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(54) **Title**: SYNTHETIC GENES

(57) **Abstract**: The invention provides synthetic nucleic acid sequences encoding proteins of interest that are particularly adapted to express well in plants. The claimed synthetic sequences utilize plant- optimized codons roughly in the same frequency at which they are utilized, on average, in genes naturally occurring in the plant species. The invention further includes synthetic DNA sequence for herbicide tolerance, water and/or heat stress tolerance, healthy oil modifications and for transformation marker genes and selectable marker genes are used. DNA construct and transgenic plants containing the synthetic sequences are taught as are methods and compositions for using the plants in agriculture.

SYNTHETIC GENES

BACKGROUND OF THE INVENTION

[0001] To achieve desired expression levels of heterologous proteins in transgenic plants it has been found beneficial to alter the native, sometimes referred to as wild-type or original, DNA coding sequence in various ways, e.g. so that codon usage more closely matches the codon usage of the host plant species, and/or so the G+C content of the coding sequence more closely matches the G+C level typically found in coding sequences of the host plant species, and/or so that certain sequences that destabilize mRNA are removed. Expression in plants of *Bacillus thuringiensis* (B.t.) crystal protein insect toxins, for example, has been improved using one or more of these approaches. See, for example, US Patent No. 5380301, US Patent No. 5625136, US Patent No. 6218188, US Patent No. 6340593, US Patent No. 6673990, US Patent No. 7741118. Codon degeneracy allows one to make synthetic DNA sequences that encode a protein of interest using codons that differ from those used in the original DNA coding sequence.

[0002] In regard to removing sequences that may destabilize mRNA, US Patent No. 7741118 discloses a list of 16 polyadenylation signal sequences (column 15, Table II) and calls for reducing the number of such sequences in synthetic coding sequences that are intended for expression in plants. The polyadenylation signal sequences listed in US 7741118, Table II are listed below in Table 1:

Table 1. Polyadenylation signal sequences listed in US 7741118, Table II.

1	AATAAA	6	ATACTA	11	ATACAT	16	CATAAA
2	AATAAT	7	ATAAAA	12	AAAATA		
3	AACCAA	8	ATGAAA	13	ATTAAA		
4	ATATAA	9	AAGCAT	14	AATTAA		
5	AATCAA	10	ATTAAT	15	AATACA		

[0003] US 7741118 also calls for preferably removing the sequence ATTTA (known as the Shaw-Kamen sequence), because it has been identified as potentially destabilizing mRNA.

[0004] Contrary to the teaching of US 7741118, we have found that reduction in the number of the polyadenylation signal sequences identified in Table 1 above is neither necessary nor sufficient to enable enhanced expression of synthetic genes in plants.

SUMMARY OF THE INVENTION

[0005] Table 2 below identifies 20 potential polyadenylation signal sequences that occur frequently in maize genes.

Table 2. Potential polyadenylation signal sequences found in maize genes

1	ATATAT	6	TATTTT	11	TAATAA	16	TATTAT
2	TTGTTT	7	TTTTTT	12	ATTTAT	17	TGTTTG
3	TTTTGT	8	ATTTTT	13	TATATT	18	TTATAT
4	TGTTTT	9	TTATTT	14	TTTTAT	19	TGTAAT
5	TATATA	10	TTTATT	15	ATATTT	20	AAATAA

[0006] Table 3 below identifies 20 potential polyadenylation signal sequences that occur frequently in soybean genes.

Table 3. Potential polyadenylation signal sequences found in soybean genes.

1	ATTTTT	6	TTTTAT	11	AAATTT	16	ATATAT
2	TATTTT	7	AATTTT	12	AAATAA	17	ATTATT
3	TTATTT	8	TTTTTA	13	ATATTT	18	ATTTTA
4	TTTATT	9	TAATTT	14	TTTGTT	19	TTTAAT
5	TTTTTT	10	TTAATT	15	TTGTTT	20	TTTTAA

[0007] The present invention provides a synthetic DNA sequence for expressing a protein of interest in maize cells which comprises:

a) a codon-optimized DNA sequence encoding the protein of interest,

b) at least one polyadenylation signal sequence chosen from the group consisting of Class I and Class II wherein;

Class I is chosen from the group consisting of AATAAA, AATAAT, AACCAA, ATATAA, AATCAA, ATACTA, ATAAAA, ATGAAA, AAGCAT, ATTAAT, ATACAT, AAAATA, ATTAAA, AATTAA, AATACA, and CATAAA; and

Class II is chosen from the group consisting of ATATAT, TTGTTT, TTTTGT, TGTTTT, TATATA, TATTTT, TTTTTT, ATTTTT, TTATTT, TTTATT, TAATAA, ATTTAT, TATATT, TTTTAT, ATATTT, TATTAT, TGTTTG, TTATAT, TGTAAT, and AAATAA; and

wherein said codon-optimized DNA sequence contains-at least one polyadenylation signal sequence from Class II and wherein said synthetic DNA sequence contains fewer Class II polyadenylation signal sequences than the protein's native DNA sequence and contains the same number of Class I polyadenylation signal sequences compared to the native DNA sequence.

[0008] The present invention also provides a synthetic DNA sequence for expressing a protein of interest in soybean cells which comprises:

a) a codon-optimized DNA sequence encoding the protein of interest,

b) at least one polyadenylation signal sequence chosen from the group consisting of Class I and Class III wherein;

Class I is chosen from the group consisting of AATAAA, AATAAT, AACCAA, ATATAA, AATCAA, ATACTA, ATAAAA, ATGAAA, AAGCAT, ATTAAT, ATACAT, AAAATA, ATTAAA, AATTAA, AATACA, and CATAAA; and

Class III is chosen from the group consisting of ATTTTT, TATTTT, TTATTT, TTTATT, TTTTTT, TTTTAT, AATTTT, TTTTTA, ATATAT, TAATTT, TTAATT, AAATTT, AAATAA, ATATTT, TTTGTT TTGTTT, ATTATT, ATTTTA, TTTAAT, and TTTTAA, and

wherein said codon-optimized DNA sequence contains at least one polyadenylation signal sequence from Class III and wherein said synthetic DNA sequence contains fewer Class III polyadenylation signal sequences than the protein's native DNA sequence and contains the same number of Class I polyadenylation signal sequences compared to the native DNA sequence.

[0009] The invention also provides a method of making a synthetic DNA sequence that encodes a protein of interest which comprises (a) starting with an amino acid sequence of a protein of interest derived from naturally occurring polypeptide(s) encoded by native sequence(s) that comprise at least one polyadenylation signal sequence listed in Table 2, and (b) making a synthetic DNA sequence that encodes said amino acid sequence and contains fewer polyadenylation signal sequences listed in Table 2 compared to the corresponding coding sequence of the native sequence(s) and contains the same number of polyadenylation signal

sequences listed in Table 1.

[0010] In another embodiment the invention provides a method of making a synthetic DNA sequence that encodes a protein of interest which comprises (a) starting with an amino acid sequence of a protein of interest derived from naturally occurring polypeptide(s) encoded by native sequence(s) that comprise at least one polyadenylation signal sequence-listed in Table 3, and (b) making a synthetic DNA sequence that encodes said amino acid sequence and contains fewer polyadenylation signal sequences listed in Table 3 compared to the corresponding coding sequence of the native sequence(s) and contains the same number of polyadenylation signal sequences listed in Table 1.

[0011] In some embodiments the synthetic DNA sequences provided by the invention are devoid of the polyadenylation signal sequences listed in Table 2 and/or Table 3, or the number of polyadenylation signal sequences identified in Table 2 and/or Table 3 is reduced as much as possible consistent with maintaining the same number of polyadenylation signal sequences identified in Table 1 and maintaining the Table 1 sequences in their original positions in the sequence.

[0012] In some embodiments the synthetic DNA sequences provided by the invention encode an insecticidal protein, optionally derived from *Bacillus thuringiensis,* as well as DNA sequences useful for herbicide tolerance, water and/or heat stress tolerance, healthy oil modifications and for transformation marker genes and selectable marker genes.

[0013] The synthetic DNA sequences of the invention may be used in a DNA construct for expression of a protein of interest, where the construct comprises a 5' non-translated sequence, a synthetic DNA sequence of the invention, and a 3' non-translated region, and said 5' non-translated sequence contains a promoter that functions in plants, and said 3' non-translated sequence comprises a transcription termination and polyadenylation signal.

[0014] The invention also provides a transgenic plant containing the synthetic DNA sequences of the invention.

[0015] Also provided is a method of controlling pests in a plant which comprises expressing a synthetic DNA sequence of the invention in the plant where the synthetic DNA sequence encodes an insect toxin, for example a *Bacillus thuringiensis* Cry protein.

[0016] Also provided is a method for herbicide tolerance in a plant which comprises expressing a synthetic DNA sequence of the invention in the plant where the synthetic DNA

sequence encodes a known herbicide tolerance enzyme, for example the aryloxyalkanoate dioxygenase (AAD1) see WO/2005/107437, or phosphinothricin acetytransferase, or 5-enolpyruvylshikimate-3-phosphate synthase enzymes.

[0017] Also provided is a method for modifying oil profiles in a plant which comprises expressing one or more synthetic DNA sequences of the invention in the plant where the synthetic DNA sequence encodes one or more known enzymes for modifying oil profiles in plants, for example fatty acid desaturase.

[0018] Also provided is a method for stress tolerance in a plant which comprises expressing a synthetic DNA sequence of the invention in the plant where the synthetic DNA sequence encodes known stress tolerance genes for water and/or heat stress, for example the stress associated protein (SAP1); US Patent Publication No: 2010/0275327, and 1-Cys peroxiredoxin (Per1) proteins(Mowla, *et al.*, 2002, **Planta** 215:716-726).

[0019] Also provided is a method adding reporter genes in a plant which comprises expressing a synthetic DNA sequence of the invention in the plant where the synthetic DNA sequence encodes a known transformation marker protein functional in plants, for example green fluorescence protein (GFP) or beta glucuronidase enzyme.

[0020] Also provided is a method of controlling pests in grain or seed which comprises obtaining said grain or seed from plants containing a synthetic gene of the invention that expresses an insect toxin, and a method of controlling pests in meal or flour which comprises obtaining said meal or flour from grain containing a synthetic gene of the invention that expresses an insect toxin.

[0021] Also provided is a composition derived from transgenic plants containing synthetic DNA of the invention wherein said composition is a commodity product selected from the group consisting of meal, flour, protein concentrate, or oil.

[0022] In some cases the number of polyadenylation signals listed in Table 1 can be maintained in synthetic DNA sequences of the invention by deleting occurrences of AATAAA and substituting another polyadenylation signal sequence listed in Table 1. This is exemplified in Example 1, SEQ ID NO:5.

DESCRIPTION OF THE SEQUENCES

[0023] SEQ ID NO:1 is the native DNA sequence encoding *Bacillus thuringiensis* Cry1Fa core toxin.

[0024] SEQ ID NO:2 is *Bacillus thuringiensis* Cry1Fa core toxin sequence.

[0025] SEQ ID NO:3 is a synthetic DNA sequence encoding *Bacillus thuringiensis* Cry1Fa core toxin using codons optimized for maize and Table 1 sequences are maintained.

[0026] SEQ ID NO:4 is *Bacillus thuringiensis* Cry1Fa core toxin sequence.

[0027] SEQ ID NO:5 is a synthetic DNA sequence in accordance with the invention encoding-*Bacillus thuringiensis* Cry1Fa core toxin using codons optimized for maize and with sequences identified in Table 2 removed and Table 1 sequences are maintained.

[0028] SEQ ID NO 6 is *Bacillus thuringiensis* Cry1Fa core toxin sequence.

[0029] SEQ ID NO:7 is the native DNA sequence encoding *Bacillus thuringiensis* Cry34Ab1 toxin.

[0030] SEQ ID NO:8 is *Bacillus thuringiensis* Cry34Ab1 toxin sequence.

[0031] SEQ ID NO:9 is a synthetic DNA sequence encoding *Bacillus thuringiensis* Cry34Ab1 toxin using codons optimized for maize and Table 1 sequences are maintained.

[0032] SEQ ID NO:10 is *Bacillus thuringiensis* Cry34Ab1 toxin sequence.

[0033] SEQ ID NO:11 is a synthetic DNA sequence in accordance with the invention encoding *Bacillus thuringiensis* Cry34Ab1 toxin using codons optimized for maize and with sequences identified in Table 2 removed and Table 1 sequences are maintained.

[0034] SEQ ID NO:12 is *Bacillus thuringiensis* Cry34Ab1 toxin sequence.

[0035] SEQ ID NO:13 is the native DNA sequence encoding *Bacillus thuringiensis* Cry35Ab1 toxin.

[0036] SEQ ID NO:14 is *Bacillus thuringiensis* Cry35Ab1 toxin sequence.

[0037] SEQ ID NO:15 is a synthetic DNA sequence encoding *Bacillus thuringiensis* Cry35Ab1 toxin using codons optimized for maize and Table 1 sequences are maintained

[0038] SEQ ID NO:16 is *Bacillus thuringiensis* Cry35Ab1 toxin sequence.

[0039] SEQ ID NO:17 is a synthetic DNA sequence in accordance with the invention encoding *Bacillus thuringiensis* Cry35Ab1 toxin using codons optimized for maize and with sequences identified in Table 2 removed and Table 1 sequences are maintained.

[0040] SEQ ID NO:18 is *Bacillus thuringiensis* Cry35Ab1 toxin sequence.

[0041] SEQ ID NO:19 is the native DNA sequence encoding *Bacillus thuringiensis* Cry1Ab1 core toxin.

[0042] SEQ ID NO:20 is *Bacillus thuringiensis* Cry1Ab1 core toxin sequence.

[0043] SEQ ID NO:21 is a synthetic DNA sequence encoding *Bacillus thuringiensis* Cry1Ab1 core toxin using codons optimized for maize and Table 1 sequences are maintained.

[0044] SEQ ID NO:22 is *Bacillus thuringiensis* Cry1Ab1 core toxin sequence.

[0045] SEQ ID NO:23 is a synthetic DNA sequence in accordance with the invention encoding-*Bacillus thuringiensis* Cry1Ab1 core toxin using codons optimized for maize and with sequences identified in Table 2 removed and Table 1 sequences are maintained.

[0046] SEQ ID NO:24 is *Bacillus thuringiensis* Cry1Ab1 core toxin sequence.

[0047] SEQ ID NO:25 is the native DNA sequence encoding *Bacillus thuringiensis* Cry1Ca core toxin.

[0048] SEQ ID NO:26 is encoding *Bacillus thuringiensis* Cry1Ca core toxin sequence.

[0049] SEQ ID NO:27 is a synthetic DNA sequence encoding *Bacillus thuringiensis* Cry1Ca core toxin using codons optimized for maize and Table 1 sequences are maintained.

[0050] SEQ ID NO:28 is encoding *Bacillus thuringiensis* Cry1Ca core toxin sequence.

[0051] SEQ ID NO:29 is a synthetic DNA sequence in accordance with the invention encoding *Bacillus thuringiensis* Cry1Ca core toxin using codons optimized for maize and with sequences identified in Table 2 removed and Table 1 sequences are maintained.

[0052] SEQ ID NO:30 is encoding *Bacillus thuringiensis* Cry1Ca core toxin sequence.

[0053] SEQ ID NO:31 is the native DNA sequence encoding *Bacillus thuringiensis* Cry6Aa toxin.

[0054] SEQ ID NO:32 is *Bacillus thuringiensis* Cry6Aa toxin sequence.

[0055] SEQ ID NO:33 is a synthetic DNA sequence encoding *Bacillus thuringiensis* Cry6Aa toxin using codons optimized for maize and Table 1 sequences are maintained.

[0056] SEQ ID NO:34 is *Bacillus thuringiensis* Cry6Aa toxin sequence.

[0057] SEQ ID NO:35 is a synthetic DNA sequence in accordance with the invention encoding *Bacillus thuringiensis* Cry6Aa toxin using codons optimized for maize and with sequences identified in Table 2 removed and Table 1 sequences are maintained.

[0058] SEQ ID NO:36 is *Bacillus thuringiensis* Cry6Aa toxin sequence.

[0059] SEQ ID NO:37 is the native DNA sequence encoding *Sphingobiurn herbicidovorans*

AAD1 protein.

[0060] SEQ ID NO:38 is *Sphingobiurn herbicidovorans* AAD1 protein sequence.

[0061] SEQ ID NO:39 is a synthetic DNA sequence encoding *Sphingobiurn herbicidovorans* AAD1 protein using codons optimized for maize and Table 1 & Table 2 sequences are maintained.

[0062] SEQ ID NO:40 is *Sphingobiurn herbicidovorans* AAD1 protein sequence.

[0063] SEQ ID NO:41 is a synthetic DNA sequence in accordance with the invention encoding *Sphingobiurn herbicidovorans* AAD1 protein using codons optimized for maize and with sequences identified in Table 2 removed and Table 1 sequences are maintained.

[0064] SEQ ID NO:42 is *Sphingobiurn herbicidovorans* AAD1 protein sequence.

[0065] SEQ ID NO:43 is the native DNA sequence encoding *Aspergillus nidulans* delta-9 fatty acid desaturase protein.

[0066] SEQ ID NO:44 is *Aspergillus nidulans* delta-9 fatty acid desaturase protein sequence.

[0067] SEQ ID NO:45 is a synthetic DNA sequence encoding *Aspergillus nidulans* delta-9 fatty acid desaturase protein using codons optimized for maize and Table 1 & Table 2 sequences are maintained.

[0068] SEQ ID NO:46 is *Aspergillus nidulans* delta-9 fatty acid desaturase protein sequence.

[0069] SEQ ID NO:47 is a synthetic DNA sequence in accordance with the invention encoding *Aspergillus nidulans* delta-9 fatty acid desaturase protein using codons optimized for maize and with sequences identified in Table 2 removed and Table 1 sequences are maintained.

[0070] SEQ ID NO:48 is *Aspergillus nidulans* delta-9 fatty acid desaturase protein.

[0071] SEQ ID NO:49 is the native DNA sequence encoding *Xerophyta viscosa* SAP1 protein.

[0072] SEQ ID NO:50 is *Xerophyta viscosa* SAP1 protein sequence.

[0073] SEQ ID NO:51 is a synthetic DNA sequence encoding *Xerophyta viscosa* SAP1 protein using codons optimized for maize and Table 1 & Table 2 sequences are maintained

[0074] SEQ ID NO:52 is *Xerophyta viscosa* SAP1 protein sequence.

[0075] SEQ ID NO:53 is a synthetic DNA sequence in accordance with the invention encoding *Xerophyta viscosa* SAP1 protein using codons optimized for maize and with sequences identified in Table 2 removed and Table 1 sequences are maintained

[0076] SEQ ID NO:54 is *Xerophyta viscosa* SAP1 protein sequence.

[0077] SEQ ID NO:55 is the native DNA sequence encoding *Aequorea victoria* GFP1 protein.

[0078] SEQ ID NO:56 is *Aequorea victoria* GFP1 protein sequence.

[0079] SEQ ID NO:57 is a synthetic DNA sequence encoding *Aequorea victoria* GFP1 protein using codons optimized for maize and Table 1 & Table 2 sequences are maintained.

[0080] SEQ ID NO:58 is *Aequorea victoria* GFP1 protein sequence.

[0081] SEQ ID NO:59 is a synthetic DNA sequence in accordance with the invention encoding *Aequorea victoria* GFP1 protein using codons optimized for maize and with sequences identified in Table 2 removed and Table 1 sequences are maintained.

[0082] SEQ ID NO:60 is *Aequorea victoria* GFP1 protein sequence.

[0083] SEQ ID NO:61 is the native DNA sequence encoding *Leptosphaeria nodo*rum delta-9 fatty acid desaturase protein.

[0084] SEQ ID NO:62 is *Leptosphaeria nodo*rum delta-9 fatty acid desaturase protein sequence.

[0085] SEQ ID NO:63 is a synthetic DNA sequence encoding *Leptosphaeria nodorum* delta-9 fatty acid desaturase protein using codons optimized for maize and Table 1 & Table 2 sequences are maintained

[0086] SEQ ID NO:64 is *Leptosphaeria nodo*rum delta-9 fatty acid desaturase protein sequence.

[0087] SEQ ID NO:65 is a synthetic DNA sequence in accordance with the invention encoding *Leptosphaeria nodorum* delta-9 fatty acid desaturase protein using codons optimized for maize and with sequences identified in Table 2 removed and Table 1 sequences are maintained

[0088] SEQ ID NO:66 is *Leptosphaeria nodo*rum delta-9 fatty acid desaturase protein sequence.

[0089] SEQ ID NO:67 is the native DNA sequence encoding *Xerophyta viscosa* PER1 protein.

[0090] SEQ ID NO:68 is *Xerophyta viscosa* PER1 protein sequence.

[0091] SEQ ID NO:69 is a synthetic DNA sequence encoding *Xerophyta viscosa* PER1 protein using codons optimized for maize and Table 1 & Table 2 sequences are maintained.

[0092] SEQ ID NO:70 is *Xerophyta viscosa* PER1 protein sequence.

[0093] SEQ ID NO:71 is a synthetic DNA sequence in accordance with the invention encoding-*Xerophyta viscosa* PER1 protein using codons optimized for maize and with sequences identified in Table 2 removed and Table 1 sequences are maintained.

[0094] SEQ ID NO:72 is *Xerophyta viscosa* PER1 protein sequence.

DETAILED DESCRIPTION OF THE INVENTION

[0095] The invention provides synthetic nucleic acid sequences encoding proteins of interest. The synthetic coding sequences are particularly adapted for use in expressing the proteins of interest in transgenic plants.

[0096] A protein of interest is any protein or polypeptide that occurs in nature, or any naturally occurring variant including but not limited to processed forms of such proteins. The protein of interest also may be a protein formed by combining portions or fragments of more than one naturally occurring protein such as by mixing and matching functional protein domains.

[0097] A preferred group of proteins of interest is one in which the resulting phenotype is an agronomic trait or reporter protein useful for creating agronomic traits. These include but are not limited to resistance to insects, tolerance to herbicides, tolerance to water and/or heat stress, and oil profile modification.

[0098] A more preferred group of proteins of interest is one in which the resulting phenotype is an agronomic trait. Another preferred group is one in which the resulting phenotype provides herbicide tolerance. Another preferred group is one in which the resulting phenotype provides stress tolerance. Another preferred group is one in which the resulting phenotype provides a modified oil profile for healthier food. A more highly preferred group is one in which the protein of interest is a Cry protein that provides insect resistance.

[0099] The native / wild-type DNA sequences encoding the protein of interest must be identified and analyzed to determine whether polyadenylation signal sequences listed in Tables 1 and 2 and/or 3 are present. In accordance with the invention, for coding sequences intended for use in maize, the number of polyadenylation signal sequences listed in Table 2 is reduced compared to the number present in the native sequence. For coding sequences intended for use in soybean, the number of polyadenylation signal sequences listed in Table 3 is reduced. It is very important to remove the polyadenylation signal sequences listed in Tables 2 and 3, particularly where they occur in nested multimeric form.

[00100] In addition to removing polyadenylation signal sequences listed in Tables 2 and 3, it may be desirable to remove occurrences of the Shaw-Kamen sequence, ATTTA.

[00101] In addition to removing polyadenylation signal sequences and Shaw-Kamen sequences, we prefer to build synthetic DNA coding sequences that utilize codons roughly in the same frequency at which they are utilized, on average, in genes naturally occurring in the plant species in which the synthetic DNA sequence will be used. Table 4 gives suitable target percentages for codon usage in synthetic genes intended for use in various specific crops as well as for use in dicots generally or plants generally.

Table 4. Target rescaled codon compositions of synthetic plant genes.

Amino Acid	Codon	Maize %	Soybean %	Amino Acid	Codon	Maize %	Soybean %
ALA (A)	GCA	18.0	33.1	LEU (L)	CTA	0	0
	GCC	34.0	24.5		CTC	29.9	22.4
	GCG	24.0	0		CTG	33.3	16.3
	GCT	24.0	42.3		CTT	19.5	31.5
ARG (R)	AGA	18.8	36.0		TTA	0	0
	AGG	32.5	32.2		TTG	17.2	29.9
	CGA	0	0	LYS (K)	AAA	22.0	42.5
	CGC	30.0	15		AAG	78.0	57.5
	CGG	18.8	0	MET (M)	ATG	100	100
	CGT	0	16.9	PHE (F)	TTC	71.0	49.2
ASN (N)	AAC	68.0	50.0		TTT	29.0	50.8
	AAT	32.0	50.0	PRO (P)	CCA	26.0	39.8
ASP (D)	GAC	63.0	38.1		CCC	24.0	20.9
	GAT	37.0	61.9		CCG	28.0	0.0
CYS (C)	TGC	68.0	50.0		CCT	22.0	39.3
	TGT	32.0	50.0	SER (S)	AGC	25.3	16.0
END	TAA	0	0		AGT	0.0	18.2
	TAG	0	0		TCA	17.6	21.9
	TGA	100	100		TCC	25.3	18.0
GLN (Q)	CAA	38.0	55.5		TCG	15.4	0
	CAG	62.0	44.5		TCT	16.5	25.8
GLU (E)	GAA	29.0	50.5	THR (T)	ACA	21.0	32.4
	GAG	71.0	49.5		ACC	37.0	30.2
GLY (G)	GGA	19.0	31.9		ACG	22.0	0.0
	GGC	42.0	19.3		ACT	20.0	37.4
	GGG	19.0	18.4	TRP (W)	TGG	100	100
	GGT	20.0	30.4	TYR (Y)	TAC	73.0	48.2
HIS (H)	CAC	62.0	44.8		TAT	27.0	51.8

	CAT	38.0	55.2	VAL (V)	GTA	0	11.5
ILE (I)	ATA	14.0	23.4		GTC	34.8	17.8
	ATC	58.0	29.9		GTG	42.4	32.0
	ATT	28.0	46.7		GTT	22.8	38.7

TRANSGENIC PLANTS

[00102] A preferred embodiment of the subject invention is the transformation of plants with genes encoding insect toxins. The transformed plants that express insect toxin genes are resistant to attack by an insect target pest by virtue of the presence of controlling amounts of the subject insecticidal protein or its variants in the cells of the transformed plant. By incorporating genetic material that encodes the insecticidal properties of the *B.t.* insecticidal toxins into the genome of a plant eaten by a particular insect pest, the adult or larvae die after consuming the food plant. Numerous members of the monocotyledonous and dicotyledonous classifications have been transformed. Transgenic agronomic crops as well as fruits and vegetables are of commercial interest. Such crops include but are not limited to maize, rice, soybeans, canola, sunflower, alfalfa, sorghum, wheat, cotton, peanuts, tomatoes, potatoes, and the like. Several techniques exist for introducing foreign genetic material into plant cells, and for obtaining plants that stably maintain and express the introduced gene. Such techniques include acceleration of genetic material coated onto microparticles directly into cells (US Patent No. 4945050 and US Patent No. 5141131). Plants may be transformed using *Agrobacterium* technology, see US Patent No. 5177010, European Patent No. EP131624B1, European Patent No. EP159418B1, European Patent No. EP176112B1, US Patent No. 5149645, EP120516B1, US Patent No. 5464763, US Patent No. 4693976, European Patent No. EP116718B1, European Patent No. EP290799B1, European Patent No. EP320500B1, European Patent No. EP604662B1, US Patent No. 7060876, US Patent No. 6037526, US Patent No. 6376234, European Patent No. EP292435B1, US Patent No. 5231019, US Patent No. 5463174, US Patent No. 4762785, US Patent No. 5608142, and US Patent No. 5159135. Other transformation technology includes WHISKERS™ technology, see US Patent No. 5302523 and US Patent No. 5464765. Electroporation technology has also been used to transform plants, see WO1987006614, US Patent No. 5472869, US Patent No. 5384253, WO199209696, US Patent No. 6074877, WO1993021335, and US Patent No. 5679558. In addition to numerous technologies for transforming plants, the type of tissue which is contacted

with the foreign genes may vary as well. Such tissue would include but would not be limited to embryogenic tissue, callus tissue type I and type II, hypocotyl, meristem, and the like. Almost all plant tissues may be transformed during dedifferentiation using appropriate techniques within the skill of an artisan.

[00103] Known techniques of inserting DNA into plants include transformation with T-DNA delivered by *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* as the transformation agent. The use of T-DNA-containing vectors for the transformation of plant cells has been intensively researched and sufficiently described in European Patent No. EP120516B1; Lee and Gelvin (2008) **Plant Physiol.** 146:325-332; Fraley *et al.* (1986) **Crit. Rev. Plant Sci.** 4:1-46; and An *et al.* (1985) **EMBO J.** 4:277-284; and is well established in the field. Additionally, fusion of plant protoplasts with liposomes containing the DNA to be delivered, direct injection of the DNA, biolistics transformation (microparticle bombardment), or electroporation, as well as other possible methods, may be employed.

[00104] Once the inserted DNA has been integrated into the plant genome, it is relatively stable throughout subsequent generations. The vector used to transform the plant cell normally contains a selectable marker gene encoding a protein that confers on the transformed plant cells resistance to a herbicide or an antibiotic, such as Bialaphos, Kanamycin, G418, Bleomycin, or Hygromycin, *inter alia*. The individually employed selectable marker gene should accordingly permit the selection of transformed cells while the growth of cells that do not contain the inserted DNA is suppressed by the selective compound.

[00105] In a preferred embodiment of the subject invention, plants are transformed with genes wherein the codon usage of the protein coding region has been optimized for plants. See, for example, US Patent No. 5380831. Also, advantageously, plants encoding a truncated toxin, e.g. a functional protein domain, maybe used. The truncated toxin typically encodes about 55% to about 80% of the native full length toxin. Methods for creating synthetic *B.t.* genes for use in plants are known in the art (Stewart 2007, **Frontiers in Drug Design and Discovery** 1:297-341).

[00106] Regardless of transformation technique, the gene is preferably incorporated into a gene transfer vector adapted to express the protein of interest in the plant cell by including in the vector a plant promoter. In addition to plant promoters, promoters from a variety of sources can be used efficiently in plant cells to express foreign genes. For example, promoters of bacterial origin, such as the octopine synthase promoter, the nopaline synthase promoter, the mannopine

synthase promoter; promoters of viral origin, such as the 35S and 19S promoters of cauliflower mosaic virus (CaMV), and the like may be used. Plant-derived promoters include, but are not limited to ribulose-1,6-bisphosphate (RUBP) carboxylase small subunit (ssu), beta-conglycinin promoter, phaseolin promoter, ADH (alcohol dehydrogenase) promoter, heat-shock promoters, ADF (actin depolymerization factor) promoter, and tissue specific promoters. Promoters may also contain certain enhancer sequence elements that may improve the transcription efficiency. Typical enhancers include but are not limited to ADH1-intron 1 and ADH1-intron 6. Constitutive promoters may be used. Constitutive promoters direct continuous gene expression in nearly all cells types and at nearly all times (*e.g.* actin, ubiquitin, CaMV 35S). Tissue specific promoters are responsible for gene expression in specific cell or tissue types, such as the leaves or seeds (*e.g.* zein, oleosin, napin, ACP (Acyl Carrier Protein)), and these promoters may also be used. Promoters may also be used that are active during a certain stage of the plants' development as well as active in specific plant tissues and organs. Examples of such promoters include but are not limited to promoters that are root specific, pollen-specific, embryo specific, corn silk specific, cotton fiber specific, seed endosperm specific, phloem specific, and the like.

[00107] Under certain circumstances it may be desirable to use an inducible promoter. An inducible promoter is responsible for expression of genes in response to a specific signal, such as: physical stimulus (*e.g.* heat shock genes); light (*e.g.* RUBP carboxylase); hormone (*e.g.* glucocorticoid); antibiotic (*e.g.* tetracycline); metabolites; and stress (*e.g.* drought). Other desirable transcription and translation elements that function in plants may be used, such as 5' untranslated leader sequences, RNA transcription termination sequences and poly-adenylate addition signal sequences. Numerous plant-specific gene transfer vectors are known to the art.

[00108] Transgenic crops containing insect resistance (IR) traits are prevalent in corn and cotton plants throughout North America, and usage of these traits is expanding globally. Commercial transgenic crops combining IR and herbicide tolerance (HT) traits have been developed by numerous seed companies. These include combinations of IR traits conferred by *B.t.* insecticidal proteins and HT traits such as tolerance to Acetolactate Synthase (ALS) inhibitors such as sulfonylureas, imidazolinones, triazolopyrimidine, sulfonanilides, and the like, Glutamine Synthetase (GS) inhibitors such as Bialaphos, glufosinate, and the like, 4-HydroxyPhenylPyruvate Dioxygenase (HPPD) inhibitors such as mesotrione, isoxaflutole, and the like, 5-EnolPyruvylShikimate-3-Phosphate Synthase (EPSPS) inhibitors such as glyphosate

and the like, and Acetyl-Coenzyme A Carboxylase (ACCase) inhibitors such as haloxyfop, quizalofop, diclofop, and the like. Other examples are known in which transgenically provided proteins provide plant tolerance to herbicide chemical classes such as phenoxy acids herbicides and pyridyloxyacetates auxin herbicides (see WO2007053482), or phenoxy acids herbicides and aryloxyphenoxypropionates herbicides (see US Patent Application No. 20090093366). The ability to control multiple pest problems through IR traits is a valuable commercial product concept, and the convenience of this product concept is enhanced if insect control traits and weed control traits are combined in the same plant. Further, improved value may be obtained via single plant combinations of IR traits conferred by a *B.t.* insecticidal protein such as that of the subject invention with one or more additional HT traits such as those mentioned above, plus one or more additional input traits (*e.g.*other insect resistance conferred by *B.t.*-derived or other insecticidal proteins, insect resistance conferred by mechanisms such as RNAi and the like, nematode resistance, disease resistance, stress tolerance, improved nitrogen utilization, and the like), or output traits (*e.g* high oils content, healthy oil composition, nutritional improvement, and the like). Such combinations may be obtained either through conventional breeding (breeding stack) or jointly as a novel transformation event involving the simultaneous introduction of multiple genes (molecular stack or co-transformation). Benefits include the ability to manage insect pests and improved weed control in a crop plant that provides secondary benefits to the producer and/or the consumer. Thus, the subject invention can be used in connection with a variety of traits to provide a complete agronomic package of improved crop quality with the ability to flexibly and cost effectively control any number of agronomic issues.

[00109] All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety to the extent they are not inconsistent with the explicit teachings of this specification. Unless specifically indicated or implied, the terms "a", "an", and "the" signify "at least one" as used herein. By "isolated" applicants mean that the nucleotide or polypeptide molecules have been removed from their native environment and have been placed in a different environment by the hand of man.

[00110] Embodiments of the present invention are further defined in the following Examples. It should be understood that these Examples are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can

make various changes and modifications of the embodiments of the invention to adapt it to various usages and conditions. Thus, various modifications of the embodiments of the invention, in addition to those shown and described herein, will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

[00111] All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted. All temperatures are in degrees Celsius.

EXAMPLE 1

Synthetic Coding Region Encoding *Bacillus thuringiensis* Cry1Fa Core Toxin

[00112] Comparative Sequence. The native DNA sequence encoding the Cry1Fa core toxin is given in SEQ ID NO:1. This sequence was analyzed to determine which sequences identified in Table 1 are present in SEQ ID NO:1 and their locations. The amino acid sequence encoded by SEQ ID NO:1 was then reverse translated using the target codon frequencies given in the column of Table 4 for synthetic genes to be used in maize. The resulting DNA sequence was analyzed and codons were changed where necessary to remove unwanted open reading frames and remove unwanted restriction sites, and restore sequences identified in Table 1. The amino acid sequence encoded by SEQ ID NO:1 was preserved. The resulting DNA sequence is given in SEQ ID NO:3.

[00113] SEQ ID NO:3 was analyzed and codons were changed to remove potential polyadenylation signal sequences identified in Table 2, while maintaining the same number of sequences identified in Table 1. The resulting sequence, which embodies the present invention, is given in SEQ ID NO:5. Table 5 shows that the number and locations of polyadenylation signal sequences identified in Table 1 are maintained in SEQ ID NO:5, with the exception that the two occurrences of AATAAA, one at nt 426 and one at nt 582, in SEQ ID NO:1 were replaced with AATCAA, which maintains the number and location of polyadenylation signal sequences identified in Table 1, but substitutes a less problematic sequence for each of the two AATAAA sequences. Table 6 shows that the number of polyadenylation signal sequences identified in Table 2 are reduced in SEQ ID NO:5. Because there is overlap in the sequences identified in Tables 2 and 3 (sequences 1, 2, 6, 7, 8, 9, 10, 14, 13,and 20 in Table 2 correspond to sequences 16, 15, 2, 5, 1, 3, 4, 6, 13,and 12, respectively, in Table 3) it is also true that the

number of polyadenylation signal sequences identified in Table 3 are reduced in SEQ ID NO:5.

[00114] The synthetic coding region of SEQ ID NO:5 was optimized for expression in maize.

[00115] A construct for use in expressing the synthetic coding region of SEQ ID NO:5 is made by combining the synthetic coding region of SEQ ID NO:5 with a 5' non-translated region comprising a promoter that functions in plant cells and a 3' non-translated region comprising a transcription termination and polyadenylation sequence.

[00116] In one embodiment of such a construct, production of the primary mRNA transcript comprising SEQ ID NO:5 was driven by a copy of a maize ubiquitin1 promoter with its native intron1 (US Patent No. 5510474). A fragment comprising a 3' untranslated region from a maize peroxidase 5 gene (ZmPer5 3'UTR v2; US Patent No. 6699984) was used to terminate transcription. A binary plant transformation plasmid, pDAB111440, containing the aforementioned gene expression cassette, was constructed and utilized in the production of transgenic maize plants. Plasmid pDAB111440 further comprises a herbicide resistance gene comprising a coding region for aryloxyalknoate dioxygenase (AAD-1 v3; US Patent No. 7838733(B2), and Wright *et al.* (2010) **Proc. Natl. Acad. Sci. U.S.A.** 107:20240-5) under the transcriptional control of a sugarcane bacilliform badnavirus (ScBV) promoter (Schenk *et al.* (1999) **Plant Molec. Biol.** 39:1221-30). A fragment comprising a 3' untranslated region from a maize lipase gene (ZmLip 3'UTR; US Patent No. 7179902) was used to terminate transcription.

Table 5. Table 1 sequences found in the native Cry1Fa core toxin coding region (SEQ ID NO:1) and in the redesigned version (SEQ ID NO:5)

	Table 1 Sequence	No. Sites in Native Cry1Fa core sequence (SEQ ID NO:1)	nt Location in Native Cry1Fa core sequence (SEQ ID NO:1)	No. Sites in redesigned Cry1Fa core sequence (SEQ ID NO:5)	nt Location in redesigned Cry1Fa core sequence (SEQ ID NO:5)
1	AATAAA	2	426; 582	0	NA*
2	AATAAT	5	7; 46; 358; 430; 562	5	7; 46; 358; 430; 562
3	AACCAA	0	NA	0	NA
4	ATATAA	1	1520	1	1520
5	AATCAA	2	19; 628	4	19; 426; 582; 628
6	ATACTA	1	1508	1	1508
7	ATAAAA	0	NA	0	NA
8	ATGAAA	2	314; 1211	2	314; 1211
9	AAGCAT	0	NA	0	NA

10	ATTAAT	2	579; 1690	2	579; 1690
11	ATACAT	0	NA	0	NA
12	AAAATA	0	NA	0	NA
13	ATTAAA	2	66; 1266	2	66; 1266
14	AATTAA	2	368; 779	2	368; 779
15	AATACA	3	400; 1369; 1693	3	400; 1369; 1693
16	CATAAA	0	NA	0	NA
	Total	**22**		**22**	

*NA = Not Applicable

Table 6. Table 2 sequences found in the native Cry1Fa core toxin coding region (SEQ ID NO:1) and in the redesigned version (SEQ ID NO:5)

	Table 2 Sequence	No. Sites in Native Cry1Fa core sequence (SEQ ID NO:1)	nt Location in Native Cry1Fa core sequence (SEQ ID NO:1)	No. Sites in redesigned Cry1Fa core Sequence (SEQ ID NO:5)	nt Location in redesigned Cry1Fa core Sequence (SEQ ID NO:5)
1	ATATAT	1	104	0	NA*
2	TTGTTT	3	39; 612; 907	0	NA
3	TTTTGT	1	1089	0	NA
4	TGTTTT	2	1086; 1334	0	NA
5	TATATA	1	1771	0	NA
6	TATTTT	0	NA	0	NA
7	TTTTTT	0	NA	0	NA
8	ATTTTT	1	1615	0	NA
9	TTATTT	2	172; 217	0	NA
10	TTTATT	0	NA	0	NA
11	TAATAA	4	357; 416; 561; 581	0	NA
12	ATTTAT	3	319; 497; 793	0	NA
13	TATATT	1	322	0	NA
14	TTTTAT	3	192; 464; 1063	0	NA
15	ATATTT	0	NA	0	NA
16	TATTAT	0	NA	0	NA
17	TGTTTG	2	613; 908	0	NA
18	TTATAT	2	321; 1770	0	NA
19	TGTAAT	0	NA	0	NA
20	AAATAA	2	45; 429	0	NA
	Total	**28**		0	**NA**

*NA = Not Applicable

EXAMPLE 2

Synthetic Coding Region Encoding *Bacillus thuringiensis* Cry34A Toxin

[00117] Comparative Sequences. The native DNA sequence encoding the Cry34A toxin is given in SEQ ID NO:7. This sequence was analyzed to determine which sequences identified in Table 1 are present in SEQ ID NO:7 and their locations. The native DNA sequence was translated into the corresponding amino acid sequence using the standard genetic code. The amino acid sequence encoded by SEQ ID NO:7 was then reverse translated using the target codon frequencies given in the column of Table 7 for synthetic genes to be used in maize. The resulting DNA sequence was analyzed and codons were changed where necessary to remove unwanted open reading frames and remove unwanted restriction sites, and restore all sequences identified in Table 1. The amino acid sequence encoded by SEQ ID NO:7 was preserved. The resulting DNA sequence is given in SEQ ID NO:9. DNA having the sequence of SEQ ID NO:9 is synthesized.

[00118] SEQ ID NO:9 was analyzed and codons were changed to remove potential polyadenylation signal sequences identified in Table 2, while maintaining the same number of sequences identified in Table 1. The resulting sequence, which embodies the present invention, is given in SEQ ID NO:11. Table 7 shows that the number and locations of polyadenylation signals sequences identified in Table 1 are maintained in SEQ ID NO:11. Table 8 shows that the number of polyadenylation signal sequences identified in Tables 2 and 3 are reduced in SEQ ID NO:5.

[00119] DNA of SEQ ID NO:5 is synthesized, and expression levels observed in plant cells transformed to express this sequence are compared with expression levels observed in plant cells transformed to express SEQ ID NO:1 and SEQ ID NO:3.

[00120] The synthetic coding region of SEQ ID NO:5 was optimized for expression in maize.

[00121] A construct for use in expressing the synthetic coding region of SEQ ID NO:5 is made by combining the synthetic coding region of SEQ ID NO:5 with a 5' non-translated region comprising a promoter that functions in plant cells and a 3' non-translated region comprising a transcription termination and polyadenylation sequence.

Table 7. Table 1 sequences found in the native Cry34Ab1 coding region (SEQ ID NO:7) and the redesigned version (SEQ ID NO:11)

	Table 1 Sequence	No. Sites in native Cry34Ab1 sequence (SEQ ID NO:7)	nt Location in native Cry34Ab1 sequence (SEQ ID NO:7)	No. Sites in redesigned Cry34Ab1 sequence (SEQ ID NO:11)	nt Location in redesigned Cry34Ab1 sequence (SEQ ID NO:11)
1	AATAAA	2	247; 268	2	247; 268
2	AATAAT	1	31	1	31
3	AACCAA	0	NA*	0	NA
4	ATATAA	0	NA	0	NA
5	AATCAA	2	146; 310	2	146; 310
6	ATACTA	1	329	1	329
7	ATAAAA	1	65	1	65
8	ATGAAA	1	281	1	281
9	AAGCAT	0	NA	0	NA
10	ATTAAT	0	NA	0	NA
11	ATACAT	1	47	1	47
12	AAAATA	0	NA	0	NA
13	ATTAAA	1	127	1	127
14	AATTAA	1	126	1	126
15	AATACA	0	NA	0	NA
16	CATAAA	1	361	1	361
	Total	**12**		**12**	

*NA = Not Applicable

Table 8. Table 2 sequences found in the native Cry34Ab1 coding region (SEQ ID NO:7) and in the redesigned version (SEQ ID NO:11)

	Table 2 Sequence	No. Sites in native Cry34Ab1 sequence (SEQ ID NO:7)	nt Location in native Cry34Ab1 sequence (SEQ ID NO:7)	No. Sites in redesigned Cry34Ab1 sequence (SEQ ID NO:11)	nt Location in redesigned Cry34Ab1 sequence (SEQ ID NO:11)
1	ATATAT	1	181	0	NA*
2	TTGTTT	0	NA	0	NA
3	TTTTGT	0	NA	0	NA
4	TGTTTT	0	NA	0	NA
5	TATATA	1	180	0	NA
6	TATTTT	1	220	0	NA
7	TTTTTT	0	NA	0	NA
8	ATTTTT	0	NA	0	NA
9	TTATTT	0	NA	0	NA

10	TTTATT	0	NA	0	NA
11	TAATAA	2	33; 246	2	33; 246
12	ATTTAT	0	NA	0	NA
13	TATATT	2	182; 218	0	NA
14	TTTTAT	1	156	0	NA
15	ATATTT	1	219	0	NA
16	TATTAT	1	184	0	NA
17	TGTTTG	0	NA	0	NA
18	TTATAT	1	217	0	NA
19	TGTAAT	0	NA	0	NA
20	AAATAA	1	30	1	30
	Total	**12**		**3**	

*NA = Not Applicable

EXAMPLE 3

Synthetic Coding Region Encoding *Bacillus thuringiensis* Cry35Ab1 Toxin

[00122] Comparative Sequences. The native DNA sequence encoding the Cry35Ab1 toxin is given in SEQ ID NO:13. This sequence was analyzed to determine which sequences identified in Table 1 are present in SEQ ID NO:13 and their locations. The amino acid sequence encoded by SEQ ID NO:13 was then reverse translated using the target codon frequencies given in the column of Table 4 for synthetic genes to be used in maize. The resulting DNA sequence was analyzed and codons were changed where necessary to remove unwanted open reading frames and remove unwanted restriction enzyme recognition sites, while maintaining all sequences identified in Table 1. The amino acid sequence encoded by SEQ ID NO:13 was preserved. The resulting DNA sequence is given in SEQ ID NO:15. This sequence will be synthesized and used for comparison with a synthetic coding region designed in accordance with the invention.

[00123] SEQ ID NO:15 was analyzed and codons were changed to remove potential polyadenylation signal sequences identified in Table 2, while maintaining the same number of sequences identified in Table 1, except that two of the occurrences of AATAAA, one at nt 228 and one at nt 276 of SEQ ID NO:8 were changed to AATCAA. The resulting sequence, which embodies the present invention, is given in SEQ ID NO:17. Table 9 shows that the number and location of polyadenylation signal sequences identified in Table 1 are maintained in SEQ ID NO:17. Table 10 shows that the number of polyadenylation signal sequences identified in

Tables 2 and 3 are reduced in SEQ ID NO:17 compared to SEQ ID NO:13.

[00124] DNA of SEQ ID NO:17 is synthesized, and expression levels observed in plant cells transformed to express this sequence are compared with expression levels observed in plant cells transformed to express SEQ ID NO:13 and SEQ ID NO:15.

[00125] The synthetic coding region of SEQ ID NO:17 was optimized for expression in maize.

[00126] A construct for use in expressing the synthetic coding region of SEQ ID NO:17 is made by combining the synthetic coding region of SEQ ID NO:17 with a 5' non-translated region comprising a promoter that functions in plant cells and a 3' non-translated region comprising a transcription termination and polyadenylation sequence.

Table 9. Table 1 sequences found in the native Cry35Ab1 coding region (SEQ ID NO:13) and in the redesigned version (SEQ ID NO:17)

	Table 1 Sequence	No. Sites in native Cry 35Ab1 sequence (SEQ ID NO:13)	nt Location in native Cry35Ab1 sequence (SEQ ID NO:13)	No. Sites in redesigned Cry35Ab1 sequence (SEQ ID NO:17)	nt Location in redesigned Cry35Ab1 sequence (SEQ ID NO:17)
1	AATAAA	5	13; 100; 228; 276; 810	3	13; 100; 810
2	AATAAT	4	193; 217; 385; 864	4	193; 217; 385; 864
3	AACCAA	0	NA*	0	NA
4	ATATAA	1	966	1	966
5	AATCAA	3	394; 750; 914	5	228; 276; 394; 750; 914
6	ATACTA	1	8	1	8
7	ATAAAA	5	101; 224; 277; 575; 811	5	101; 224; 277; 575; 811
8	ATGAAA	5	23; 671; 769; 806; 854	5	23; 671; 769; 806; 854
9	AAGCAT	0	NA	0	NA
10	ATTAAT	1	522	1	522
11	ATACAT	1	734	1	734
12	AAAATA	7	226; 578; 618; 838; 862; 873; 1137	7	226; 578; 618; 838; 862; 873; 1137

13	ATTAAA	4	462; 589; 834; 1131	4	462; 589; 834; 1131
14	AATTAA	5	461; 521; 588; 833; 1130	5	461; 521; 588; 833; 1130
15	AATACA	3	261; 303; 733	3	261; 303; 733
16	CATAAA	0	NA	0	NA
	Total	**45**		**45**	

*NA = Not Applicable

Table 10. Table 2 sequences found in the native Cry35Ab1 coding region (SEQ ID NO:13) and in the redesigned version (SEQ ID NO:17)

	Table 2 Sequence	No. Sites in native Cry35Ab1 sequence (SEQ ID NO:13)	nt Location in native Cry35Ab1 sequence (SEQ ID NO:13)	No. Sites in redesigned Cry35Ab1 sequence (SEQ ID NO:17)	nt Location in redesigned Cry35Ab1 sequence (SEQ ID NO:17)
1	ATATAT	1	168	0	NA*
2	TTGTTT	0	NA	0	NA
3	TTTTGT	0	NA	0	NA
4	TGTTTT	0	NA	0	NA
5	TATATA	1	959	0	NA
6	TATTTT	2	609; 1144	0	NA
7	TTTTTT	0	NA	0	NA
8	ATTTTT	1	1145	0	NA
9	TTATTT	3	63; 145; 1143	1	1143
10	TTTATT	2	144; 1056	0	NA
11	TAATAA	2	12; 216	1	12
12	ATTTAT	0	NA	0	NA
13	TATATT	2	169; 607	0	NA
14	TTTTAT	1	143	0	NA
15	ATATTT	1	608	0	NA
16	TATTAT	4	171; 549; 604; 1141	1	1141
17	TGTTTG	0	NA	0	NA
18	TTATAT	2	606; 958	0	NA
19	TGTAAT	1	300	0	NA
20	AAATAA	8	26; 192; 227; 275; 384; 809; 863; 1097	2	809; 863
	Total	**31**		**5**	

*NA = Not Applicable

EXAMPLE 4

Synthetic Coding Region Encoding *Bacillus thuringiensis* Cry1Ab Core Toxin

[00127] Comparative Sequences. The native DNA sequence encoding Cry1Ab core toxin is given in SEQ ID NO:19. This sequence was analyzed to determine which sequences identified in Table 1 are present in SEQ ID NO:19 and their locations. The amino acid sequence encoded by SEQ ID NO:19 was then reverse translated using the target codon frequencies given in the column of Table 4 for synthetic genes to be used in maize. The resulting DNA sequence was analyzed and codons were changed where necessary to remove unwanted open reading frames and remove unwanted restriction enzyme recognition sites, while maintaining all sequences identified in Table 1. The amino acid sequence encoded by SEQ ID NO:19 was preserved. The resulting DNA sequence is given in SEQ ID NO:21.

[00128] SEQ ID NO:21 was analyzed and codons were changed to remove potential polyadenylation signal sequences identified in Table 2, while maintaining the same number of sequences identified in Table 1. The resulting sequence, which embodies the present invention, is given in SEQ ID NO:23. Table 11 shows that the number and location of polyadenylation signal sequences identified in Table 1 are maintained in SEQ ID NO:23. Table 12 shows that the number of polyadenylation signal sequences identified in Tables 2 and 3 are reduced in SEQ ID NO:23 compared to SEQ ID NO:19.

[00129] The synthetic coding region of SEQ ID NO:23 was optimized for expression in maize.

[00130] A construct for use in expressing the synthetic coding region of SEQ ID NO:23 was made by combining the synthetic coding region of SEQ ID NO:23 with a 5' non-translated region comprising a promoter that functions in plant cells and a 3' non-translated region comprising a transcription termination and polyadenylation sequence.

[00131] In one embodiment of such a construct, production of the primary mRNA transcript comprising SEQ ID NO:23 was driven by a copy of a maize ubiquitin1 promoter with its native intron1 (US Patent No. 5510474). A fragment comprising a 3' untranslated region from a maize peroxidase 5 gene (ZmPer5 3'UTR v2; US Patent No. 6699984) was used to terminate transcription. A binary plant transformation plasmid, pDAB111449, containing the aforementioned gene expression cassette, was constructed and utilized in the production of transgenic maize plants. Plasmid pDAB111449 further comprises a herbicide resistance gene

comprising a coding region for aryloxyalknoate dioxygenase (AAD-1 v3; US Patent No. 7838733(B2), and Wright *et al.* (2010) **Proc. Natl. Acad. Sci. U.S.A.** 107:20240-5) under the transcriptional control of a sugarcane bacilliform badnavirus (ScBV) promoter (Schenk *et al.* (1999) **Plant Molec. Biol.** 39:1221-30). A fragment comprising a 3' untranslated region from a maize lipase gene (ZmLip 3'UTR; US Patent No. 7179902) was used to terminate transcription.

Table 11. Table 1 sequences found in the native Cry1Ab core toxin coding region (SEQ ID NO:19) and in the redesigned version (SEQ ID NO:23)

	Table 1 Sequence	**No. Sites in Native Cry1Ab core sequence (SEQ ID NO:19)**	**nt Location in Native Cry1Ab core sequence (SEQ ID NO:19)**	**No. Sites in redesigned Cry1Ab core sequence (SEQ ID NO:23)**	**nt Location in redesigned Cry1Ab core sequence (SEQ ID NO:23)**
1	AATAAA	0	NA*	0	NA
2	AATAAT	3	960, 1126, 1387	3	960, 1126, 1387
3	AACCAA	2	253, 280	2	253, 280
4	ATATAA	2	185, 1391	2	185, 1391
5	AATCAA	2	688, 1129	3	688, 1129, 1639
6	ATACTA	0	NA	0	NA
7	ATAAAA	0	NA	0	NA
8	ATGAAA	1	1232	1	1232
9	AAGCAT	0	NA	0	NA
10	ATTAAT	1	1636	1	1636
11	ATACAT	2	1366, 1613	2	1366, 1613
12	AAAATA	0	NA	0	NA
13	ATTAAA	3	249, 704, 785	3	249, 704, 785
13	AATTAA	0	NA	0	NA
15	AATACA	0	NA	0	NA
16	CATAAA	0	NA	0	NA
	Total	**16**	**NA**	**17**	**NA**

*NA = Not Applicable

Table 12. Table 2 sequences found in the native Cry1Ab coding region (SEQ ID NO:19) and in the redesigned version (SEQ ID NO:23)

	Table 2 Sequence	No. Sites in Native Cry1Ab core sequence (SEQ ID NO:19)	nt Location in Native Cry1Ab core sequence (SEQ ID NO:19)	No. Sites in redesigned Cry1Ab core sequence (SEQ ID NO:23)	nt Location in redesigned Cry1Ab core sequence (SEQ ID NO:23)
1	ATATAT	0	NA*	0	NA
2	TTGTTT	1	42	0	NA
3	TTTTGT	0	NA	0	NA
4	TGTTTT	0	NA	0	NA
5	TATATA	2	1097, 1792	0	NA
6	TATTTT	0	NA	0	NA
7	TTTTTT	0	NA	0	NA
8	ATTTTT	2	199, 1649	0	NA
9	TTATTT	0	NA	0	NA
10	TTTATT	1	470	0	NA
11	TAATAA	2	1340, 1386	0	NA
12	ATTTAT	2	503, 799	0	NA
13	TATATT	0	NA	0	NA
14	TTTTAT	0	NA	0	NA
15	ATATTT	1	110	0	NA
16	TATTAT	2	937, 940	0	NA
17	TGTTTG	1	530	0	NA
18	TTATAT	2	1096, 1791	0	NA
19	TGTAAT	0	NA	0	NA
20	AAATAA	2	959, 1125	1	959
	Total	**18**		**1**	

*NA = Not Applicable

EXAMPLE 5

Synthetic Coding Region Encoding *Bacillus thuringiensis* Cry1Ca Core Toxin

[00132] Comparative Sequences. The native DNA sequence encoding the Cry35A core toxin is given in SEQ ID NO:25. This sequence was analyzed to determine which sequences identified in Table 1 are present in SEQ ID NO:25 and their locations. The amino acid sequence encoded by SEQ ID NO:25 was then reverse translated using the target codon frequencies given in the column of Table 4 for synthetic genes to be used in maize. The resulting DNA sequence was analyzed and codons were changed where necessary to remove unwanted open reading frames,

and remove unwanted restriction enzyme recognition sites, while maintaining all sequences identified in Table 1. The amino acid sequence encoded by SEQ ID NO:25 was preserved. The resulting DNA sequence is given in SEQ ID NO:27. This sequence will be synthesized and used for comparison with a synthetic gene designed in accordance with the invention.

[00133] SEQ ID NO:27 was analyzed and codons were changed to remove potential polyadenylation signal sequences identified in Table 2, while maintaining the same number of sequences identified in Table 1. The resulting sequence, which embodies the present invention, is given in SEQ ID NO:29. Table 13 shows that the number and location of polyadenylation signal sequences identified in Table 1 are maintained in SEQ ID NO:29. Table 14 shows that the number of polyadenylation signal sequences identified in Tables 2 and 3 are reduced in SEQ ID NO:29 compared to SEQ ID NO:25.

[00134] DNA of SEQ ID NO:29 is synthesized, and expression levels observed in plant cells transformed to express this sequence are compared with expression levels observed in plant cells transformed to express SEQ ID NO:25 and SEQ ID NO:27.

[00135] The synthetic gene of SEQ ID NO:29 was optimized for expression in maize.

[00136] A construct for use in expressing the synthetic gene of SEQ ID NO:29 is made by combining the synthetic gene of SEQ ID NO:29 with a 5' non-translated region comprising a promoter that functions in plant cells and a 3' non-translated region comprising a transcription terminator and polyadenylation sequence.

Table 13. Table 1 sequences found in the native Cry1Ca core toxin coding region (SEQ ID NO:25) and in the redesigned version (SEQ ID NO:29)

	Table 1 Sequence	No. Sites in Native Cry1Ca core sequence (SEQ ID NO:25)	nt Location in Native Cry1Ca core sequence (SEQ ID NO:25)	No. Sites in redesigned Cry1Ca core sequence (SEQ ID NO:29)	nt Location in redesigned Cry1Ca core sequence (SEQ ID NO:29)
1	AATAAA	0	NA*	0	NA
2	AATAAT	2	646, 916	2	646, 916
3	AACCAA	0	NA	1	1042
4	ATATAA	2	684, 1757	2	684, 1757
5	AATCAA	1	1405	1	1405
6	ATACTA	0	NA	0	NA
7	ATAAAA	1	1826	1	1826
8	ATGAAA	2	254, 569	2	254, 569
9	AAGCAT	1	335	1	335

10	ATTAAT	7	177, 246, 250, 813, 817, 1402, 1534	7	177, 246, 250, 813, 817, 1402, 1534
11	ATACAT	0	NA	0	NA
12	AAAATA	0	NA	0	NA
13	ATTAAA	4	245, 249, 816, 1401	4	245, 249, 816, 1401
13	AATTAA	1	642	1	642
15	AATACA	1	1381	1	1381
16	CATAAA	0	NA	0	NA
	Total	**22**		**23**	

*NA = Not Applicable

Table 14. Table 2 sequences found in the native Cry1Ca core toxin coding region (SEQ ID NO:25) and in the redesigned version (SEQ ID NO:29)

	Table 2 Sequence	**No. Sites in Native Cry1Ca core sequence (SEQ ID NO:25)**	**nt Location in Native Cry1Ca core sequence (SEQ ID NO:25)**	**No. Sites in redesigned Cry1Ca core sequence (SEQ ID NO:29)**	**nt Location in redesigned Cry1Ca core sequence (SEQ ID NO:29)**
1	ATATAT	4	323, 325, 908, 1024	0	NA*
2	TTGTTT		NA	0	NA
3	TTTTGT	3	186, 1302, 1512	0	NA
4	TGTTTT	0	NA	0	NA
5	TATATA	3	324, 1023, 1819	0	NA
6	TATTTT	1	1346	0	NA
7	TTTTTT	1	1326	0	NA
8	ATTTTT	2	529, 959	0	NA
9	TTATTT	1	901	0	NA
10	TTTATT	2	900, 962	0	NA
11	TAATAA	0	NA	0	NA
12	ATTTAT	1	899	0	NA
13	TATATT	2	510, 909	0	NA
14	TTTTAT	2	470, 961	0	NA
15	ATATTT	1	110	0	NA
16	TATTAT	0	NA	0	NA
17	TGTTTG	0	NA	0	NA
18	TTATAT	1	1818	0	NA
19	TGTAAT	1	525	0	NA
20	AAATAA	1	645	1	645
	Total	**26**		**1**	

*NA = Not Applicable

EXAMPLE 6

Synthetic Coding Region Encoding *Bacillus thuringiensis* Cry6Aa Toxin

[00137] Comparative Sequences. The native DNA sequence encoding the Cry6Aa toxin is given in SEQ ID NO:31. This sequence was analyzed to determine which sequences identified in Table 1 are present in SEQ ID NO:31 and their locations. The amino acid sequence encoded by SEQ ID NO:31 was then reverse translated using the target codon frequencies given in the column of Table 4 for synthetic genes to be used in maize. The resulting DNA sequence was analyzed and codons were changed where necessary to remove unwanted open reading frames, and remove unwanted restriction enzyme recognition sites, while maintaining all sequences identified in Table 1. The amino acid sequence encoded by SEQ ID NO:31 was preserved. The resulting DNA sequence is given in SEQ ID NO:33. This sequence will be synthesized and used for comparison with a synthetic gene designed in accordance with the invention.

[00138] SEQ ID NO:33 was analyzed and codons were changed to remove potential polyadenylation signal sequences identified in Table 2, while maintaining the number of sequences identified in Table 1. The resulting sequence, which embodies the present invention, is given in SEQ ID NO:35. Table 15 shows that the number and location of polyadenylation signal sequences identified in Table 1 are maintained in SEQ ID NO:35. Table 16 shows that the number of polyadenylation signal sequences identified in Tables 2 and 3 are reduced in SEQ ID NO:35 compared to SEQ ID NO:31.

[00139] DNA of SEQ ID NO:35 is synthesized, and expression levels observed in plant cells transformed to express this sequence are compared with expression levels observed in plant cells transformed to express SEQ ID NO:31 and SEQ ID NO:33.

[00140] The synthetic coding region of SEQ ID NO:35 was optimized for expression in maize.

[00141] A construct for use in expressing the synthetic coding region of SEQ ID NO:35 is made by combining the synthetic coding region of SEQ ID NO:35 with a 5' non-translated region comprising a promoter that functions in plant cells and a 3' non-translated region comprising a transcription terminator and polyadenylation sequence.

Table 15. Table 1 sequences found in the native Cry6Aa coding region (SEQ ID NO:31) and in the redesigned version (SEQ ID NO:35)

	Table 1 Sequence	No. Sites in Native Cry6Aa sequence (SEQ ID NO:31)	nt Location in Native Cry6Aa sequence (SEQ ID NO:31)	No. Sites in redesigned Cry6Aa sequence (SEQ ID NO:35)	nt Location in redesigned Cry6Aa sequence (SEQ ID NO:35)
1	AATAAA	1	292	1	292
2	AATAAT	6	430, 1309, 1360, 1384, 1402, 1420	6	430, 1309, 1360, 1384, 1402, 1420
3	AACCAA	0	NA*	0	NA
4	ATATAA	2	824, 1344	2	824, 1344
5	AATCAA	5	103, 634, 832, 1234, 1270	5	103, 634, 832, 1234, 1270
6	ATACTA	0	NA	0	NA
7	ATAAAA	3	269, 293, 826	3	269, 293, 826
8	ATGAAA	1	794	1	794
9	AAGCAT	0	NA	0	NA
10	ATTAAT	2	919, 1183	2	919, 1183
11	ATACAT	0	NA	1	1275
12	AAAATA	3	530, 806, 1358	3	530, 806, 1358
13	ATTAAA	5	51, 56, 188, 495, 963	5	51, 56, 188, 495, 963
13	AATTAA	7	52, 57, 316, 463, 496, 718, 964	7	52, 57, 316, 463, 496, 718, 964
15	AATACA	2	922, 1238	3	922, 1238, 1274
16	CATAAA	1	664	1	664
	Total	**38**		**40**	

*NA = Not Applicable

Table 16. Table 2 sequences found in the native Cry6Aa coding region (SEQ ID NO:31) and in the redesigned version (SEQ ID NO:35)

	Table 2 Sequence	No. Sites in Native Cry6Aa sequence (SEQ ID NO:31)	nt Location in Native Cry6Aa sequence (SEQ ID NO:31)	No. Sites in redesigned Cry6Aa sequence (SEQ ID NO:35)	nt Location in redesigned Cry6Aa sequence (SEQ ID NO:35)
1	ATATAT	4	147, 218, 1275, 1372	0	NA*
2	TTGTTT	1	788	0	NA
3	TTTTGT		NA	0	NA
4	TGTTTT	0	NA	0	NA
5	TATATA	1	941	0	NA
6	TATTTT	2	388, 489	0	NA
7	TTTTTT		NA	0	NA
8	ATTTTT	2	236, 555	0	NA

9	TTATTT	1	113	0	NA
10	TTTATT	1	109, 257	0	NA
11	TAATAA	5	66, 429, 1383, 1401, 1419	0	NA
12	ATTTAT	3	108, 299, 938	0	NA
13	TATATT	2	148, 1373	0	NA
14	TTTTAT	2	1314, 1365	0	NA
15	ATATTT	1	387	0	NA
16	TATTAT	1	111	0	NA
17	TGTTTG	0	NA	0	NA
18	TTATAT	4	247, 301, 940, 1190	0	NA
19	TGTAAT	1	1204	0	NA
20	AAATAA	2	1308, 1359	1	1359
	Total	**33**		**1**	

*NA = Not Applicable

EXAMPLE 7

Synthetic Coding Region Encoding *Sphingobiurn herbicidovorans* AAD1

[00142] Comparative Sequences. The native DNA sequence encoding the AAD1 protein is given in SEQ ID NO:37. This sequence was analyzed to determine which sequences identified in Table 1 are present in SEQ ID NO:37 and their locations. The amino acid sequence encoded by SEQ ID NO:37 was then reverse translated using the target codon frequencies given in the column of Table 4 for synthetic genes to be used in maize. The resulting DNA sequence was analyzed and codons were changed where necessary to remove unwanted open reading frames, and remove unwanted restriction enzyme recognition sites, while maintaining all sequences identified in Table 1. The amino acid sequence encoded by SEQ ID NO:37 was preserved. The resulting DNA sequence is given in SEQ ID NO:39. This sequence will be synthesized and used for comparison with a synthetic gene designed in accordance with the invention.

[00143] SEQ ID NO:39 was analyzed and codons were changed to remove potential polyadenylation signal sequences identified in Table 2, while maintaining the number of sequences identified in Table 1. The resulting sequence, which embodies the present invention, is given in SEQ ID NO:41. Table 17 shows that the number and location of polyadenylation signal sequences identified in Table 1 are maintained in SEQ ID NO:41. Table 18 shows that the number of polyadenylation signal sequences identified in Tables 2 and 3 are reduced in SEQ ID NO:41 compared to SEQ ID NO:37.

[00144] DNA of SEQ ID NO:41 is synthesized, and expression levels observed in plant cells transformed to express this sequence are compared with expression levels observed in plant cells transformed to express SEQ ID NO:37 and SEQ ID NO:39.

[00145] The synthetic coding region of SEQ ID NO:41 was optimized for expression in maize.

[00146] A construct for use in expressing the synthetic coding region of SEQ ID NO:41 is made by combining the synthetic coding region of SEQ ID NO:41 with a 5' non-translated region comprising a promoter that functions in plant cells and a 3' non-translated region comprising a transcription terminator and polyadenylation sequence.

Table 17. Table 1 sequences found in the native AAD1 coding region (SEQ ID NO:37) and in the redesigned version (SEQ ID NO:41)

	Table 1 Sequence	No. Sites in Native AAD1 sequence (SEQ ID NO:37)	Nt Location in Native AAD1 sequence (SEQ ID NO:37)	No. Sites in redesigned AAD1 sequence (SEQ ID NO:41)	nt Location in redesigned AAD1 sequence (SEQ ID NO:41)
1	AATAAA	0	NA*	0	NA
2	AATAAT	0	NA	0	NA
3	AACCAA	0	NA	1	652
4	ATATAA	0	NA	0	NA
5	AATCAA	0	NA	0	NA
6	ATACTA	0	NA	0	NA
7	ATAAAA	0	NA	0	NA
8	ATGAAA	0	NA	0	NA
9	AAGCAT	0	NA	0	NA
10	ATTAAT	0	NA	0	NA
11	ATACAT	0	NA	0	NA
12	AAAATA	0	NA	0	NA
13	ATTAAA	0	NA	0	NA
14	AATTAA	0	NA	0	NA
15	AATACA	0	NA	0	NA
16	CATAAA	0	NA	0	NA
	Total	**0**		**1**	

*NA = Not Applicable

Table 18. Table 2 sequences found in the native AAD1 coding region (SEQ ID NO:37) and in the redesigned version (SEQ ID NO:41)

	Table 2 Sequence	No. Sites in Native AAD1 sequence (SEQ ID NO:37)	nt Location in Native AAD1 sequence (SEQ ID NO:37)	No. Sites in redesigned AAD1 sequence (SEQ ID NO:41)	nt Location in redesigned AAD1 sequence (SEQ ID NO:41)
1	ATATAT	0	NA*	0	NA
2	TTGTTT	0	NA	0	NA
3	TTTTGT	0	NA	0	NA
4	TGTTTT	0	NA	0	NA
5	TATATA	0	NA	0	NA
6	TATTTT	1	166	0	NA
7	TTTTTT	0	NA	0	NA
8	ATTTTT	0	NA	0	NA
9	TTATTT	0	NA	0	NA
10	TTTATT	0	NA	0	NA
11	TAATAA	0	NA	0	NA
12	ATTTAT	0	NA	0	NA
13	TATATT	0	NA	0	NA
14	TTTTAT	0	NA	0	NA
15	ATATTT	0	NA	0	NA
16	TATTAT	0	NA	0	NA
17	TGTTTG	0	NA	0	NA
18	TTATAT	0	NA	0	NA
19	TGTAAT	0	NA	0	NA
20	AAATAA	0	NA	0	NA
	Total	**1**		0	

*NA = Not Applicable

EXAMPLE 8

Synthetic Coding Region Encoding *Aspergillus nidulans* Delta-9 Desaturase

[00147] Comparative Sequences. The native DNA sequence encoding the *Aspergillus nidulans* Delta-9 Desaturase protein is given in SEQ ID NO:43. This sequence was analyzed to determine which sequences identified in Table 1 are present in SEQ ID NO:43 and their locations. The amino acid sequence encoded by SEQ ID NO:43 was then reverse translated using the target codon frequencies given in the column of Table 4 for synthetic genes to be used in maize. The resulting DNA sequence was analyzed and codons were changed where necessary to remove unwanted open reading frames and remove unwanted restriction enzyme recognition sites, while maintaining all sequences identified in Table 1. The amino acid sequence encoded by SEQ ID NO:43 was preserved. The resulting DNA sequence is given in SEQ ID NO:45.

This sequence will be synthesized and used for comparison with a synthetic gene designed in accordance with the invention.

[00148] SEQ ID NO:45 was analyzed and codons were changed to remove potential polyadenylation signal sequences identified in Table 2, while maintaining the number of sequences identified in Table 1. The resulting sequence, which embodies the present invention, is given in SEQ ID NO:47. Table 1 shows that the number and location of polyadenylation signal sequences identified in Table 1 are maintained in SEQ ID NO:47. Table 20 shows that the number of polyadenylation signal sequences identified in Tables 2 and 3 are reduced in SEQ ID NO:47 compared to SEQ ID NO:43.

[00149] DNA of SEQ ID NO:47 is synthesized, and expression levels observed in plant cells transformed to express this sequence are compared with expression levels observed in plant cells transformed to express SEQ ID NO:43 and SEQ ID NO:45.

[00150] The synthetic coding region of SEQ ID NO:47 was optimized for expression in maize.

[00151] A construct for use in expressing the synthetic coding region of SEQ ID NO:47 is made by combining the synthetic coding region of SEQ ID NO:47 with a 5' non-translated region comprising a promoter that functions in plant cells and a 3' non-translated region comprising a transcription termination and polyadenylation sequence.

Table 19. Table 1 sequences found in the native *Aspergillus nidulans* Delta-9 Desaturase coding region (SEQ ID NO:43) and in the redesigned version (SEQ ID NO:47)

	Table 1 Sequence	No. Sites in Native Asp- Δ9 sequence (SEQ ID NO:43)	nt Location in Native Asp- Δ9 sequence (SEQ ID NO:43)	No. Sites in redesigned Asp- Δ9 sequence (SEQ ID NO:47)	nt Location in redesigned Asp- Δ9 Sequence (SEQ ID NO:47)
1	AATAAA	0	NA*	0	NA
2	AATAAT	0	NA	0	NA
3	AACCAA	1	1326	1	1326
4	ATATAA	0	NA	0	NA
5	AATCAA	0	NA	0	NA
6	ATACTA	0	NA	0	NA
7	ATAAAA	0	NA	0	NA
8	ATGAAA	0	NA	0	NA
9	AAGCAT	1	94	1	94
10	ATTAAT	0	NA	0	NA
11	ATACAT	0	NA	0	NA
12	AAAATA	0	NA	0	NA
13	ATTAAA	0	NA	0	NA
14	AATTAA	0	NA	0	NA
15	AATACA	0	NA	0	NA
16	CATAAA	0	NA	0	NA
	Total	**2**		**2**	

*NA = Not Applicable

Table 20. Table 2 sequences found in the native *Aspergillus nidulans* Delta-9 Desaturase coding region (SEQ ID NO:43) and in the redesigned version (SEQ ID NO:47)

	Table 2 Sequence	No. Sites in Native Asp- Δ9 sequence (SEQ ID NO:43)	nt Location in Native Asp- Δ9 Sequence (SEQ ID NO:43)	No. Sites in redesigned Asp- Δ9 Sequence (SEQ ID NO:47)	nt Location in redesigned Asp- Δ9 Sequence (SEQ ID NO:47)
1	ATATAT	0	NA*	0	NA
2	TTGTTT	0	NA	0	NA
3	TTTTGT	0	NA	0	NA
4	TGTTTT	0	NA	0	NA
5	TATATA	0	NA	0	NA
6	TATTTT	1	166	0	NA
7	TTTTTT	0	NA	0	NA
8	ATTTTT	0	NA	0	NA
9	TTATTT	0	NA	0	NA
10	TTTATT	0	NA	0	NA

11	TAATAA	0	NA	0	NA
12	ATTTAT	0	NA	0	NA
13	TATATT	0	NA	0	NA
14	TTTTAT	0	NA	0	NA
15	ATATTT	1	479	0	NA
16	TATTAT	0	NA	0	NA
17	TGTTTG	0	NA	0	NA
18	TTATAT	0	NA	0	NA
19	TGTAAT	0	NA	0	NA
20	AAATAA	0	NA	0	NA
	Total	**1**		0	

*NA = Not Applicable

EXAMPLE 9

Synthetic Coding Region Encoding *Xerophyta viscosa* SAP1

[00152] Comparative Sequences. The native DNA sequence encoding the *Xerophyta viscosa* SAP1 protein is given in SEQ ID NO:49. This sequence was analyzed to determine which sequences identified in Table 1 are present in SEQ ID NO:49 and their locations. The amino acid sequence encoded by SEQ ID NO:49 was then reverse translated using the target codon frequencies given in the column of Table 4 for synthetic genes to be used in maize. The resulting DNA sequence was analyzed and codons were changed where necessary to remove unwanted open reading frames and remove unwanted restriction enzyme recognition sites, while maintaining all sequences identified in Table 1. The amino acid sequence encoded by SEQ ID NO:49 was preserved. The resulting DNA sequence is given in SEQ ID NO:51. This sequence will be synthesized and used for comparison with a synthetic gene designed in accordance with the invention.

[00153] SEQ ID NO:52 was analyzed and codons were changed to remove potential polyadenylation signal sequences identified in Table 2, while maintaining the number of sequences identified in Table 1. The resulting sequence, which embodies the present invention, is given in SEQ ID NO:53. Table 1 shows that the number and location of polyadenylation signal sequences identified in Table 1 are maintained in SEQ ID NO:53. Table 21 shows that the number of polyadenylation signal sequences identified in Tables 2 and 3 are reduced in SEQ ID NO:53 compared to SEQ ID NO:49.

[00154] DNA of SEQ ID NO:53 is synthesized, and expression levels observed in plant cells

transformed to express this sequence are compared with expression levels observed in plant cells transformed to express SEQ ID NO:49 and SEQ ID NO:51.

[00155] The synthetic coding region of SEQ ID NO:53 was optimized for expression in maize.

[00156] A construct for use in expressing the synthetic coding region of SEQ ID NO:53 is made by combining the synthetic coding region of SEQ ID NO:53 with a 5' non-translated region comprising a promoter that functions in plant cells and a 3' non-translated region comprising a transcription terminator and polyadenylation sequence.

Table 21. Table 1 sequences found in the native *Xerophyta viscosa* SAP1 coding region (SEQ ID NO:49) and in the redesigned version (SEQ ID NO:53)

	Table 1 Sequence	No. Sites in Native XvSAP1 sequence (SEQ ID NO:49)	nt Location in Native XvSAP1 sequence (SEQ ID NO:49)	No. Sites in redesigned XvSAP1 sequence (SEQ ID NO:53)	nt Location in redesigned XvSAP1 sequence (SEQ ID NO:53)
1	AATAAA	0	NA*	0	NA
2	AATAAT	0	NA	0	NA
3	AACCAA	0	NA	0	NA
4	ATATAA	0	NA	0	NA
5	AATCAA	0	NA	0	NA
6	ATACTA	0	NA	0	NA
7	ATAAAA	0	NA	0	NA
8	ATGAAA	0	NA	1	25
9	AAGCAT	0	NA	0	NA
10	ATTAAT	0	NA	0	NA
11	ATACAT	0	NA	0	NA
12	AAAATA	0	NA	0	NA
13	ATTAAA	0	NA	0	NA
14	AATTAA	0	NA	0	NA
15	AATACA	0	NA	0	NA
16	CATAAA	0	NA	0	NA
	Total	**0**		**1**	

*NA = Not Applicable

Table 22. Table 2 sequences found in native the Native *Xerophyta viscosa* SAP1 coding region (SEQ ID NO:49) and in the redesigned version (SEQ ID NO:53)

	Table 2 Sequence	No. Sites in Native XvSAP1 sequence (SEQ ID NO:49)	nt Location in Native XvSAP1 sequence (SEQ ID NO:49)	No. Sites in redesigned XvSAP1 sequence (SEQ ID NO:53)	nt Location in redesigned XvSAP1 sequence (SEQ ID NO:53)
1	ATATAT	0	NA*	0	NA
2	TTGTTT	0	NA	0	NA
3	TTTTGT	0	NA	0	NA
4	TGTTTT	0	NA	0	NA
5	TATATA	0	NA	0	NA
6	TATTTT	1	755	0	NA
7	TTTTTT	0	NA	0	NA
8	ATTTTT	1	756	0	NA
9	TTATTT	0	NA	0	NA
10	TTTATT	0	NA	0	NA
11	TAATAA	0	NA	0	NA
12	ATTTAT	0	NA	0	NA
13	TATATT	0	NA	0	NA
14	TTTTAT	0	NA	0	NA
15	ATATTT	1	754	0	NA
16	TATTAT	1	665	0	NA
17	TGTTTG	1	696	0	NA
18	TTATAT	0	NA	0	NA
19	TGTAAT	0	NA	0	NA
20	AAATAA	0	NA	0	NA
	Total	**5**		0	

*NA = Not Applicable

EXAMPLE 10

Synthetic Coding Region Encoding *Aequorea victoria* GFP1

[00157] Comparative Sequences. The native DNA sequence encoding the *Aequorea victoria* GFP1 is given in SEQ ID NO:55. This sequence was analyzed to determine which sequences identified in Table 1 are present in SEQ ID NO:55 and their locations. The amino acid sequence encoded by SEQ ID NO:55 was then reverse translated using the target codon frequencies given in the column of Table 4 for synthetic genes to be used in maize. The resulting DNA sequence was analyzed and codons were changed where necessary to remove unwanted open reading frames and remove unwanted restriction enzyme recognition sites, while maintaining all

sequences identified in Table 1. The amino acid sequence encoded by SEQ ID NO:55 was preserved. The resulting DNA sequence is given in SEQ ID NO:57. This sequence will be synthesized and used for comparison with a synthetic gene designed in accordance with the invention.

[00158] SEQ ID NO:57 was analyzed and codons were changed to remove potential polyadenylation signal sequences identified in Table 2, while maintaining the number of sequences identified in Table 1. The resulting sequence, which embodies the present invention, is given in SEQ ID NO:59. Table 1 shows that the number and location of polyadenylation signal sequences identified in Table 1 are maintained in SEQ ID NO:59. Table 23 shows that the number of polyadenylation signal sequences identified in Tables 2 and 3 are reduced in SEQ ID NO:59 compared to SEQ ID NO:55.

[00159] DNA of SEQ ID NO:59 is synthesized, and expression levels observed in plant cells transformed to express this sequence are compared with expression levels observed in plant cells transformed to express SEQ ID NO:55 and SEQ ID NO:57.

[00160] The synthetic coding region of SEQ ID NO:59 was optimized for expression in maize.

[00161] A construct for use in expressing the synthetic coding region of SEQ ID NO:59 is made by combining the synthetic coding region of SEQ ID NO:59 with a 5' non-translated region comprising a promoter that functions in plant cells and a 3' non-translated region comprising a transcription terminator and polyadenylation sequence.

Table 23. Table 1 sequences found in the native *Aequorea victoria* GFP1 coding region (SEQ ID NO:55) and in the redesigned version (SEQ ID NO:59)

	Table 1 Sequence	No. Sites in Native GFP1 sequence (SEQ ID NO:55)	nt Location in Native GFP1 sequence (SEQ ID NO:55)	No. Sites in redesigned GFP1 sequence (SEQ ID NO:59)	nt Location in redesigned GFP1 sequence (SEQ ID NO:59)
1	AATAAA	0	NA*	0	NA
2	AATAAT	0	NA	0	NA
3	AACCAA	1	467	1	467
4	ATATAA	0	NA	0	NA
5	AATCAA	0	NA	0	NA
6	ATACTA	0	NA	0	NA
7	ATAAAA	0	NA	0	NA
8	ATGAAA	1	237	1	237

9	AAGCAT	0	NA	0	NA
10	ATTAAT	0	NA	0	NA
11	ATACAT	1	450	1	450
12	AAAATA	1	551	1	551
13	ATTAAA	1	511	1	511
14	AATTAA	0	NA	0	NA
15	AATACA	1	425	1	425
16	CATAAA	0	NA	1	480
	Total	**6**		**7**	

*NA = Not Applicable

Table 24. Table 2 sequences found in the native the *Aequorea victoria* GFP1 coding region (SEQ ID NO:55) and in the redesigned version (SEQ ID NO:59)

	Table 2 Sequence	**No. Sites in Native GFP1 sequence (SEQ ID NO:55)**	**nt Location in Native GFP1 sequence (SEQ ID NO:55)**	**No. Sites in redesigned GFP1 sequence (SEQ ID NO:59)**	**nt Location in redesigned GFP1 sequence (SEQ ID NO:59)**
1	ATATAT	0	NA*	0	NA
2	TTGTTT	0	NA	0	NA
3	TTTTGT	0	NA	0	NA
4	TGTTTT	0	NA	0	NA
5	TATATA	0	NA	0	NA
6	TATTTT	1	293	0	NA
7	TTTTTT	0	NA	0	NA
8	ATTTTT	0	NA	0	NA
9	TTATTT	1	137	0	NA
10	TTTATT	1	136	0	NA
11	TAATAA	0	NA	0	NA
12	ATTTAT	0	NA	0	NA
13	TATATT	1	291	0	NA
14	TTTTAT	1	135	0	NA
15	ATATTT	1	292	0	NA
16	TATTAT	0	NA	0	NA
17	TGTTTG	0	NA	0	NA
18	TTATAT	0	NA	0	NA
19	TGTAAT	0	NA	0	NA
20	AAATAA	0	NA	0	NA
	Total	**6**		0	

*NA = Not Applicable

EXAMPLE 11

Synthetic Coding Region Encoding *Leptosphaeria nodorum* FAD9

[00162] Comparative Sequences. The native DNA sequence encoding the *Leptosphaeria nodorum* FAD9 protein is given in SEQ ID NO:61. This sequence was analyzed to determine which sequences identified in Table 1 are present in SEQ ID NO:61 and their locations. The amino acid sequence encoded by SEQ ID NO:61 was then reverse translated using the target codon frequencies given in the column of Table 4 for synthetic genes to be used in maize. The resulting DNA sequence was analyzed and codons were changed where necessary to remove unwanted open reading frames and remove unwanted restriction enzyme recognition sites, while maintaining all sequences identified in Table 1. The amino acid sequence encoded by SEQ ID NO:61 was preserved. The resulting DNA sequence is given in SEQ ID NO:63. This sequence will be synthesized and used for comparison with a synthetic gene designed in accordance with the invention.

[00163] SEQ ID NO:63 was analyzed and codons were changed to remove potential polyadenylation signal sequences identified in Table 2, while maintaining the number of sequences identified in Table 1. The resulting sequence, which embodies the present invention, is given in SEQ ID NO:65. Table 1 shows that the number and location of polyadenylation signal sequences identified in Table 1 are maintained in SEQ ID NO:65. Table 25 shows that the number of polyadenylation signal sequences identified in Tables 2 and 3 are reduced in SEQ ID NO:65 compared to SEQ ID NO:61.

[00164] DNA of SEQ ID NO:65 is synthesized, and expression levels observed in plant cells transformed to express this sequence are compared with expression levels observed in plant cells transformed to express SEQ ID NO:61 and SEQ ID NO:63.

[00165] The synthetic coding region of SEQ ID NO:65 was optimized for expression in maize.

[00166] A construct for use in expressing the synthetic coding region of SEQ ID NO:65 is made by combining the synthetic coding region of SEQ ID NO:65 with a 5' non-translated region comprising a promoter that functions in plant cells and a 3' non-translated region comprising a transcription terminator and polyadenylation sequence.

Table 25. Table 1 sequences found in the native *Leptosphaeria nodorum* FAD9 coding region (SEQ ID NO:61) and in the redesigned version (SEQ ID NO:65)

	Table 1 Sequence	No. Sites in Native Ln FAD9 sequence (SEQ ID NO:61)	nt Location in Native Ln FAD9 sequence (SEQ ID NO:61)	No. Sites in redesigned Ln FAD9 sequence (SEQ ID NO:65)	nt Location in redesigned Ln FAD9 sequence (SEQ ID NO:65)
1	AATAAA	0	NA*	0	NA
2	AATAAT	0	NA	0	NA
3	AACCAA	0	NA	0	NA
4	ATATAA	0	NA	0	NA
5	AATCAA	0	NA	0	NA
6	ATACTA	0	NA	0	NA
7	ATAAAA	0	NA	0	NA
8	ATGAAA	0	NA	0	NA
9	AAGCAT	0	NA	0	NA
10	ATTAAT	0	NA	0	NA
11	ATACAT	0	NA	0	NA
12	AAAATA	0	NA	0	NA
13	ATTAAA	0	NA	0	NA
14	AATTAA	0	NA	0	NA
15	AATACA	0	NA	0	NA
16	CATAAA	0	NA	0	NA
	Total	**0**		**0**	

*NA = Not Applicable

Table 26. Table 2 sequences found in the native *Leptosphaeria nodorum* FAD9 coding region (SEQ ID NO:61) and redesigned version (SEQ ID NO:65)

	Table 2 Sequence	No. Sites in Native Ln FAD9 sequence (SEQ ID NO:61)	nt Location in Native Ln FAD9 sequence (SEQ ID NO:61)	No. Sites in redesigned Ln FAD9 sequence (SEQ ID NO:65)	nt Location in redesigned Ln FAD9 sequence (SEQ ID NO:65)
1	ATATAT	0	NA*	0	NA
2	TTGTTT	0	NA	0	NA
3	TTTTGT	0	NA	0	NA
4	TGTTTT	1	1275	0	NA
5	TATATA	0	NA	0	NA
6	TATTTT	0	NA	0	NA
7	TTTTTT	0	NA	0	NA
8	ATTTTT	0	NA	0	NA
9	TTATTT	0	NA	0	NA
10	TTTATT	1	1090	0	NA
11	TAATAA	0	NA	0	NA

12	ATTTAT	0	NA	0	NA
13	TATATT	0	NA	0	NA
14	TTTTAT	0	NA	0	NA
15	ATATTT	0	NA	0	NA
16	TATTAT	1	416	0	NA
17	TGTTTG	0	NA	0	NA
18	TTATAT	0	NA	0	NA
19	TGTAAT	0	NA	0	NA
20	AAATAA	0	NA	0	NA
	Total	**3**		0	

*NA = Not Applicable

EXAMPLE 12
Synthetic Coding Region Encoding *Xerophyta viscosa PER1*

[00167] Comparative Sequences. The native DNA sequence encoding the *Xerophyta viscosa PER1* protein is given in SEQ ID NO:67. This sequence was analyzed to determine which sequences identified in Table 1 are present in SEQ ID NO:67 and their locations. The amino acid sequence encoded by SEQ ID NO:67 was then reverse translated using the target codon frequencies given in the column of Table 4 for synthetic genes to be used in maize. The resulting DNA sequence was analyzed and codons were changed where necessary to remove unwanted open reading frames and remove unwanted restriction enzyme recognition sites, while maintaining all sequences identified in Table 1. The amino acid sequence encoded by SEQ ID NO:67 was preserved. The resulting DNA sequence is given in SEQ ID NO:69. This sequence will be synthesized and used for comparison with a synthetic gene designed in accordance with the invention.

[00168] SEQ ID NO:69 was analyzed and codons were changed to remove potential polyadenylation signal sequences identified in Table 2, while maintaining the number of sequences identified in Table 1. The resulting sequence, which embodies the present invention, is given in SEQ ID NO:71. Table 1 shows that the number and location of polyadenylation signal sequences identified in Table 1 are maintained in SEQ ID NO:71. Table 27 shows that the number of polyadenylation signal sequences identified in Tables 2 and 3 are reduced in SEQ ID NO:71 compared to SEQ ID NO:67.

[00169] DNA of SEQ ID NO:71 is synthesized, and expression levels observed in plant cells transformed to express this sequence are compared with expression levels observed in plant cells

transformed to express SEQ ID NO:67 and SEQ ID NO:69.

[00170] The synthetic coding region of SEQ ID NO:71 was optimized for expression in maize.

[00171] A construct for use in expressing the synthetic coding region of SEQ ID NO:71 is made by combining the synthetic coding region of SEQ ID NO:71 with a 5' non-translated region comprising a promoter that functions in plant cells and a 3' non-translated region comprising a transcription terminator and polyadenylation sequence.

Table 27. Table 1 sequences found in the native *Xerophyta viscosa* PER1 coding region (SEQ ID NO:67) and in the redesigned version (SEQ ID NO:71)

	Table 1 Sequence	No. Sites in Native XvPER1 sequence (SEQ ID NO:67)	nt Location in Native XvPER1 sequence (SEQ ID NO:67)	No. Sites in redesigned XvPER1 sequence (SEQ ID NO:71)	nt Location in redesigned XvPER1 sequence (SEQ ID NO:71)
1	AATAAA	0	NA*	0	NA
2	AATAAT	0	NA	0	NA
3	AACCAA	0	NA	0	NA
4	ATATAA	0	NA	0	NA
5	AATCAA	0	NA	0	NA
6	ATACTA	0	NA	0	NA
7	ATAAAA	1	605	1	605
8	ATGAAA	0	NA	0	NA
9	AAGCAT	0	NA	0	NA
10	ATTAAT	0	NA	0	NA
11	ATACAT	0	NA	0	NA
12	AAAATA	1	282	1	282
13	ATTAAA	0	NA	0	NA
14	AATTAA	0	NA	0	NA
15	AATACA	0	NA	0	NA
16	CATAAA	0	NA	0	NA
	Total	**2**		**2**	

*NA = Not Applicable

Table 28. Table 2 sequences found in the native the *Xerophyta viscosa* PER1 coding region (SEQ ID NO:67 and in the redesigned version (SEQ ID NO:71)

	Table 2 Sequence	No. Sites in Native XvPER1 sequence (SEQ ID NO:67)	nt Location in Native XvPER1 sequence (SEQ ID NO:67)	No. Sites in redesigned XvPER1 sequence (SEQ ID NO:71)	nt Location in redesigned XvPER1 sequence (SEQ ID NO:71)
1	ATATAT	0	NA*	0	NA
2	TTGTTT	0	NA	0	NA
3	TTTTGT	0	NA	0	NA
4	TGTTTT	0	NA	0	NA
5	TATATA	0	NA	0	NA
6	TATTTT	0	NA	0	NA
7	TTTTTT	0	NA	0	NA
8	ATTTTT	0	NA	0	NA
9	TTATTT	0	NA	0	NA
10	TTTATT	0	NA	0	NA
11	TAATAA	0	NA	0	NA
12	ATTTAT	0	NA	0	NA
13	TATATT	0	NA	0	NA
14	TTTTAT	0	NA	0	NA
15	ATATTT	0	NA	0	NA
16	TATTAT	0	NA	0	NA
17	TGTTTG	0	NA	0	NA
18	TTATAT	0	NA	0	NA
19	TGTAAT	0	NA	0	NA
20	AAATAA	0	NA	0	NA
	Total	**0**		0	

*NA = Not Applicable

EXAMPLE 13
WHISKERS®Transformation of Maize with Xv SAP1

[00172] A standard WHISKERS transformation vector was constructed in which the *Arabidopsis thaliana* promoter, Rd29A, was placed 5' to the XvSAP1 redesigned coding region sequence of the invention (SEQ ID NO:53). These sequences were flanked by *Zea maize* PER5, 3' and 5' untranslated regions to stabilize expression of the redesigned coding region. A *pat* selection cassette (See, for example, US 5648477) driven by the rice actin1 promoter was placed 3' to the XvSAP1 expression cassette.

[00173] Vector DNA was digested with appropriate restriction enzymes to release a fragment

containing the bacterial ampicillin resistance gene present in the vector backbone, and to produce a linear DNA fragment suitable for WHISKERS™-mediated transformation. Purification of the linear fragment containing the XvSAP1 and *pat* expression cassettes was accomplished on a preparative scale by high pressure liquid chromatography (HPLC). This plant transformation DNA was delivered into maize Hi-II suspension cell cultures via WHISKERS™-mediated transformation (essentially as described in US Patent Nos. 5302523 and 5464765; US Patent Publication No. 2008/0182332; and Petolino and Arnold (2009) (Methods Molec. Biol. 526:59-67).

[00174] Transformants were placed in selective medium after which transformed isolates were obtained over the course of approximately 8 weeks. The selection medium was an LS based medium (LS Basal medium, N6 vitamins, 1.5 mg/L 2,4-D, 0.5 gm/L MES (2-(N-morpholino)ethanesulfonic acid monohydrate; *Phyto*Technologies Labr.), 30.0 gm/L sucrose, 6 mM L-proline, 1.0 mg/L $AgNO_3$, 250 mg/L cefotaxime, 2.5 gm/L Gellan gum, pH 5.7) containing Bialaphos (Gold BioTechnology). The embryos were transferred to selection media containing 3 mg/L Bialaphos until embryogenic isolates were obtained. Recovered isolates were bulked up by transferring to fresh selection medium at 2-week intervals for regeneration and further analysis.

[00175] For regeneration, the cultures were transferred to "28" induction medium (MS salts and vitamins, 30 gm/L sucrose, 5 mg/L Benzylaminopurine, 0.25 mg/L 2, 4-D, 3 mg/L Bialaphos, 250 mg/L cefotaxime, 2.5 gm/L Gellan gum, pH 5.7) for 1 week under low-light conditions (14 $\mu Em^{-2}s^{-1}$) then 1 week under high-light conditions (approximately 89 $\mu Em^{-2}s^{-1}$). Tissues were subsequently transferred to "36" regeneration medium (same as induction medium except lacking plant growth regulators). When plantlets reached 3-5 cm in length, they were transferred to glass culture tubes containing SHGA medium (Schenk and Hildebrandt salts and vitamins (1972); *Phyto*Technologies Labr.), 1.0 gm/L *myo*-inositol, 10 gm/L sucrose and 2.0 gm/L Gellan gum, pH 5.8) to allow for further growth and development of the shoot and roots. Plants were transplanted to the same soil mixture as described earlier herein and grown to flowering in the greenhouse. Controlled pollinations for seed production were conducted.

EXAMPLE 14
Agrobacterium transformation

[00176] Standard cloning methods are used in the construction of binary plant transformation and expression plasmids. Restriction endonucleases and T4 DNA Ligase are obtained from NEB. Plasmid preparations are performed using the NucleoSpin® Plasmid Preparation kit or the NucleoBond® AX Xtra Midi kit (both from Macherey-Nagel), following the instructions of the manufacturers. DNA fragments are purified using the QIAquick® PCR Purification Kit or the QIAEX II® Gel Extraction Kit (both from Qiagen) after gel isolation.

[00177] Synthetic genes in accordance with the invention may be synthesized by a commercial vendor (*e.g.* DNA2.0, Menlo Park, CA) and supplied as cloned fragments in standard plasmid vectors, or may be obtained by standard molecular biology manipulation of other constructs containing appropriate nucleotide sequences.

[00178] In a non-limiting example, a basic cloning strategy may be to subclone full length coding sequences (CDS) into a plant expression plasmid at *NcoI* and *SacI* restriction sites. The resulting plant expression cassettes containing the appropriate coding region under the control of plant expression elements, (*e.g.*, plant expressible promoters, 3' terminal transcription termination and polyadenylate addition determinants, and the like) are subcloned into a binary vector plasmid, utilizing, for example, Gateway® technology or standard restriction enzyme fragment cloning procedures. LR Clonase™ (Invitrogen) for example, may be used to recombine the full length and modified gene plant expression cassettes into a binary plant transformation plasmid if the Gateway® technology is utilized. It is convenient to employ a binary plant transformation vector that harbors a bacterial gene that confers resistance to the antibiotic spectinomycin when the plasmid is present in *E. coli* and *Agrobacterium* cells. It is also convenient to employ a binary vector plasmid that contains a plant-expressible selectable marker gene that is functional in the desired host plants. Examples of plant-expressible selectable marker genes include but are not limited those that encode the aminoglycoside phosphotransferase gene (*aphII*) of transposon Tn5, which confers resistance to the antibiotics kanamycin, neomycin and G418, as well as those genes which code for resistance or tolerance to glyphosate; hygromycin; methotrexate; phosphinothricin (bialaphos), imidazolinones, sulfonylureas and triazolopyrimidine herbicides, such as chlorosulfuron, bromoxynil, dalapon and the like.

[00179] Electro-competent cells of *Agrobacterium tumefaciens* strain Z707S (a streptomycin-resistant derivative of Z707; Hepburn *et al.*, 1985, **J. Gen. Microbiol.** 131:2961-2969.) are prepared and transformed using electroporation (Weigel and Glazebrook, 2002, **Arabidopsis: A Laboratory Manual**). After electroporation, 1 mL of YEP broth (gm/L: yeast extract, 10; peptone, 10; NaCl, 5) are added to the cuvette and the cell-YEP suspension is transferred to a 15 mL culture tube for incubation at 28° in a water bath with constant agitation for 4 hours. The cells are plated on YEP plus agar (25 gm/L) with spectinomycin (200 μg/mL) and streptomycin (250 μg/mL) and the plates are incubated for 2-4 days at 28°. Well separated single colonies are selected and streaked onto fresh YEP + agar plates with spectinomycin and streptomycin as before, and incubated at 28° for 1-3 days.

[00180] The presence of the synthetic gene insert in the binary plant transformation vector is performed by PCR analysis using vector-specific primers with template plasmid DNA prepared from selected *Agrobacterium* colonies. The cell pellet from a 4 mL aliquot of a 15 mL overnight culture grown in YEP with spectinomycin and streptomycin as before is extracted using Qiagen Spin® Mini Preps, performed per manufacturer's instructions. Plasmid DNA from the binary vector used in the *Agrobacterium* electroporation transformation is included as a control. The PCR reaction is completed using Taq DNA polymerase from Invitrogen per manufacture's instructions at 0.5X concentrations. PCR reactions are carried out in a MJ Research Peltier Thermal Cycler programmed with the following conditions: Step 1) 94° for 3 minutes; Step 2) 94° for 45 seconds; Step 3) 55° for 30 seconds; Step 4) 72° for 1 minute per kb of expected product length; Step 5) 29 times to Step 2; Step 6) 72° for 10 minutes. The reaction is maintained at 4° after cycling. The amplification products are analyzed by agarose gel electrophoresis (*e.g.* 0.7 % to 1% agarose, w/v) and visualized by ethidium bromide staining. A colony is selected whose PCR product is identical to the plasmid control.

[00181] Alternatively, the plasmid structure of the binary plant transformation vector containing the synthetic gene insert is performed by restriction digest fingerprint mapping of plasmid DNA prepared from candidate *Agrobacterium* isolates by standard molecular biology methods well known to those skilled in the art of *Agrobacterium* manipulation.

[00182] Those skilled in the art of obtaining transformed plants *via Agrobacterium*-mediated transformation methods will understand that other *Agrobacterium* strains besides Z707S may be used to advantage, and the choice of strain may depend upon the identity of the host plant species

to be transformed.

EXAMPLE 15
Production of insecticidal proteins in dicot plants

[00183] *Arabidopsis* Transformation. *Arabidopsis thaliana* Col-01 is transformed using the floral dip method (Weigel and Glazebrook, *supra*). The selected *Agrobacterium* colony is used to inoculate 1 mL to 15 mL cultures of YEP broth containing appropriate antibiotics for selection. The culture is incubated overnight at 28° with constant agitation at 220 rpm. Each culture is used to inoculate two 500 mL cultures of YEP broth containing appropriate antibiotics for selection and the new cultures are incubated overnight at 28° with constant agitation. The cells are pelleted at approximately 8700 x g for 10 minutes at room temperature, and the resulting supernatant is discarded. The cell pellet is gently resuspended in 500 mL of infiltration media containing: 1/2x Murashige and Skoog salts (Sigma-Aldrich)/Gamborg's B5 vitamins (Gold BioTechnology, St. Louis, MO), 10% (w/v) sucrose, 0.044 μM benzylaminopurine (10 μL/liter of 1 mg/mL stock in DMSO) and 300 μL/liter Silwet L-77. Plants approximately 1 month old are dipped into the media for 15 seconds, with care taken to assure submergence of the newest inflorescence. The plants are then laid on their sides and covered (transparent or opaque) for 24 hours, washed with water, and placed upright. The plants are grown at 22°, with a 16-hour light/8-hour dark photoperiod. Approximately 4 weeks after dipping, the seeds are harvested.

[00184] *Arabidopsis* Growth and Selection. Freshly harvested T1 seed is allowed to dry for at least 7 days at room temperature in the presence of desiccant. Seed is suspended in a 0.1% agar/water (Sigma-Aldrich) solution and then stratified at 4° for 2 days. To prepare for planting, Sunshine Mix LP5 (Sun Gro Horticulture Inc., Bellevue, WA) in 10.5 inch x 21 inch germination trays (T.O. Plastics Inc., Clearwater, MN) is covered with fine vermiculite, sub-irrigated with Hoagland's solution (Hoagland and Arnon, 1950) until wet, then allowed to drain for 24 hours. Stratified seed is sown onto the vermiculite and covered with humidity domes (KORD Products, Bramalea, Ontario, Canada) for 7 days. Seeds are germinated and plants are grown in a Conviron (Models CMP4030 or CMP3244; Controlled Environments Limited, Winnipeg, Manitoba, Canada) under long day conditions (16 hours light/8 hours dark) at a light intensity of 120-150 μmol/m^2sec under constant temperature (22°) and humidity (40-50%). Plants are

initially watered with Hoagland's solution and subsequently with deionized water to keep the soil moist but not wet.

[00185] The domes are removed 5-6 days post sowing and plants are sprayed with a chemical selection agent to kill plants germinated from nontransformed seeds. For example, if the plant expressible selectable marker gene provided by the binary plant transformation vector is a *pat* or *bar* gene (Wehrmann *et al.*, 1996, **Nat. Biotech**. 14:1274-1278), transformed plants may be selected by spraying with a 1000X solution of Finale (5.78% glufosinate ammonium, Farnam Companies Inc., Phoenix, AZ.). Two subsequent sprays are performed at 5-7 day intervals. Survivors (plants actively growing) are identified 7-10 days after the final spraying and transplanted into pots prepared with Sunshine Mix LP5. Transplanted plants are covered with a humidity dome for 3-4 days and placed in a Conviron under the above-mentioned growth conditions.

[00186] Those skilled in the art of dicot plant transformation will understand that other methods of selection of transformed plants are available when other plant expressible selectable marker genes (*e.g.* herbicide tolerance genes) are used.

[00187] Insect Bioassays of transgenic *Arabidopsis.* Transgenic *Arabidopsis* lines expressing Cry proteins are demonstrated to be active against sensitive insect species in artificial diet overlay assays. Protein extracted from transgenic and non-transgenic *Arabidopsis* lines is quantified by appropriate methods and sample volumes are adjusted to normalize protein concentration. Bioassays are conducted on artificial diet as described above. Non-transgenic *Arabidopsis* and/or buffer and water are included in assays as background check treatments.

EXAMPLE 16

Agrobacterium transformation for generation of superbinary vectors

[00188] The *Agrobacterium* superbinary system is conveniently used for transformation of monocot plant hosts. Methodologies for constructing and validating superbinary vectors are well disclosed and incorporated herein by reference (Operating Manual for Plasmid pSB1, Version 3.1, available from Japan Tobacco, Inc., Tokyo, Japan). Standard molecular biological and microbiological methods are used to generate superbinary plasmids. Verification/validation of the structure of the superbinary plasmid is done using methodologies as described above for binary vectors, and may be modified as suggested in the Operating Manual for Plasmid pSB1.

EXAMPLE 17

Production of insecticidal proteins in monocot plants

[00189] *Agrobacterium*-Mediated Transformation of Maize. Seeds from a High II F_1 cross (Armstrong *et al.,* 1991, **Maize Genet. Coop. Newsletter** 65:92-93) are planted into 5-gallon-pots containing a mixture of 95% Metro-Mix 360 soilless growing medium (Sun Gro Horticulture, Bellevue, WA) and 5% clay/loam soil. The plants are grown in a greenhouse using a combination of high pressure sodium and metal halide lamps with a 16:8 hour Light:Dark photoperiod. For obtaining immature F_2 embryos for transformation, controlled sib-pollinations are performed. Immature embryos are isolated at 8-10 days post-pollination when embryos are approximately 1.0 to 2.0 mm in size.

[00190] Infection and co-cultivation. Maize ears are surface sterilized by scrubbing with liquid soap, immersing in 70% ethanol for 2 minutes, and then immersing in 20% commercial bleach (0.1% sodium hypochlorite) for 30 minutes before being rinsed with sterile water. A suspension of *Agrobacterium* cells containing a superbinary vector is prepared by transferring 1-2 loops of bacteria grown on YEP solid medium containing 100 mg/L spectinomycin, 10 mg/L tetracycline, and 250 mg/L streptomycin at 28° for 2-3 days into 5 mL of liquid infection medium (LS Basal Medium (Linsmaier and Skoog, 1965, **Physiol. Plant.** 18:100-127), N6 vitamins (Chu *et al.,* 1975, Scientia Sinica 18:659-668), 1.5 mg/L 2,4-Dichlorophenoxyacetic acid (2,4-D), 68.5 gm/L sucrose, 36.0 gm/L glucose, 6 mM L-proline, pH 5.2) containing 100 μM acetosyringone. The solution was vortexed until a uniform suspension was achieved, and the concentration is adjusted to a final density of about 200 Klett units, using a Klett-Summerson colorimeter with a purple filter, or an optical density of approximately 0.4 at 550 nm. Immature embryos are isolated directly into a micro centrifuge tube containing 2 mL of the infection medium. The medium is removed and replaced with 1 mL of the *Agrobacterium* solution with a density of 200 Klett units, and the *Agrobacterium* and embryo solution is incubated for 5 minutes at room temperature and then transferred to co-cultivation medium (LS Basal Medium, N6 vitamins, 1.5 mg/L 2,4-D, 30.0 gm/L sucrose, 6 mM L-proline, 0.85 mg/L $AgNO_3$,, 100 μM acetosyringone, 3.0 gm/L Gellan gum (*Phyto*Technology Laboratories., Lenexa, KS), pH 5.8) for 5 days at 25° under dark conditions.

[00191] After co-cultivation, the embryos are transferred to selective medium after which

transformed isolates are obtained over the course of approximately 8 weeks. For selection of maize tissues transformed with a superbinary plasmid containing a plant expressible *pat* or *bar* selectable marker gene, an LS based medium (LS Basal medium, N6 vitamins, 1.5 mg/L 2,4-D, 0.5 gm/L MES (2-(N-morpholino)ethanesulfonic acid monohydrate; *Phyto*Technologies Labr.), 30.0 gm/L sucrose, 6 mM L-proline, 1.0 mg/L $AgNO_3$, 250 mg/L cefotaxime, 2.5 gm/L Gellan gum, pH 5.7) is used with Bialaphos (Gold BioTechnology). The embryos are transferred to selection media containing 3 mg/L Bialaphos until embryogenic isolates were obtained. Recovered isolates are bulked up by transferring to fresh selection medium at 2-week intervals for regeneration and further analysis.

[00192] Those skilled in the art of maize transformation will understand that other methods of selection of transformed plants are available when other plant expressible selectable marker genes (*e.g.* herbicide tolerance genes) are used.

[00193] Regeneration and seed production. For regeneration, the cultures are transferred to "28" induction medium (MS salts and vitamins, 30 gm/L sucrose, 5 mg/L Benzylaminopurine, 0.25 mg/L 2, 4-D, 3 mg/L Bialaphos, 250 mg/L cefotaxime, 2.5 gm/L Gellan gum, pH 5.7) for 1 week under low-light conditions (14 $\mu Em^{-2}s^{-1}$) then 1 week under high-light conditions (approximately 89 $\mu Em^{-2}s^{-1}$). Tissues are subsequently transferred to "36" regeneration medium (same as induction medium except lacking plant growth regulators). When plantlets grow to 3-5 cm in length, they were transferred to glass culture tubes containing SHGA medium (Schenk and Hildebrandt salts and vitamins (1972); *Phyto*Technologies Labr.), 1.0 gm/L *myo*-inositol, 10 gm/L sucrose and 2.0 gm/L Gellan gum, pH 5.8) to allow for further growth and development of the shoot and roots. Plants are transplanted to the same soil mixture as described earlier herein and grown to flowering in the greenhouse. Controlled pollinations for seed production are conducted.

[00194] Alternatively, binary vectors may be used to produce transgenic maize plants that contain one or more chimeric genes stably-integrated into the plant genome and comprising a coding region disclosed herein. For example, plants comprising at least one coding region of SEQ ID NOs:5, 11, 17, 23, 29, 35, 41, 47, 53, 59, 65, or 71 are produced following *Agrobacterium*-mediated transformation. Maize transformation methods employing binary transformation vectors are known in the art. In one embodiment, transformed tissues are selected by their ability to grow on haloxyfop-containing medium and are screened for protein

production, as appropriate.

[00195] Ear sterilization and embryo isolation. Maize immature embryos were obtained from plants of *Zea mays* inbred line B104 grown in the greenhouse and self- or sib-pollinated to produce ears. The ears were harvested approximately 9 to 12 days post-pollination. On the experimental day, de-husked ears were surface-sterilized by immersion in a 20% solution of sodium hypochlorite (6.15%) and shaken for 20 to 30 min, followed by three rinses in sterile water. After sterilization, immature zygotic embryos (1.5 to 2.4 mm) were aseptically dissected from each ear and randomly distributed into microcentrifuge tubes containing liquid Inoculation Medium. Inoculation Medium contains: 2.2 gm/L MS salts (Frame *et al.,* 2011, ***Genetic Transformation Using Maize Immature Zygotic Embryos.*** **IN Plant Embryo Culture Methods and Protocols: Methods in Molecular Biology. T. A. Thorpe and E. C. Yeung, (Eds), SPRINGER SCIENCE AND BUSINESS MEDIA, LLC.** pp 327-341); 1X ISU Modified MS Vitamins (Frame *et al.*, 2011 *supra*); 68.4 gm/L sucrose; 36 gm/L glucose; 115 mg/L L-proline; 100 mg/L *myo*-inositol; and 200 μM acetosyringone (prepared in DMSO); at pH 5.4. For a given set of experiments, embryos from pooled ears were used for each transformation.

[00196] *Agrobacterium* Culture Initiation. Glycerol stocks of *Agrobacterium* strain DAt13192 (International PCT Publication No. WO2012016222(A2)) containing the binary transformation vector pDAB111440 (Example 1) were streaked on AB minimal medium plates (Watson, *et al.*, (1975) **J. Bacteriol**. 123:255-264) containing appropriate antibiotics and were grown at 20°C for 3 to 4 days. A single colony was picked and streaked onto YEP plates (gm/L: yeast extract, 10; Peptone, 10; NaCl 5) containing the same antibiotics and was incubated at 20°C for 1-2 days.

[00197] *Agrobacterium* culture and Co-cultivation. *Agrobacterium* colonies were taken from a YEP plate, suspended in 10 mL of Inoculation Medium in a 50 mL disposable tube, and the cell density was adjusted to an OD_{550} of 0.2 to 0.4 (Optical Density measured at 550 nm; a measure of cell growth) using a spectrophotometer. The *Agrobacterium* cultures were incubated on a rotary shaker at 125 rpm (room temperature) while embryo dissection was performed. Immature zygotic embryos (previously isolated from the sterilized maize kernels and placed in 1 mL of Inoculation Medium) were washed once in the same medium. Two ml of the *Agrobacterium* suspension was added to each tube of embryos and the tubes were placed on a

shaker platform for 10 to 15 minutes. The embryos were transferred onto Co-cultivation Medium, oriented with the scutellum facing up, and incubated at 25°C, under 24-hour light at 50 μEm^{-2} sec^{-1} light intensity for 3 days. Co-cultivation Medium, contains 4.33 gm/L MS salts; 1X ISU Modified MS Vitamins; 30 gm/L sucrose; 700 mg/L L-proline; 3.3 mg/L Dicamba in KOH (3,6-dichloro-o-anisic acid or 3,6-dichloro-2-methoxybenzoic acid); 100 mg/L *myo*-inositol; 100 mg/L Casein Enzymatic Hydrolysate; 15 mg/L $AgNO_3$; 100 µM acetosyringone in DMSO; and 3 gm/L GELZAN™ (SIGMA-ALDRICH); at pH 5.8.

[00198] Callus Selection and Regeneration of Putative Events. Following the co-cultivation period, embryos were transferred to Resting Medium and incubated under 24-hour light at 50 $\mu Em^{-2}sec^{-1}$ light intensity and at 25°C for 3 days. Resting Medium contains 4.33 gm/L MS salts; 1X ISU Modified MS Vitamins; 30 gm/L sucrose; 700 mg/L L-proline; 3.3 mg/L Dicamba in KOH; 100 mg/L *myo*-inositol; 100 mg/L Casein Enzymatic Hydrolysate; 15 mg/L $AgNO_3$; 0.5 gm/L MES (2-(N-morpholino)ethanesulfonic acid monohydrate; *PHYTO*TECHNOLOGIES LABR.; Lenexa, KS); 250 mg/L Carbenicillin; and 2.3 gm/L GELZAN™; at pH 5.8. Embryos were transferred onto Selection Medium 1 (which consists of the Resting Medium (above) with 100 nM R-Haloxyfop acid (0.0362 mg/L)), and incubated in either dark and/or under 24-hour light at 50 $\mu Em^{-2}sec^{-1}$ light intensity for 7 to 14 days at 28°C. Proliferating embryogenic calli were transferred onto Selection Medium 2 (which consists of Resting Medium (above), with 500 nM R-Haloxyfop acid (0.1810 mg/L)), and were incubated in 24-hour light at 50 $\mu Em^{-2}sec^{-1}$ light intensity for 14 to 21 days at 28°C. This selection step allowed transgenic callus to further proliferate and differentiate.

[00199] Proliferating, embryogenic calli were transferred onto PreRegeneration Medium and cultured under 24-hour light at 50 $\mu Em^{-2}sec^{-1}$ light intensity for 7 days at 28°C. PreRegeneration Medium contains 4.33 gm/L MS salts; 1X ISU Modified MS Vitamins; 45 gm/L sucrose; 350 mg/L L-proline; 100 mg/L *myo*-inositol; 50 mg/L Casein Enzymatic Hydrolysate; 1.0 mg/L $AgNO_3$; 0.25 gm/L MES; 0.5 mg/L naphthaleneacetic acid in NaOH; 2.5 mg/L abscisic acid in ethanol; 1 mg/L 6-benzylaminopurine; 250 mg/L Carbenicillin; 2.5 gm/L GELZAN™; and 500 nM R-Haloxyfop acid; at pH 5.8. Embryogenic calli with shoot-like buds were transferred onto Regeneration Medium and cultured under 24-hour light at 50 μEm^{-2} sec^{-1} light intensity for 7 days. Regeneration Medium I contains 4.33 gm/L MS salts; 1X ISU Modified MS Vitamins; 60 gm/L sucrose; 100 mg/L *myo*-inositol; 125 mg/L Carbenicillin; 3.0

gm/L GELZAN™; and 500 nM R-Haloxyfop acid; at pH 5.8. Small shoots with primary roots were transferred to Shoot/Root medium in PHYTATRAYS (*PHYTO*TECHNOLOGIES LABR.; Lenexa, KS) and were incubated under 16:8 hr. light:dark at 140 to 190 μEm^{-2} sec^{-1} light intensity for 7 days at 27°C. Shoot/Root Medium contains 4.33 gm/L MS salts; 1X ISU Modified MS Vitamins; 30 gm/L sucrose; 100 mg/L *myo*-inositol; 3.5 gm/L GELZAN™; at pH 5.8. Putative transgenic plantlets were analyzed for transgene copy number by quantitative real-time PCR or other standard molecular analysis techniques, and were transferred to soil.

[00200] Transfer and establishment of T_0 plants in the greenhouse for seed production. Transformed plant tissues selected by their ability to grow on medium containing 500 nM R-Haloxyfop acid were transplanted into METRO-MIX 360 soilless growing medium (SUN GRO HORTICULTURE) and hardened-off in a growth room. Plants were then transplanted into SUNSHINE CUSTOM BLEND 160 soil mixture and grown to flowering in the greenhouse. Controlled pollinations for seed production are conducted.

[00201] Leaf tissues of selected T_0 plants were sampled at the V-3 to V-5 stage. Two 6 mm diameter leaf samples were stored in a 96 well cluster tube rack at -80°C until the day of analysis. Two DAISY™ steel BB's and 200 µL of extraction buffer (PBS solution containing 0.05% of Tween 20 and 5 µL/ml of SIGMA protease inhibitor cocktail (catalog number 9599)) were added to each tube. The samples were milled in a KLECO bead mill (Visalia, CA) for 3 minutes, on maximum setting. Samples were centrifuged at 3,000 x g for 5 minutes, then 100 µL of the supernatant were transferred to an empty sample tube. Another 100 µL of extraction buffer was added to the plant sample and bead-milled an 3 additional minutes. After centrifuging again, 100 µL of this extract was combined with the first 100 µL. The combined supernatants were mixed and analyzed on the same day as the extraction.

[00202] Proteins extracted from measured areas of leaf tissue were analyzed for expression of Cry1Fa protein and AAD-1 protein by standard ELISA (Enzyme-Linked Immunosorbant Assay) or protein immunoblots (western blots). For Cry1Fa ELISA detection, reagents from an ENVIROLOGIX ELISA kit (Cat. No. AP 016 NW V10; Portland, ME) were used according to the manufacturer's instructions. AAD-1 detection was performed by standard ELISA methodologies (for example, as taught in Ausubel *et al.* (1995 and updates) **Current Protocols in Molecular Biology**, John Wiley and Sons, New York) using rabbit antibodies prepared against purified AAD-1 protein.

[00203] The ELISA results obtained from extracts of pDAB111440-transformed plants are disclosed in Table 29. Protein levels are expressed as ng of the subject protein detected per square centimeter of leaf area harvested.

Table 29. Expression levels of Cry1Fa and AAD-1 proteins extracted from maize plants transformed with plasmid pDAB111440, as detected by ELISA methods.

Sample ID	Cry1Fa ng/cm^2	AAD-1 ng/cm^2
111440[3]-001.001	2.30	14.0
111440[3]-015.001	3.80	0.0
111440[3]-023.001	3.80	320.0
111440[3]-020.001	5.40	190.0
111440[3]-011.001	17.00	0.0

[00204] Protein extracts of the five pDAB111440-transformed plants listed in Table 29 (as well as extract from a non-transformed negative control plant) were prepared as above and probed with Cry1Fa antibody on immunoblots (western blots). Immunoblot procedures were essentially as described by Gallagher *et al.* (2008; **Immunoblotting and Immunodetection. Current Protocols in Immunology** 8.10.1 – 8.10.28). Protein samples (80 µL) were mixed with 20 µL of INVITROGEN NuPAGE LDS Sample Buffer , heated at >90°C for five min, loaded on an INVITROGEN NuPAGE 4-12% Bis-Tris gel, and run in MOPS SDS Running Buffer (200 Volts for 45 minutes). BIORAD PRECISION PLUS Dual Color Standards were loaded in a separate lane. Proteins were transferred to 0.2 µM nitrocellulose membrane by means of an INVITROGEN iBLOT Gel Transfer system according to the manufacturer's instructions. The membrane was blocked with INVITROGEN WESTERN BREEZE BLOCKING MIX, then reacted with Primary antibody (anti-Cry1F Purified Rabbit Antibody No. D0609RA07-A0; Strategic Diagnostics Inc., Newark, DE), followed by Secondary antibody (INVITROGEN Biotinylated goat anti-rabbit antibody.) This was followed by INVITROGEN HRP-Streptavidin conjugate and reacted bands were detected by PIERCE SUPERSIGNAL WEST PICO LUMINOL ENHANCER AND STABLE PEROXIDE (No. 34080).

[00205] Positive control lanes contained 0.5 ng or 1.0 ng of purified Cry1Fa core toxin protein produced by expression of a full length Cry1Fa coding region in a *Pseudomonas fluorescens* expression system (*See*, for example, US Patent Application No.20100269223A1). The full-length Cry1Fa protein was trypsin treated to release the Cry1Fa core toxin segment of calculated molecular size 68 kDa, which was used as the positive control standard on the immunoblot. No

antibody-reacting bands were detected in the extract from the negative control plant, while all five transgenic plant extracts contained a single predominant band (roughly equal in intensity to the control Cry1Fa proteins) of estimated size somewhat larger than 75 kDa.

[00206] Methods of controlling insect pests . When an insect comes into contact with an effective amount of toxin delivered via transgenic plant expression the results are typically death of the insect, or the insects do not feed upon the source which makes the toxins available to the insects.

CLAIMS

1. A synthetic DNA sequence for expressing a protein of interest in maize cells which comprises:

a) a codon-optimized DNA sequence encoding the protein of interest,

b) at least one polyadenylation signal sequence chosen from the group consisting of Class I and Class II wherein;

Class I is chosen from the group consisting of AATAAA, AATAAT, AACCAA, ATATAA, AATCAA, ATACTA, ATAAAA, ATGAAA, AAGCAT, ATTAAT, ATACAT, AAAATA, ATTAAA, AATTAA, AATACA, and CATAAA; and

Class II is chosen from the group consisting of ATATAT, TTGTTT, TTTTGT, TGTTTT, TATATA, TATTTT, TTTTTT, ATTTTT, TTATTT, TTTATT, TAATAA, ATTTAT, TATATT, TTTTAT, ATATTT, TATTAT, TGTTTG, TTATAT, TGTAAT, and AAATAA; and

wherein said codon-optimized DNA sequence contains at least one polyadenylation signal sequence from Class II and wherein said synthetic DNA sequence contains fewer Class II polyadenylation signal sequences than the protein's native DNA sequence and contains the same number of class I polyadenylation signal sequences compared to said native DNA sequence.

2. The synthetic DNA sequence of **Claim 1** that is substantially devoid of class II polyadenylation signal sequences.

3. The synthetic DNA sequence of **Claim 1** that is devoid of class II polyadenylation signal sequences.

4. The synthetic DNA sequence of **Claim 1** wherein said synthetic DNA sequence encodes a native protein selected from the group consisting of insecticidal proteins, herbicide tolerance proteins, stress tolerance-related proteins, and oil profile modification proteins.

5. The synthetic DNA sequence of **Claim 4** wherein said synthetic DNA sequence encodes an insecticidal protein.

6. The synthetic DNA sequence of **Claim 4** wherein said synthetic DNA sequence encodes aryloxyalkanoate dioxygenase 1 protein.

7. A synthetic DNA sequence for expressing a protein of interest in soybean cells which comprises:

a) a codon-optimized DNA sequence encoding the protein of interest,

b) at least one polyadenylation signal sequence chosen from the group consisting of Class I and Class III wherein;

Class I is chosen from the group consisting of AATAAA, AATAAT, AACCAA, ATATAA, AATCAA, ATACTA, ATAAAA, ATGAAA, AAGCAT, ATTAAT, ATACAT, AAAATA, ATTAAA, AATTAA, AATACA, and CATAAA; and

Class III is chosen from the group consisting of ATTTTT, TATTTT, TTATTT, TTTATT, TTTTTT, TTTTAT, AATTTT, TTTTTA, ATATAT, TAATTT, TTAATT, AAATTT, AAATAA, ATATTT, TTTGTT TTGTTT, ATTATT, ATTTTA, TTTAAT, and TTTTAA, and

wherein said codon-optimized DNA sequence contains at least one polyadenylation signal sequence from Class III and wherein said synthetic DNA sequence contains fewer Class III polyadenylation signal sequences than the protein's native DNA sequence and contains the same number of Class I polyadenylation signal sequences compared to said native DNA sequence.

8. The synthetic DNA sequence of **Claim 7** that is substantially devoid of class III polyadenylation signal sequences.

9. The synthetic DNA sequence of **Claim 7** that is devoid of class III polyadenylation signal sequences.

10. The synthetic DNA sequence of **Claim** wherein said synthetic DNA sequence encodes a native protein selected from the group consisting of insecticidal proteins, herbicide tolerance proteins, stress tolerance-related proteins, and oil profile modification proteins.

11. The synthetic DNA sequence of **Claim 10** wherein said synthetic DNA sequence encodes an insecticidal protein.

12. The synthetic DNA sequence of **Claim 10** wherein said synthetic DNA sequence encodes aryloxyalkanoate dioxygenase 1 protein.

13. A synthetic DNA sequence chosen from the group consisting of SEQ ID NO:5, SEQ ID NO:11, SEQ ID NO:17, SEQ ID NO:23, SEQ ID NO:29, SEQ ID NO:35, SEQ ID NO:41, SEQ ID NO:47, SEQ ID NO:53, SEQ ID NO:59, SEQ ID NO:65, and SEQ ID NO:71.

14. A DNA construct for expression of a protein of interest comprising a 5' non-translated sequence, a coding sequence for a protein of interest, and a 3' non-translated region, wherein said 5' nontranslated sequence contains a promoter functional in a plant cell, said coding sequence is the synthetic DNA coding sequence of **Claim 1**, and wherein said 3' nontranslated sequence comprises a transcription termination sequence and a polyadenylation signal.

15. A DNA construct for expression of a protein of interest comprising a 5' non-translated sequence, a coding sequence for a protein of interest, and a 3' non-translated region, wherein said 5' nontranslated sequence contains a promoter functional in a plant cell, said coding sequence is the synthetic DNA coding sequence of **Claim 7**, and wherein said 3' nontranslated sequence comprises transcription termination sequence and a polyadenylation signal.

16. A transgenic plant containing the synthetic DNA sequence of **Claim 1.**

17. A transgenic plant containing the synthetic DNA sequence of **Claim 7.**

18. A method of controlling pests in grain or seed which comprises obtaining said grain or seed from plants containing the synthetic DNA of **Claim 5**.

19. A method of controlling pests in grain or seed which comprises obtaining said grain or seed from plants containing the synthetic DNA of **Claim 11**.

20. A method of controlling pests in grain or seed which comprises obtaining said grain or seed from plants containing the synthetic DNA of **Claim 5**.

21. A method of controlling pests in meal or flour which comprises obtaining said meal or flour from the grain containing the synthetic DNA of **Claim 5**.

22. A composition derived from the transgenic plant of **Claim 16** wherein said composition is a commodity product selected from the group consisting of meal, flour, protein concentrate, or oil.

23. A composition derived from the transgenic plant of **Claim17** wherein said composition is a commodity product selected from the group consisting of meal, flour, protein concentrate, or oil.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/33458

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 5/04, C12N 5/10, C12N 15/82 (2012.01)
USPC - 536/23.6, 800/300, 435/419, 435/468
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - C12N 5/04, C12N 5/10, C12N 15/82 (2012.01)
USPC - 536/23.6, 800/300, 435/419, 435/468

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 800/278, 279, 300.1, 301, 302, 312; 435/91.1, 413, 418, 426
(Text Search)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(PGPB,USPT,USOC,EPAB,JPAB; Dilaog Classic (Files 654, 652, 349, 348, 340, 35, 65, 155).
Search terms -- polyadenylation signal sequences, transgenic plants,synthetic DNA, soybean, maize, untranslated ends, promoters, transcription terminators, seed, meal, aryloxyalnanoate dioxygenase, insecticide gene, AATAAT, ATATAA, AATCAA, ATACTA, ATAAA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 7,741,118 B1 (FISCHHOFF et al.) 22 June 2010 (22.06.2010) Table I-II; claim 1; abstract; col 10, ln 8-28; col 11, ln 7-30; col 13, ln 56-65; col 14, ln 15-19	1-12 and 14-23
A	US 2009/0270327 A1 (BAUM et al.) 29 October 2009 (29.10.2009) para [0139]	1-12 and 14-23
A	US 2009/0260107 A1 (ENGLISH et al.) 15 October 2009 (15.10.2009) table 25; para [0494]	1-12 and 14-23
A	US 2004/0168213 A1 (VERBSKY et al.) 26 August 2004 (26.08.2004) para [0059], [0103]	1-12 and 14-23
A	US 2010/0138953 A1 (FLASINSKI) 03 June 2010 (03.06.2010) para [0194], [0208]-[0214]	1-12 and 14-23
A	2009/0093366 A1 (WRIGHT et al.) 09 April 2009 (09.04.2009) para [0228]	1-12 and 14-23
A	US 2007/0028321 A1 (MANJUNATH et al.) 01 February 2007 (01.02.2007) para [0133], [0286], [0448].	1-12 and 14-23

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
07 September 2012 (07.09.2012)	20 SEP 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/33458

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-12 and 14-23, drawn to a synthetic DNA sequence for expression a protein of interest in maize cells which comprises: a) a codon-optimized DNA sequence encoding the protein of interest, b) at least one polyadenylation signal sequence chosen from the group consisting of Class I and Class II wherein; Class I is chosen from the group consisting of AAT AAA, AA T AA T, AACCAA,
ATATAA, AATCAA, ATACTA, ATAAAA, ATGAAA, AAGCAT, ATTAAT, ATACAT, AAAATA, ATTAAA, AATTAA, AATACA, and CATAAA; and Class II is chosen from the group consisting of ATATAT, TTGTTT, TTTTGT, TGTTTT, TATATA, TATTTT, TTTTTT, ATTTTT, TTATTT, TTTATT, TAATAA, ATTTAT, TATATT, TTTTAT, ATATTT, TATTAT, TGTTTG, TTATAT, TGTAAT,and AAATAA;

SEE CONTINUATION SHEET.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-12 and 14-23.

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/33458

Continuation of Box III - Observations where unity of invention is lacking:

and wherein said codon-optimized DNA sequence contains at least one polyadenylation signal sequence from Class II and wherein said synthetic DNA sequence contains fewer Class II polyadenylation signal sequences than the protein's native DNA sequence and contains the same number of class I polyadenylation signal sequences compared to said native DNA sequence; and A synthetic DNA sequence for expressing a protein of interest in soybean cells which comprises: a) a codon-optimized DNA sequence encoding the protein of interest, b) at least one polyadenylation signal sequence chosen from the group consisting of Class I and Class III wherein; Class I is chosen from the group consisting of AAT AAA, AA T AA T, AACCAA, ATATAA, AATCAA, ATACTA, ATAAAA, ATGAAA, AAGCAT, ATTAAT, ATACAT, AAAATA, ATT AAA, AATTAA, AATACA, and CATAAA; and Class III is chosen from the group consisting of ATTTTT, TATTTT, TTATTT, TTTATT, TTTTTT, TTTTAT, AATTTT, TTTTTA, ATATAT, TAATTT, TTAATT, AAATTT, AAA TAA, ATATTT, TTTGTT TTGTTT, ATTATT, ATTTTA, TTT AA T, and TTTTAA, and wherein said codon-optimized DNA sequence contains at least one polyadenylation signal sequence from Class III and wherein said synthetic DNA sequence contains fewer Class III polyadenylation signal sequences than the protein's native DNA sequence and contains the same number of Class I polyadenylation signal sequences compared to said native DNA sequence.

Group II+: Claim 13, drawn to a synthetic DNA sequence chosen from the group consisting of SEQ ID NO:5, SEQ ID NO:11, SEQ IDNO:17, SEQ IDNO:23, SEQ ID NO:29, SEQ ID NO:35, SEQ ID NO:41, SEQ ID NO:47, SEQ ID NO:53, SEQ ID NO:59, SEQ ID NO:65, and SEQ ID NO:71.
The first invention encompasses SEQ ID NO: 5. Should an additional fee(s) be paid, Applicant is invited to elect an additional SEQ ID NO(s) to be searched.

The inventions listed as Groups I and II+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Group I does not include the inventive concept of a synthetic DNA sequence chosen from the group consisting of SEQ ID NO:5, SEQ ID NO:11, SEQ IDNO:17, SEQ IDNO:23, SEQ ID NO:29, SEQ ID NO:35, SEQ ID NO:41, SEQ ID NO:47, SEQ ID NO:53, SEQ ID NO:59, SEQ ID NO:65, and SEQ ID NO:71, as required by Group II+.

Group II+ does not include the inventive concept of a synthetic DNA sequence for expression a protein of interest in maize cells which comprises: a) a codon-optimized DNA sequence encoding the protein of interest, b) at least one polyadenylation signal sequence chosen from the group consisting of Class I and Class II wherein; Class I is chosen from the group consisting of AAT AAA, AA T AA T, AACCAA,
ATATAA, AATCAA, ATACTA, ATAAAA, ATGAAA, AAGCAT, ATTAAT, ATACAT, AAAATA, ATTAAA, AATTAA, AATACA, and CATAAA; and Class II is chosen from the group consisting of ATATAT, TTGTTT, TTTTGT, TGTTTT, TATATA, TATTTT, TTTTTT, ATTTTT, TTATTT, TTTATT, TAATAA, ATTTAT, TATATT, TTTTAT, ATATTT, TATTAT, TGTTTG, TTATAT, TGTAAT, and AAATAA; and wherein said codon-optimized DNA sequence contains at least one polyadenylation signal sequence from Class II and wherein said synthetic DNA sequence contains fewer Class II polyadenylation signal sequences than the protein's native DNA sequence and contains the same number of class I polyadenylation signal sequences compared to said native DNA sequence; and A synthetic DNA sequence for expressing a protein of interest in soybean cells which comprises: a) a codon-optimized DNA sequence encoding the protein of interest, b) at least one polyadenylation signal sequence chosen from the group consisting of Class I and Class III wherein; Class I is chosen from the group consisting of AAT AAA, AA T AA T, AACCAA, ATATAA, AATCAA, ATACTA, ATAAAA, ATGAAA, AAGCAT, ATTAAT, ATACAT, AAAATA, ATT AAA, AATTAA, AATACA, and CATAAA; and Class III is chosen from the group consisting of ATTTTT, TATTTT, TTATTT, TTTATT, TTTTTT, TTTTAT, AATTTT, TTTTTA, ATATAT, TAATTT, TTAATT, AAATTT, AAA TAA, ATATTT, TTTGTT TTGTTT, ATTATT, ATTTTA, TTT AA T, and TTTTAA, and wherein said codon-optimized DNA sequence contains at least one polyadenylation signal sequence from Class III and wherein said synthetic DNA sequence contains fewer Class III polyadenylation signal sequences than the protein's native DNA sequence and contains the same number of Class I polyadenylation signal sequences compared to said native DNA sequence, as required by Group I.

Groups I and II+ share the technical feature of a synthetic DNA sequence.
Groups II+ share the technical feature of a synthetic DNA sequence comprising a specific DNA sequence.
However, this shared technical feature does not represent a contribution over the prior art as being anticipated by US 7,741,118 B1 to Fischhoff et al. (published on 22 June 2010) (hereinafter 'Fischhoff') that teaches a synthetic DNA sequence for expression of a protein in plant cells, wherein the synthetic DNA sequence comprises a specific DNA sequence comprising a codon-optimized DNA sequence encoding the protein of interest and at least one polyadenylation signal (SEQ ID NO: 5; fig 4A-4C; table I, VII; col 6, ln 50-55; col 11, ln 7-30-'Codons used in the replacement of wild-type codons should preferably avoid the TA or CG doublet wherever possible. Codons are selected from a plant preferred codon table (such as Table I below) so as to avoid codons which are rarely found in plant genomes, and efforts should be made to select codons to preferably adjust the G+C content to about 50%'; col 20, ln 51 to col 23, ln 30-'The strategy employed was to use the 5'-two thirds of the synthetic HD-1 gene (first 1350 bases, up to the SacI site) and to dramatically modify the final 590 bases (through amino acid 645) of the HD-73 in a manner consistent with the algorithm used to design the synthetic HD-1 gene','The resulting gene has two potential polyadenylation sites (compared to 18 in the WT) and no ATTTA sequence (12 in the WT). The G+C content has increased from 37% to 48%. A total of 59 individual base pair changes were made using the primers in Table VI', 'Table VII below compares the codon usage of the wild-type gene of B.t.k. HD-73 versus the synthetic gene of this example for amino acids 451-645 and codon usage of naturally occurring genes of dicotyledonous plants'; col 26, ln 45-62-'this synthetic HD-73 gene still was expressed at least 100-fold better than the wild-type HD-73 gene').

As said shared technical feature was known at the time of the invention, this cannot be considered special technical feature that would otherwise unify the groups.

Groups I and II+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.