and metabolite levels. We will present an update of our work and in the process we will paint a comprehensive picture of the molecular and genetic makeup of this population. This will be invaluable for food security applications and also for developing a better understanding of the mechanisms and factors that influence multiple transgene integration and expression in important cereal crops.

Introduction

Vitamins play a vital role in human health. Deficiency of most vitamins can impair immune system function. Carotenoids are colored pigments found in plants that are precursors of vitamin A in human body. Vitamin A deficiency (VAD) is an immunodeficiency disorder characterized by widespread alterations in immunity (Semba, 1994). VAD is a major cause of premature death in developing nations, particularly among childern. About 800,000 deaths in children and women of reproductive age are attributable to vitamin A deficiency each year which, along with the direct effects on eye disease, account for 1.8% of global disability-adjusted life years (WHO, 2002; Ezzati *et al*., 2002). Vitamin E deficiency depresses immunoglobulin response to antigens, lymphocytic proliferative responses to mitogens and antigens, delayed dermal hypersensitivity reactions, and general host resistance (Beisel, 1981). Vitamin C deficiency also impairs phagocyte function and cellular immunity (Chandra, 1986).

Folate has many roles in the human body, including a critical role in the transfer of methyl groups to amino acids and DNA. Low folate intake is associated with increased risks of breast, lung (amongst former smokers), cervical, and mouth and throat cancers. (Adzersen *et al*., 2003; Shen *et al*., 2003; Hernandez *et al*., 2003; Pelucchi *et al*., 2003).

Material and methods

Cloning of the genes

We designed primers for PCR-cloning of genes (Table 1) from the sequences available in the Genbank (NCBI database). Total RNAs were isolated using RNeasy® Plant Min Kit (QIAGEN, Hilden, Germany). First-strand cDNAs were synthesized according to the protocol of Omniscript® Reverse Transcription Kit (QIAGEN, Hilden, Germany) and used as templates for PCR amplification. Genes from bacterial origin were cloned from genomic DNA. All genes were cloned into pGEM®-T vector and subjected to sequence analysis. By using Blast sequence alignment we confirmed sequence identity (100%) of the cloned genes with those described in the NCBI Genbank.

Vector construction

The full length sequences of all genes (Table 1) were excised from pGEM®-T vector and ligated into appropriate vectors (Table 2). The vector constructs containing plasmids and inserted genes, were then resequenced.

Table1. General description of genes for transformation experiments

Traits	Genes	Gene products	Source organisms	Genbank Accession no.
Carotenoids	*psy*	Phytoene synthase	*Zea mays*	U32636
	crtI	Phytoene desaturase	*Erwinia uredovora*	D90087
	lcy	Lycopene beta cyclase	*Gentiana lutea*	ABD017367
	bch	Beta-carotene hydroxylase	*Gentiana lutea*	AB027187
	crtW	Beta-carotene oxygenase	*Paracoccus species*	D58420
	bch antisence	Beta-carotene hydroxylase	*Zea mays*	AY84495
Vitamin C (Ascorbate)	*dhar*	Dehydroascorbate reductase	*Oryza sativa*	AY074786
Vitamin E (Tocopherols)	*pds1*	HPP dioxygenase (HPPD)	*Arabidopsis thaliana*	AF060481
	sdx1/ vte1	Tocopherol cyclase		AF302188
	hpt1/ vte2	Homogentisate Phytylpernyltransferase (HPT)		AY089963
	vte3	MPBQ methyltransferase (MPBQ MT)		AY089963
	vte4	Gamma -Tocopherol methyltransferase (gamma-TMT)		AF104220
Folate	*folE*	GTP cyclohydrolase-1	*Escheria coli*	X63910

Table 2. Genes with restriction sites and plasmids for vector construction

Traits	Genes	Restriction sites	Plasmids
Carotenoids	*psy*	*Bam*HI/*Eco*RI	p326
	crtI	*Xba*I/*Eco*RI	pHor-P
	lcy	*Bam*HI/*Eco*RI	pRP5
	bch	*Not*I	pTO126
	crtW	*Bam*HI/*Eco*RI	pGZ63
	bch antisence	*Xba*I/*Eco*RI	pHor-P
Vitamin C (Ascorbate)	dhar	XbaI/SacI	pHor-P
Vitamin E (Tocopherols)	pds1	BamHI/KpnI	pRP5
	sdx1/ vte1	*Bam*HI/*Sac*I	pGZ63
	hpt1/ vte2	*Bam*HI/*Eco*RI	p326
	vte3	*Xba*I/*Eco*RI	pHor-P
	vte4	*Bam*HI/*Sac*I	pGZ63
Folate	*folE*	*Bam*HI/*Sac*I	pHor-P

Rice and corn transformation and plant regeneration

Maize embryogenic callus (*Zea mays* L., cv W37) will be cultured as described (Mehlo *et al.*, 2000). After 10 days, bombardment will be carried out using 70 μg of coated gold particles (Christou *et al.*, 1991). The target tissue will be incubated on medium containing high osmoticum (0.2 M mannitol, 0.2 M sorbitol) for 5-6 h prior to bombardment and 10-16 h after bombardment. The particles will be coated with a molar ratio of 3:1 gene of interest and selectable marker cassette (derived from plasmid pAHC20 that contains the bar selectable marker gene; Christensen and Quail, 1996) for cotransformation (Christou *et al.*, 1991). Bombarded callus will be selected on phosphinothricin-supplemented medium as described previously (Mehlo *et al.*, 2000). A minimum of 100 independent plant lines will be generated.

Mature rice embryos (*Oryza sativa* L., cv EYI 105) will be excised and cultured as described (Sudhakar *et al.*, 1998; Valdez *et al.*, 1998). After 5 days, bombardment will be carried out using 70 μg of coated gold particles (Christou *et al.*, 1991). The target tissue will be incubated on medium containing high osmoticum (0.2 M mannitol, 0.2 M sorbitol) for 5-6 h prior to bombardment. The particles will be coated with a molar ratio of 3:1 gene of interest cassettes and selectable marker cassette (hygromycin phosphotransferase, *hpt*) for cotransformation (Sudhakar *et al.*, 1998; Valdez *et al.*, 1998). Bombarded callus will be selected on hygromycin-supplemented medium as described previously (Sudhakar *et al.*, 1998; Valdez *et al.*, 1998). A minimum of 100 independent plant lines will be generated.

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