Cloning Genes for Secondary Metabolites that Affect Seed Colour, Plant Defense, Nodulation and Human Health in Beans

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Because of its low fat/high protein content, dietary fiber, complex carbohydrates and vitamins including folic acid, the dry bean is characterized as the nearly perfect food. It also contains dietary phytoestrogens, secondary metabolites such as isoflavonoids and lignans, which may have significant impacts on human health by preventing some types of cancer, cardiovascular disease, osteoporosis and menopausal symptoms.

Secondary metabolites are compounds that are restricted to a specific plant species or specific plant organs that participate in interactions between the plant and its environment. A major class of secondary metabolites are the phenylpropanoids. Plants produce more than 8000 phenylpropanoids, which are derived from the amino acid phenylalanine through the action of various enzymes in the phenypropanoid pathway.

These phytochemicals play significant roles in the bean plant, including: to give seed coats their colours, signaling nitrogen fixing bacteria in the initiation of nodules, as defense compounds against a variety of pathogens and as UV sun screens.

Selection and/or molecular manipulation for increased levels of these compounds in bean requires information about the genes that control their synthesis. Although a great deal of information exists in other species, only a few gene sequences for genes that code for enzymes and regulatory molecules in the phenylpropanoid pathway were available for bean at the beginning of the current study.

Our objective in the current work was to clone, sequence and map approximately 30 structural and regulatory genes in the phenylpropanoid pathway in bean. This information will be used to assay the activity of the genes coding for phenylpropanoid pathway enzymes and regulatory proteins in a variety of beans using DNA microchip technology.

Since the information on bean phenylpropanoid pathway gene sequences is fragmentary, literature and databases were searched for the appropriate gene sequences from related species such as soybean, *Phaseolus coccineus* and *Medicago sativa.* The sequences were aligned and the conserved regions were used to design PCR primers. The primers were used in RT-PCRs with bean seedling RNA to amplify fragments of a number of structural genes coding for enzymes of the general, lignin/lignan and flavonoid branches of the phenylpropanoid pathway (including: PAL2, PAL3, C4H, 4CL-1, 4CL-2, COMT, LAC, IFS, CHS, CHI, DFR, F3H and F3'H; Fig 1). From thirty-seven primer sets for structural genes 23 gave PCR products that gave sequence that matched the expected gene; but only 5 out of 31 PCR primers designed for regulatory genes were successful. The construction of a test microarray of bean phenylpropanoid genes is underway. The array will be used to screen bean germplasm for variation in the levels of genes in the phenylpropanoid pathway.

Cloned Phenylpropanoid Gene Fragments in Bean

Enzyme	Gene	Source sequence			PCR product (bp)
		species	accession number	source	
Phenylalanine ammonia lyase	PAL2	*P. vulgaris*	P19142	DNA	398
	PAL3	*P. vulgaris*	P19143	DNA	397
Cinnamate 4-hydroxylase C4H	*P. vulgaris*	Y09447	mRNA		359
4-coumarate CoA ligase	4CL-1	*G. max*	AF279267	mRNA	545
	4CL-2	*G. max*	AF002259	mRNA	402
	4CL-3	*G. max*	AF002258	mRNA	550
Cinnamoyl CoA reductase CCR	*G. max*	BI426824	EST		447
Cinnamyl-alcohol dehydrogenase	CAD	*M. sativa*	L46856	mRNA	306
Caffeate O-methyltransferase	COMT	*M. sativa*	M63853	mRNA	392
Ferulate 5-hydroxylase	F5H	*G. max*	BM527849	EST	468
Laccase	LAC	*G. max*	AF527604	DNA	492
Lignin peroxidase	FBP4	*P. vulgaris*	AF149279	mRNA	695
Chalcone synthase	CHS	*P. vulgaris*	X06411	mRNA	778
Chalcone isomerase	CHI	*P. vulgaris*	Z15046	DNA	215
Flavanone 3-hydroxylase	F3H	*M. sativa*	X78994	DNA	550
Flavonoid 3'-hydroxylase F3'H	*G. max*	AB061212	mRNA		800
Dihydroflavonol 4-reductase	DFR	G. *max*	AF167556	mRNA	800
Leucoanthocyanidin reductase	LAR	*M. trunc*	AY184243	mRNA	800
2-hydroxyisoflavanone synthase	IFS	*G. max*	AF195818	DNA	535
7-O-methyltransferase	IOMT	*M. sativa*	AF000975	mRNA	404
Isoflavanone reductase	IFR	*M. sativa*	U17436	DNA	1400
Anthocyanin 5-acyltransferase	AAT	*P. cocc*	CA900148	EST	407
Vacuolar transporter	VT	*P. cocc*	CA907034	EST	453
LIM domain protein WLIM1 (lignin)	LIM1	*G. max*	BU764417	EST	1350
R2R3-MYB trans-factor AtMyb4	Myb4	*P. cocc*	CA902489	EST	315
homeodomain protein, GL2 like 1	HD-GL2	*P. cocc*	CA902455	EST	324
R2R3-MYB trans-factor AtMYB15	Myb15	*P. cocc*	CA902486	EST	600
trans factor KAP -2 (CHS)	KAP2	*P. vulgaris*	AF293344	mRNA	504

We anticipate that the information will accelerate and simplify breeding for increased levels of phenylpropanoid compounds in bean that might benefit human health and play important roles in the seed coat colour, disease resistance, stress tolerance and nitrogen fixation.