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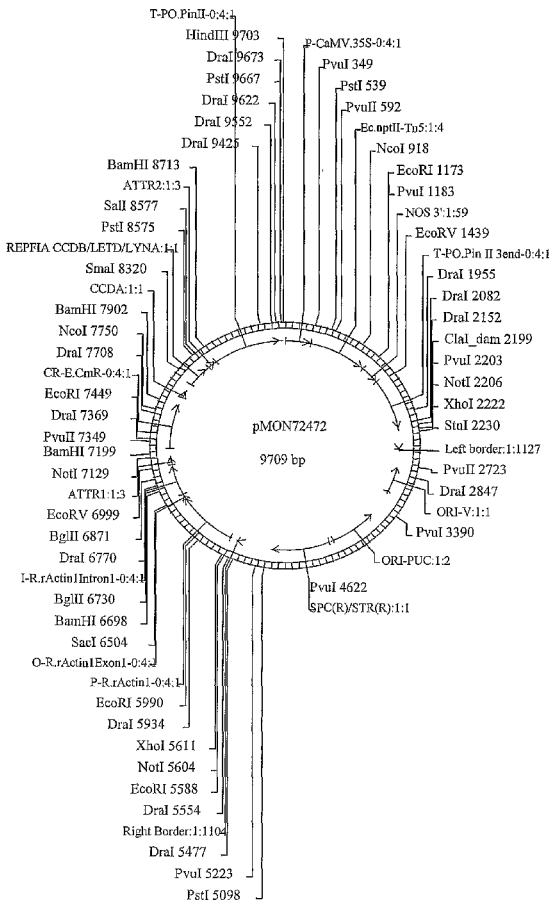
(54) Title: TRANSGENIC MAIZE WITH ENHANCED PHENOTYPE



(57) Abstract: Transgenic maize with an enhanced phenotype from insertion of heterologous DNA. Enhanced phenotypes include yield such as yield under stress conditions, quality of plant morphology, physiology or seed composition, metabolic function, cell growth, and the like. Such transgenic maize is produced by generating a plurality of transgenic events for a plurality of unique transgenic DNA constructs where each of the transgenic events comprises introducing into the genome of a parental maize line a single transgenic DNA construct comprising a promoter operably linked to heterologous DNA in sufficient quantity to produce transgenic cells which can be cultured into plants of transgenic maize having the enhanced phenotype.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Transgenic Maize With Enhanced Phenotype

Related Applications

This application claims priority to provisional application 60/337,358 filed December 4, 2001, the specification and sequence listing of which is incorporated herein by reference.

Incorporation of Sequence Listing

The sequence listing is contained in the file named "pa_00431.rpt" which is 3,661 kilobytes (measured in MS-Windows) and was created on 26 November 2002 and is located on a CD-ROM, which is filed herewith and herein incorporated by reference.

Field of the Invention

Disclosed herein is maize with enhanced phenotype, and methods of producing such maize.

Background of the invention

Maize (also known as *Zea mays* and corn) is one of the major crops grown worldwide as a primary source for animal feed, human food and industrial resource. Maize plants with improved agronomic traits such as yield, pest resistance, herbicide resistance, higher seed component, and the like are desired by both farmers and consumers of maize. Considerable effort in breeding maize, e.g. to produce hybrids, has provided significant gains in desired phenotypes.

The ability to introduce specific transgenic DNA into the genome of corn has been used to enhance transgenic maize plants with a desired trait. Researchers have utilized the genetic transformation technology to test and prove the preconceived effects of a gene for plant phenotype enhancement. In many cases, much effort has been placed on the selection of the gene to introduce into the plant as a means to increase the overall success of the experiment to produce a more desirable plant. Nonetheless, the frequency of success of enhancing the transgenic plant is low due to a number of factors including the low predictability of the effects of a specific gene on the plant's growth, development and environmental response, the low frequency of maize transformation, the lack of highly predictable control of the gene once introduced into the genome, and other undesirable effects of the transformation event and tissue culture process. Even with all these problems, transformation is still practiced with persistence and diligence to identify those transgenic plants with the expected, predetermined phenotype. Occasionally the unexpected phenotype is observed. See U.S. Patent 6, 395,966 which discloses

transgenic maize with enhanced yield resulting unexpectedly from the introduction of a gene intended to confer herbicide resistance. Other enhanced traits have been achieved by mutation, e.g. induced by a transposon or chemical or physical mutagen. See for instance, U.S. Patent 6,410,831 which discloses the production of sunflower seed with enhanced stearic acid content by random mutagenesis.

Summary of the Invention

This invention relates to the discovery that transformation by random insertion into the corn genome of genes, for the transcription of which there is no known phenotype in corn, can be used as a reliable generator of modification of the corn genome to produce unexpected but yet desired phenotypes. One aspect of the invention provides transgenic maize seed for a maize line which exhibits enhanced yield as compared to yield for a parental maize line; in another aspect the invention provide transgenic maize seed for a maize line characterized by enhanced yield under stress conditions. In another aspect the invention provides transgenic maize seed for maize lines characterized by other enhanced traits, e.g. an enhanced quality in a plant morphology, plant physiology or seed component phenotype as compared to a corresponding phenotype of a parental maize line.

Such transgenic maize seed characterized by enhanced phenotype is produced by introducing into the genome of parental maize a transgenic DNA construct comprising a promoter operably linked to heterologous DNA, where the heterologous DNA encodes a protein having an amino acid sequence with at least 60% identity to a sequence selected from the group consisting of SEQ ID NO:369 through SEQ ID NO:736. In a preferred aspect of the invention the transgenic maize is produced by introducing a transgenic DNA construct where the heterologous DNA comprises a protein coding segment of DNA having at least 60% identity to a sequence selected from the group consisting of SEQ ID NO:1 through SEQ ID NO: 368.

Other aspects of the invention provide transgenic maize seed for a maize line characterized by unique enhanced phenotype resulting from introduction of a specific heterologous DNA, e.g. shorter plants from decreased internode length, taller plants from increased internode length, early leaf senescence, sterility and elongated tassel central axis. Transgenic maize seed for shorter plants from decreased internode length can result from insertion of heterologous DNA coding for:

(a) a TOC1-like receiver domain 3 having an amino acid sequence which is at least 60% identical to SEQ ID NO:436,

(b) a HY5-like protein having an amino acid sequence which is at least 60% identical to SEQ ID NO:565, or

(c) a proline permease having an amino acid sequence which is at least 60% similar to SEQ ID NO:371.

Transgenic maize seed for taller plants from increased internode length can result from introduction of heterologous DNA coding for:

(a) a myb related transcription factor having an amino acid sequence which is at least 60% identical to SEQ ID NO:717, or

(b) an SVP-like protein having an amino acid sequence which is at least 60% identical to SEQ ID NO: 609.

Transgenic maize seed for plants with early leaf senescence can result from insertion of heterologous DNA coding for a Cytochrome P450 having an amino acid sequence which is at least 60% identical to SEQ ID NO:382.

Transgenic maize seed for sterile plants can result from insertion of heterologous DNA coding for:

(a) an RR3-like receiver domain 8 having an amino acid sequence which is at least 60% identical to SEQ ID NO:439,

(b) an ARR2-like receiver domain having an amino acid sequence which is at least 60% identical to SEQ ID NO:434,

(c) an HSF protein having an amino acid sequence which is at least 60% identical to SEQ ID NO:487, or

(d) an SVP-like protein having an amino acid sequence which is at least 60% identical to SEQ ID NO:609.

Transgenic maize seed for plants with elongated tassel central axis can result from insertion of heterologous DNA coding for an SVP-like protein having an amino acid sequence which is at least 60% identical to SEQ ID NO:609.

This invention also provides methods for introducing into a maize line an enhanced phenotype as compared to a phenotype in parental units of said maize line. The method comprises generating a population of transgenic plants comprising a variety of heterologous

DNA for the transcription of which there is no known phenotype in maize. In one aspect of the invention the population is generated for a plurality of transgenic events for a plurality of unique transgenic DNA constructs. Each transgenic event comprises introducing into the genome of a parental units a single transgenic DNA construct comprising a promoter operably linked to heterologous DNA for the transcription of which there is no known phenotype in corn. The transgenic DNA construct is introduced into the parental genome in sufficient quantity to produce transgenic cells which can be cultured into plants of transgenic maize having said enhanced phenotype. The transgenic cells are cultured into transgenic plants producing progeny transgenic seed. The population of transgenic plants are screened for observable phenotypes. And, seed is collected from transgenic plants which are selected as having an unexpected enhanced phenotype. Optionally, the method comprises repeating a cycle of germinating transgenic seed, growing subsequent generation plants from said transgenic seed, observing phenotypes of said subsequent generation plants and collecting seeds from subsequent generation plants having an enhanced phenotype.

In preferred aspects the method a large population is screened by employing at least 2 transgenic events for at least 20 unique transgenic DNA constructs, more preferably upwards of 10 or more transgenic events, say up to 100 or more transgenic events for upwards of 50 or more unique transgenic DNA constructs, say 100 or more or even 500 or more unique transgenic constructs.

Other preferred aspects of the method employ DNA construct where the heterologous DNA is operably linked to a selected promoter, e.g. the 5' end of a promoter region comprising a rice actin promoter and rice actin intron. The DNA construct may be introduced into a random location in the genome or into a preselected site in the genome.

Yet another aspect of the invention provides a method comprising crossing transgenic plants from the population of transgenic plants with at least one other maize line to produce a hybrid population of transgenic plants, observing phenotypes in the hybrid population and selecting seed from transgenic plants in the hybrid population having unexpected enhanced phenotypes.

Brief Description of Drawings

Figure 1 illustrates a vector comprising a DNA construct useful in the practice of this invention.

Detailed Description of Preferred Embodiments

Definitions – As used herein the following terms are specifically defined:

"Maize" means a variety of *Zea mays* also commonly known in some parts of the world as corn. Maize is cultivated as a crop.

"Seed" means the reproductive tissue of a plant which is formed from a fertilized ovule and from which a new plant develops. Seed contains an embryo and discrete food store (cotyledon or endosperm) surrounded by an outer covering (testa). The measure of maize seed produced is reported as yield. Maize seed contains useful industrial and food resources of protein, oil and starch.

"Phenotype" means a measurable crop trait and includes, but is not limited to, yield as compared to a parental maize line such as overall yield and yield under stress conditions such as drought, shade, fungal disease, viral disease, bacterial disease, insect infestation, nematode infestation, cold temperature exposure, heat exposure, osmotic stress, reduced nitrogen nutrient availability, reduced phosphorus nutrient availability or high plant density. Other useful phenotypes include yield as manifested by increase number of kernels per unit planted area, number of ears per unit planted area and average weight of kernels; yield as manifested by increase in biomass per unit planted area or an increase in the root/shoot ratio; or yield as manifested by increased efficiency in water use, nitrogen use or phosphate use. Other useful phenotypes include enhanced quality as compared to a parental maize line in plant morphology, physiology or seed composition such as increased internode length, shortened internode length, sterility, elongated tassel central axis, earlier leaf senescence, setting a second ear at high planting density, earlier time of germination, increased production of kernel oil and increased production of kernel protein. Still other useful phenotypes include enhanced metabolic function such as increased amino acid production, increased amino acid transport, increased protein production and increased enzyme activity; enhanced cell growth, modified cell regulation and early cell senescence; early time of germination and early flowering.

"Parental maize line" means any maize variety that provides tissue for transformation and baseline phenotype.

"Heterologous" refers to a segment of DNA that is imported into a non-natural DNA construct, e.g. regulatory DNA as well as DNA coding for a protein.

"Transgenic DNA construct" means a segment of DNA which is introduced into the genome of a parental maize line. While a transgenic DNA construct can comprise any segment of DNA that is heterologous to the insertion site, in preferred aspects of the invention the transgenic DNA construct will be designed to provide a specific function, e.g. suppress or over express a selected protein. Useful transgenic DNA constructs comprise gene regulatory segment operably linked to a protein coding segment. A gene regulatory segment can more specifically comprise promoter elements, enhancers, silencers, introns and untranslated regions. An especially useful gene regulatory segment for use in maize comprises a rice actin promoter with a rice actin intron as described more specifically below. Protein coding segment can be any coding segment that may be of interest for investigation into its effect in a transgenic plant. Exemplary protein coding segments include DNA segments encoding all or a part of any protein such as a cytochrome p450, a transporter, a lipase, a kinase, a receiver domain, a synthase, a transcription factor, a reductase, a phosphatase, a ribonuclease, an anhydrase and the like. It is also useful to use DNA segments encoding protein of unknown function.

In cases where over expression of heterologous DNA may not be satisfactory, effective or desirable in producing an observed enhanced phenotype, it is contemplated that a person or ordinary skill in the art would look to protein pathways for an alternate route to the desired enhanced phenotype. Such alternate route may include insertion of heterologous DNA coding for a protein which is upstream or downstream of the protein originally associated with the observed enhanced phenotype. Another alternate route may include insertion of heterologous DNA which is effective in suppression of a competitive protein. When suppression of protein expression is the intended objective, the heterologous DNA can be designed to produce a gene silencing effect, e.g. by an antisense or RNAi mechanism. Anti-sense suppression of genes in plants by introducing by transformation of a construct comprising DNA of the gene of interest in an anti-sense orientation is disclosed in U.S. Patents 5,107,065; 5,453,566; 5,759,829; 5,874,269; 5,922,602; 5,973,226; 6,005,167; WO 99/32619; WO 99/61631; WO 00/49035; WO 02/02798; all of which are incorporated herein by reference. Interfering RNA suppression of genes in a plant by introducing by transformation of a construct comprising DNA encoding a small (commonly less than 30 base pairs) double-stranded piece of RNA matching the RNA encoded by the gene of interest is disclosed in U.S. Patents 5,190,931; 5,272,065; 5,268,149; WO 99/61631; WO 01/75164; WO 01/92513, all of which are incorporated herein by reference.

A "non-predetermined location in genomic DNA" means a random locus in a maize chromosome in which a transgenic DNA construct is inserted by chance.

"Transformation" means a method of introducing a transgenic DNA construct into a genome and can include any of the well-known and demonstrated methods including electroporation as illustrated in U.S. Patent 5,384,253, microprojectile bombardment as illustrated in U.S. Patents 5,015,580; 5,550,318; 5,538,880; 6,160,208; 6,399,861and 6,403,865, Agrobacterium mediated transformation as illustrated in U.S. Patents 5,635,055; 5,824,877; 5,591,616; 5,981,840 and 6,384,301, and protoplast transformation as illustrated in U.S. Patents 5,508,184, all of which are incorporated herein by reference.

"Tissue from a parental maize line" means tissue which is specifically adapted for a selected method of transformation and can include cell culture or embryonic callus.

"Yield" as used herein means the production of shelled corn kernels per unit of production area, e.g. in bushels per acre or metric tons per hectare, often reported on a moisture adjusted basis, e.g. at 15.5 % moisture. As a bushel of corn is defined by law in the State of Iowa as 56 pounds by weight, a useful conversion factor for corn yield is: 100 bushels per acre is equivalent to 6.272 metric tons per hectare.

The maize seed provided by this invention is characterized by an enhanced phenotype as compared to its parental maize line. Such maize seed is preferably obtainable from a massive screening program by observing transformed plants for serendipitously imparted phenotype resulting from the introduction of a transgenic DNA construct into a non-predetermined location in the genomic DNA of tissue from a parental maize line. The transgenic DNA construct is introduced into the genome in sufficient quantity to produce transgenic cells which can be cultured into plants of transgenic maize having an enhanced phenotype as compared to the parental maize line. Such transgenic maize cells are cultured into transgenic plants which produce progeny transgenic seed. Preferably, the screening program is designed to evaluate multiple events of a plurality of distinct transgenic DNA constructs, e.g. from 2 to 20 or more transgenic events of each of from 2 to 20 or more transgenic DNA constructs, e.g. at least 50 or more or up to 100 or more transgenic DNA constructs. Although the design of a transgenic DNA construct can be based on a rational expectation of a phenotype modification, the method of the invention requires observation of an unexpected, yet desired enhanced phenotype. A useful population for screening for unexpected enhanced phenotypes may comprise 40 or more

unique transgenic plants, e.g. at least 100 transgenic plants or even up to 1000 or more unique transgenic plants. Even larger populations can be provided by crossing transgenic plants with other plant lines to provide hybrid populations of transgenic plants, such populations can comprises tens of thousands of transgenic plants for screening.

In methods of this invention transgenic plants and seeds are evaluated for desired phenotypes allowing the selection of seeds. Methods of this invention can be practiced with an optional repeating of a cycle of germinating transgenic seed, growing subsequent generation plants from said transgenic seed, observing phenotypes of said subsequent generation plants and collecting seeds from subsequent generation plants having a desirable enhanced phenotype.

Heterologous DNA

The following Table 1 describes the heterologous DNA used to produce the transgenic maize of this invention including reference to nucleic acid and polypeptide sequences which are provided in the Sequence Listing. It is contemplated that transgenic maize seed of this invention characterized by an enhanced phenotype will result from use of not only the heterologous DNA listed in Table 1 but also homolgs, orthologs and/or paralogs of such heterologous DNA or similar DNA which has been artificially modified to avoid or minimize an undesired effect but yet still produce the originally observed enhanced phenotype associated with the heterologous DNA listed in Table 1. Thus, heterologous DNA for use in this invention comprises not only DNA coding for a protein of a polypeptide listed in Table 1, e.g. with an amino acid sequence of SEQ ID NO: 369 to SEQ ID NO:738, but also DNA coding for a protein with an amino acid sequence which is at least 60% identical, e.g. at least 65%, 70% or 75% identical, in some cases more preferably at least 80%, 85%, 90% or 95% identical, to a sequence of SEQ ID NO: 369 to SEQ ID NO: 739. In another aspect of this invention the transgenic maize with an enhanced trait is provided by using heterologous DNA with a nucleic acid sequence of SEQ ID NO:1 to SEQ ID NO:368 or a homologous DNA coding for a protein of similar function but with a nucleic acid sequence which is at least 70% identical, e.g. at least 75%, 80%, 85%, 90% or 95% identical, to a sequence of SEQ ID NO:1 to SEQ ID NO:368. Sequence identity is determined over a sequence of substantially the full length of a sequence listed in Table 1. More particularly, the headings for Table 1 have the following meanings:

"NUCLEIC ACID SEQ ID NO" refers to a particular nucleic acid sequence in the Sequence Listing which defines a heterologous DNA used in a transgenic DNA construct of this invention.

"PHE ID" refers to an arbitrary number used to identify experiments using a particular heterologous DNA.

"AMINO ACID SEQ ID NO" refers to a particular amino acid sequence in the Sequence Listing corresponding to the translated protein encoded by the heterologous DNA.

"GENE NAME" refers to a common name for the heterologous DNA.

"DONOR ORGANISM" refers to the organism from which the heterologous DNA was derived.

"CODING COORDINATES" refer to peptide coding segments of the heterologous DNA.

Table 1

NUCLEIC ACID SEQ ID NO	PHE_ID	AMINO_ACID SEQ ID NO	GENE_NAME	DONOR ORGANISM	CODING COORDINATES
1	PHE0000022	369	Zm AAP6	Zea mays	96-1547
2	PHE0000023	370	Os ProT	Oryza sativa	144-1562
3	PHE0000279	371	sorghum proline permease	Sorghum bicolor	16-1341
4	PHE0000280	372	rice AA transporter	Oryza sativa	61-1485
5	PHE0000281	373	K/H specific amino acid permease	Zea mays	63-1427
6	PHE0000402	374	rice amino acid transporter-like protein	Oryza sativa	89-1426
7	PHE0000403	375	corn amino acid permease	Zea mays	116-1453
8	PHE0000404	376	rice proline transport protein	Oryza sativa	313-1731
9	PHE0000450	377	rice amino acid permease	Oryza sativa	110-1450
10	PHE0000108	378	ASH1	Arabidopsis thaliana	61-801
11	PHE0000109	379	Rice ASH1-like1	Oryza sativa	136-1008
12	PHE0000110	380	Rice MtN2-like	Oryza sativa	425-464,546-582,672-783,812-898,988-1149,1556-1675,1776-1952
13	PHE0000262	381	cytochrome P450-like protein	Zea mays	29-1495
14	PHE0000263	382	cytochrome P450	Zea mays	141-1637
15	PHE0000264	383	cytochrome P450-like	Zea mays	104-1657

NUCLEIC ACID SEQ ID NO	PHE_ID	AMINO_ACID SEQ ID NO	GENE_NAME	DONOR ORGANISM	CODING COORDINATES
16	PHE0000265	384	CYP90 protein	Zea mays	81-1589
17	PHE0000266	385	cytochrome P450 DWARF3	Zea mays	92-1648
18	PHE0000267	386	cytochrome P450	Zea mays	134-1543
19	PHE0000268	387	rice receptor protein kinase	Oryza sativa	183-476,706-735,2796-6734
20	PHE0000084	388	rice cyclin H	Oryza sativa	235-1227
21	PHE0000085	389	rice cdc2+/CDC28-related protein kinase	Oryza sativa	173-1447
22	PHE0000086	390	Cdk-activating kinase 1	Glycine max	14-1240
23	PHE0000284	391	menage a trois-like	Zea mays	164-745
24	PHE0000362	392	CDC28-related protein kinase	Zea mays	198-1484
25	PHE0000083	393	PDR5	Saccharomyces cerevisiae	1552-6087
26	PHE0000089	394	CHL1	Arabidopsis thaliana	85-1857
27	PHE0000090	395	NTR1	Oryza sativa	144-1898
28	PHE0000228	396	Synechocystis cobA	Synechocystis sp. PCC 6803	70-801
29	PHE0000229	397	Xylella tetrapyrrole methylase	Xylella fastidiosa	1-774
30	PHE0000230	398	maize uroporphyrinogen III methyltransferase	Zea mays	15-1286
31	PHE0000190	399	LEA3	Zea mays	171-755
32	PHE0000191	400	non-specific lipid transfer protein	Zea mays	70-456
33	PHE0000234	401	soy LEA protein	Glycine max	6-704
34	PHE0000235	402	dehydrin-like protein	Glycine max	33-710
35	PHE0000236	403	glycine-rich protein	Zea mays	91-558
36	PHE0000237	404	dehydrin 3	Zea mays	84-584
37	PHE0000238	405	probable lipase	Zea mays	98-967
38	PHE0000239	406	yeast GRE1	Saccharomyces cerevisiae	1024-1527
39	PHE0000240	407	yeast STF2	Saccharomyces cerevisiae	683-934
40	PHE0000241	408	yeast SIP18	Saccharomyces cerevisiae	376-855

NUCLEIC ACID SEQ ID NO	PHE_ID	AMINO_ACID SEQ ID NO	GENE_NAME	DONOR ORGANISM	CODING COORDINATES
41	PHE0000242	409	yeast YBM6	Saccharomyces cerevisiae	744-1130
42	PHE0000243	410	yeast HSP12	Saccharomyces cerevisiae	282-611
43	PHE0000038	411	corn cycD2.1	Zea mays	125-1156
44	PHE0000043	412	rice cycB1	Oryza sativa	148-1407
45	PHE0000044	413	rice cycC1	Oryza sativa	97-870
46	PHE0000045	414	rice cycB2	Oryza sativa	74-1336
47	PHE0000046	415	rice cycA1	Oryza sativa	97-1623
48	PHE0000047	416	rice cycB5	Oryza sativa	292-361,1019-1347,1447-1572,1657-1908,2059-2217,2315-2493,3276-3432
49	PHE0000050	417	corn cycA2	Zea mays	107-1222
50	PHE0000051	418	corn cycB2	Zea mays	137-1408
51	PHE0000052	419	corn cycB5	Zea mays	82-1518
52	PHE0000053	420	corn cycB4	Zea mays	254-1579
53	PHE0000054	421	corn cycD3.2	Zea mays	220-1380
54	PHE0000055	422	corn cycDx.1	Zea mays	218-1180
55	PHE0000056	423	corn cycD1.1	Zea mays	288-1334
56	PHE0000082	424	corn cycB3	Zea mays	88-1425
57	PHE0000101	425	corn cycD3.1	Zea mays	250-1422
58	PHE0000105	426	corn cycD1.2	Zea mays	229-1275
59	PHE0000106	427	corn cycA1	Zea mays	107-1633
60	PHE0000107	428	corn cycD1.3	Zea mays	206-1252
61	PHE0000382	429	corn cycB1	Zea mays	114-1385
62	PHE0000014	430	rice cycD2	Oryza sativa	13-324,623-709,813-911,1003-1204,1314-1438,1529-1774
63	PHE0000015	431	rice GCR1	Oryza sativa	312-500,1123-1154,1384-1553,2048-2163,2724-2825,2946-3002,3331-3474,3930-4000,4118-4223
64	PHE0000016	432	kn1	Zea mays	181-1257
65	PHE0000115	433	Receiver domain (RR3-like) 7	Zea mays	277-1002
66	PHE0000116	434	Receiver domain (ARR2-like) 1	Zea mays	188-2245
67	PHE0000117	435	Receiver domain (TOC1-like) 2	Zea mays	112-2238

NUCLEIC ACID SEQ ID NO	PHE_ID	AMINO_ACID SEQ ID NO	GENE_NAME	DONOR ORGANISM	CODING COORDINATES
68	PHE0000118	436	Receiver domain (TOC1-like) 3	Zea mays	84-1976
69	PHE0000119	437	Receiver domain (ARR2-like) 4	Zea mays	39-1931
70	PHE0000120	438	Receiver domain (RR11-like) 5	Zea mays	61-1812
71	PHE0000121	439	Receiver domain (RR3-like) 6	Zea mays	391-1116
72	PHE0000122	440	Receiver domain (RR3-like) 8	Zea mays	335-1066
73	PHE0000123	441	Receiver domain 9	Zea mays	55-759
74	PHE0000124	442	ZmRR2	Zea mays	154-624
75	PHE0000125	443	Receiver domain (TOC1-like) 10	Zea mays	374-722,791-2019
76	PHE0000272	444	corn GCR1	Zea mays	74-1036
77	PHE0000231	445	nucellin-like protein	Zea mays	122-1594
78	PHE0000232	446	nucellin-like protein	Zea mays	76-1605
79	PHE0000233	447	nucellin-like protein	Zea mays	195-1628
80	PHE0000269	448	soy E2F-like	Glycine max	80-1117
81	PHE0000270	449	nuclear matrix constituent protein	Zea mays	243-3371
82	PHE0000271	450	OsE2F1	Oryza sativa	93-1403
83	PHE0000067	451	yeast eIF-5A	Saccharomyces cerevisiae	569-1042
84	PHE0000068	452	yeast deoxyhypusine synthase	Saccharomyces cerevisiae	173-1336
85	PHE0000069	453	yeast L5	Saccharomyces cerevisiae	987-1880
86	PHE0000070	454	yeast ornithine decarboxylase	Saccharomyces cerevisiae	576-1976

NUCLEIC ACID SEQ ID NO	PHE_ID	AMINO_ACID SEQ ID NO	GENE_NAME	DONOR ORGANISM	CODING COORDINATES
87	PHE0000071	455	rice exportin 4-like	Oryza sativa	501-750,1257-1417,1735-1800,3104-3218,3318-3427,3525-3620,7587-7744,7828-7915,8565-8669,8774-8878,9421-9450,9544-9656,9732-9819,9961-10180,11034-11164,12058-12204,12770-12898,12975-13073,13221-13259,14674-14823
88	PHE0000072	456	yeast S-adenosylmethionine decarboxylase	Saccharomyces cerevisiae	415-1605
89	PHE0000073	457	corn S-adenosylmethionine decarboxylase 1	Zea mays	268-1365
90	PHE0000074	458	corn S-adenosylmethionine decarboxylase 2	Zea mays	581-1780
91	PHE0000204	459	deoxyhypusine synthase	Glycine max	26-1129
92	PHE0000291	460	deoxyhypusine synthase	Zea mays	54-1163
93	PHE0000292	461	corn eIF-5A	Zea mays	85-564
94	PHE0000286	462	oryzacystatin	Oryza sativa	108-527
95	PHE0000287	463	Similar to cysteine proteinase inhibitor	Oryza sativa	18-767
96	PHE0000288	464	cysteine proteinase inhibitor	Sorghum bicolor	135-461
97	PHE0000001	465	esk2-like - cellulose synthase	Zea mays	113-3061
98	PHE0000227	466	soy omega-3 fatty acid desaturase	Glycine max	138-1496
99	PHE0000258	467	AtFAD7	Arabidopsis thaliana	132-1472

NUCLEIC ACID SEQ ID NO	PHE_ID	AMINO_ACID SEQ ID NO	GENE_NAME	DONOR ORGANISM	CODING COORDINATES
100	PHE0000259	468	AtFAD8	Arabidopsis thaliana	61-1368
101	PHE0000260	469	desB	Synechocystis sp. PCC 6803	643-1719
102	PHE0000186	470	maize ferritin 2	Zea mays	3-758
103	PHE0000187	471	maize ferritin 1	Zea mays	34-795
104	PHE0000188	472	E. coli cytoplasmic ferritin	Escherichia coli	245-742
105	PHE0000102	473	AnFPPS (farnesyl-pyrophosphate synthetase)	Emericella nidulans	146-1186
106	PHE0000103	474	OsFPPS	Oryza sativa	42-1103
107	PHE0000104	475	corn FPPS 2	Zea mays	313-1377
108	PHE0000277	476	wheat G28-like	Triticum aestivum	65-877
109	PHE0000261	477	yeast glutamate decarboxylase	Saccharomyces cerevisiae	33-1790
110	PHE0000019	478	AOX1a	Oryza sativa	4531-4851,5011-5139,6072-6560,6663-6722
111	PHE0000020	479	alxA	Emericella nidulans	2189-2442,2492-2783,2843-3352
112	PHE0000095	480	HSF1	Saccharomyces cerevisiae	1017-3518
113	PHE0000096	481	Zm HSP101	Zea mays	436-1773,1878-2159,2281-2621,2711-2990,3079-3276,3371-3670
114	PHE0000097	482	HSP104	Saccharomyces cerevisiae	334-3060
115	PHE0000098	483	E. coli clpB	Escherichia coli	557-3130
116	PHE0000099	484	Synechocystis clpB	Synechocystis sp. PCC 6803	316-2931
117	PHE0000100	485	Xylella clpB	Xylella fastidiosa	187-2769
118	PHE0000192	486	soy HSF	Glycine max	23-1114
119	PHE0000193	487	soy HSF	Glycine max	93-992
120	PHE0000133	488	G protein b subunit	Zea mays	90-1229
121	PHE0000273	489	soy mlo-like	Glycine max	15-1532
122	PHE0000274	490	soy mlo-like	Glycine max	48-1841
123	PHE0000275	491	rice G alpha 1	Oryza sativa	106-1248
124	PHE0000276	492	soy G-gamma subunit	Glycine max	210-536
125	PHE0000062	493	sRAD54 - with NLS	Synechocystis sp. PCC 6803	437-3556

NUCLEIC ACID SEQ ID NO	PHE_ID	AMINO_ACID SEQ ID NO	GENE_NAME	DONOR ORGANISM	CODING COORDINATES
126	PHE0000063	494	T4 endonuclease VII (gp49) - with NLS	coliphage T4	603-1148
127	PHE0000169	495	maize p23	Zea mays	106-708
128	PHE0000170	496	maize cyclophilin	Zea mays	99-1757
129	PHE0000171	497	yeast HSP82	Saccharomyces cerevisiae	333-2462
130	PHE0000172	498	yeast SIT1	Saccharomyces cerevisiae	361-2130
131	PHE0000173	499	yeast CNS1	Saccharomyces cerevisiae	762-1919
132	PHE0000174	500	yeast HCH1	Saccharomyces cerevisiae	193-654
133	PHE0000298	501	rice p23 co-chaperone	Oryza sativa	68-706
134	PHE0000299	502	corn p23 co-chaperone	Zea mays	71-565
135	PHE0000300	503	rice p23 co-chaperone	Oryza sativa	124-642
136	PHE0000301	504	corn p23 co-chaperone	Zea mays	90-617
137	PHE0000436	505	rice cns1-like	Oryza sativa	121-1242
138	PHE0000437	506	corn HCH1-like 1	Zea mays	42-1100
139	PHE0000438	507	corn HOP-like 1	Zea mays	88-1830
140	PHE0000439	508	corn HOP-like 2	Zea mays	65-1261
141	PHE0000440	509	rice CHIP-like 1	Oryza sativa	121-939
142	PHE0000441	510	corn CHIP-like 2	Zea mays	115-939
143	PHE0000442	511	corn HSP90 1	Zea mays	55-2478
144	PHE0000443	512	rice HSP90 1	Oryza sativa	68-2500
145	PHE0000444	513	corn HSP90 2	Zea mays	63-2423
146	PHE0000445	514	sorghum HSP90 1	Sorghum bicolor	138-2285
147	PHE0000446	515	rice HSP90 2	Oryza sativa	78-2174
148	PHE0000215	516	invW	Oryza sativa	1108-1489,1813-2684,6105-6266,6417-6658,
149	PHE0000248	517	Zm lipoxygenase	Zea mays	89-2821
150	PHE0000249	518	corn allene oxide synthase	Zea mays	111-1556
151	PHE0000250	519	corn COI1-like	Zea mays	139-1911
152	PHE0000252	520	corn COI1-like	Zea mays	130-1923
153	PHE0000253	521	COI1-like	Zea mays	389-2368
154	PHE0000256	522	corn 1-aminocyclopropane-1-carboxylate oxidase	Zea mays	61-1011

NUCLEIC ACID SEQ ID NO	PHE_ID	AMINO_ACID SEQ ID NO	GENE_NAME	DONOR ORGANISM	CODING COORDINATES
155	PHE0000257	523	rice 1-aminocyclopropane-1 carboxylate synthase	Oryza sativa	2-1465
156	PHE0000432	524	corn 12-oxophytodienoate reductase 1	Zea mays	128-1240
157	PHE0000433	525	corn 12-oxo-phytodienoate reductase-like 3	Zea mays	166-1242
158	PHE0000434	526	corn 12-oxophytodienoate reductase-like 4	Zea mays	92-1210
159	PHE0000435	527	corn hydroperoxide lyase	Zea mays	83-1594
160	PHE0000484	528	soy JMT-like protien 1	Glycine max	26-1135
161	PHE0000485	529	corn JMT-like protein 1	Zea mays	39-1184
162	PHE0000486	530	corn JMT-like protein 2	Zea mays	63-1208
163	PHE0000077	531	yeast flavohemoglobin - chloroplastic	Saccharomyces cerevisiae	1695-2894
164	PHE0000039	532	nph1	Zea mays	415-3150
165	PHE0000176	533	RNAse S	Zea mays	85-771
166	PHE0000177	534	maize ecto-apyrase	Zea mays	210-2312
167	PHE0000178	535	PHO5	Saccharomyces cerevisiae	1-1404
168	PHE0000179	536	high affinity phosphate translocator	Glycine max	105-1703
169	PHE0000180	537	high affinity phosphate translocator	Zea mays	128-1750
170	PHE0000181	538	Xylella citrate synthase	Xylella fastidiosa	256-1545
171	PHE0000182	539	E. coli citrate synthase	Escherichia coli	309-1592
172	PHE0000183	540	rice citrate synthase	Oryza sativa	105-1523
173	PHE0000184	541	citrate synthase	Zea mays	56-1564
174	PHE0000185	542	citrate synthase	Glycine max	153-1691
175	PHE0000302	543	putative purple acid phosphatase precursor	Oryza sativa	22-1038

NUCLEIC ACID SEQ ID NO	PHE_ID	AMINO_ACID SEQ ID NO	GENE_NAME	DONOR ORGANISM	CODING COORDINATES
176	PHE0000303	544	acid phosphatase type 5	Zea mays	143-1186
177	PHE0000304	545	aleurone ribonuclease	Oryza sativa	47-814
178	PHE0000305	546	putative ribonuclease	Zea mays	55-888
179	PHE0000306	547	S-like RNase	Zea mays	15-770
180	PHE0000307	548	ribonuclease	Zea mays	95-781
181	PHE0000027	549	SbPhytochrome A	Sorghum bicolor	238-3633
182	PHE0000028	550	OsPhytochrome B	Oryza sativa	67-3582
183	PHE0000029	551	SbPhytochrome B	Sorghum bicolor	429-2640,3333-4140,5819-6112,7491-7713
184	PHE0000030	552	OsPhytochrome C	Oryza sativa	1036-3100,3205-4021,4418-4711,5272-5509
185	PHE0000031	553	SbPhytochrome C	Sorghum bicolor	303-3710
186	PHE0000032	554	Positive Factor 1	Oryza sativa	35-676
187	PHE0000033	555	GT-2	Oryza sativa	58-2271
188	PHE0000034	556	biliverdin IXa reductase	Synechocystis sp. PCC 6803	9-992
189	PHE0000049	557	OsPhytochrome A	Oryza sativa	4626-6690,6913-7729,8011-8307,8410-8617
190	PHE0000057	558	corn mt NDK - LIB189022Q1E 1E9	Zea mays	60-725
191	PHE0000058	559	corn cp NDK - 700479629	Zea mays	103-816
192	PHE0000059	560	corn NDK - LIB3597020Q1 K6C3	Zea mays	49-495
193	PHE0000060	561	corn NDK - 700241377	Zea mays	162-608
194	PHE0000061	562	OsGAI	Oryza sativa	216-2093
195	PHE0000064	563	corn NDPK - fC-zmemLIB39570 15Q1K6H6	Zea mays	91-624
196	PHE0000111	564	PAS domain kinase	Zea mays	358-2613
197	PHE0000126	565	corn HY5-like	Zea mays	32-541
198	PHE0000127	566	scarecrow 1 (PAT1-like)	Zea mays	295-1929
199	PHE0000128	567	scarecrow 2	Zea mays	153-1934
200	PHE0000283	568	scarecrow 6	Zea mays	520-2145

NUCLEIC ACID SEQ ID NO	PHE_ID	AMINO_ACID SEQ ID NO	GENE_NAME	DONOR ORGANISM	CODING COORDINATES
201	PHE0000293	569	gibberellin response modulator	Zea mays	131-2020
202	PHE0000294	570	scarecrow-like protein	Zea mays	266-1948
203	PHE0000308	571	helix-loop-helix protein (PIF3-like)	Zea mays	202-756
204	PHE0000318	572	scarecrow 17	Zea mays	441-2102
205	PHE0000361	573	PAT1-like scarecrow 9	Zea mays	191-1900
206	PHE0000427	574	corn SPA1-like 1	Zea mays	227-3139
207	PHE0000428	575	corn PIF3-like	Zea mays	173-856
208	PHE0000429	576	soy Athb-2-like 1	Glycine max	78-932
209	PHE0000430	577	corn SUB1-like 1	Zea mays	44-1954
210	PHE0000431	578	soy GH3 protein	Glycine max	42-1820
211	PHE0000065	579	TOR1	Saccharomyces cerevisiae	302-7714
212	PHE0000152	580	14-3-3-like protein 2	Glycine max	85-861
213	PHE0000153	581	14-3-3-like protein D	Glycine max	42-824
214	PHE0000154	582	14-3-3 protein 1	Glycine max	49-834
215	PHE0000155	583	Rice FAP1-like protein	Oryza sativa	654-1862,2310-2426,3407-3492,3590-3752,3845-3890,4476-4522,4985-5191,5306-5392,5473-5640
216	PHE0000156	584	rice TAP42-like	Oryza sativa	199-1338
217	PHE0000157	585	rice eIF-4E	Oryza sativa	58-741
218	PHE0000158	586	BMH1	Saccharomyces cerevisiae	79-882
219	PHE0000311	587	GF14-c protein	Oryza sativa	81-848
220	PHE0000312	588	14-3-3-like protein	Oryza sativa	6-785
221	PHE0000313	589	rice eIF-(iso)4F	Oryza sativa	96-713
222	PHE0000314	590	rice eIF-4F	Oryza sativa	46-726
223	PHE0000315	591	sorghum eIF-(iso)4F	Sorghum bicolor	78-707
224	PHE0000316	592	sorghum eIF-4F	Sorghum bicolor	9-668
225	PHE0000317	593	rice FIP37-like	Oryza sativa	73-1128
226	PHE0000040	594	Zm Hb1 - hemoglobin	Zea mays	172-669
227	PHE0000400	595	soy G559-like	Glycine max	301-1560

NUCLEIC ACID SEQ ID NO	PHE_ID	AMINO_ACID SEQ ID NO	GENE_NAME	DONOR ORGANISM	CODING COORDINATES
228	PHE0000091	596	Zm SET domain 2	Zea mays	101-1009
229	PHE0000092	597	Zm SET domain 1	Zea mays	528-1544
230	PHE0000114	598	Su(var) 3-9-like	Zea mays	71-814
231	PHE0000175	599	corn EZA1-like	Zea mays	34-2706
232	PHE0000282	600	SET-domain protein-like	Zea mays	478-3045
233	PHE0000075	601	retinoblastoma-related protein 1	Zea mays	37-2634
234	PHE0000076	602	C1 protein	Wheat dwarf virus	49-843
235	PHE0000006	603	RAP2.8/G9 transcription factor	Arabidopsis thaliana	81-1136
236	PHE0000007	604	rice RAP2.8/G9 transcription factor	Oryza sativa	336-1430
237	PHE0000008	605	rice RAP2.8/G9 transcription factor	Oryza sativa	572-1522
238	PHE0000012	606	rs81	Zea mays	1-747
239	PHE0000013	607	rs288	Zea mays	1-864
240	PHE0000244	608	corn SVP-like	Zea mays	177-860
241	PHE0000245	609	corn SVP-like	Zea mays	93-791
242	PHE0000246	610	soy SVP-like	Glycine max	96-713
243	PHE0000247	611	soy jointless-like	Glycine max	60-674
244	PHE0000451	612	wheat SVP-like 1	Triticum aestivum	149-736
245	PHE0000452	613	corn SVP-like 3	Zea mays	75-749
246	PHE0000453	614	corn SVP-like 5	Zea mays	304-774,956-1219
247	PHE0000087	615	STURDY	Arabidopsis thaliana	329-1267,1353-1562
248	PHE0000088	616	patatin-like protein	Nostoc PCC7120	451-2184
249	PHE0000220	617	corn RNase PH	Zea mays	86-805
250	PHE0000221	618	SKI2	Saccharomyces cerevisiae	1351-5211
251	PHE0000222	619	SKI3	Saccharomyces cerevisiae	793-5091
252	PHE0000223	620	SKI4	Saccharomyces cerevisiae	323-1201
253	PHE0000224	621	SKI6	Saccharomyces cerevisiae	1007-1747
254	PHE0000225	622	SKI7	Saccharomyces cerevisiae	279-2519

NUCLEIC ACID SEQ ID NO	PHE_ID	AMINO_ACID SEQ ID NO	GENE_NAME	DONOR ORGANISM	CODING COORDINATES
255	PHE0000226	623	rice SKI7-like	Oryza sativa	464-884,1132-1287,2103-2252,2353-2487,2957-3288,3399-3509,3596-4095,4350-4518,4783-5022,5097-5228,5315-5449
256	PHE0000309	624	SKI4-like protein	Zea mays	36-632
257	PHE0000310	625	putative 3 exoribonuclease	Zea mays	238-1098
258	PHE0000159	626	rice chloroplastic fructose-1,6-bisphosphatase	Oryza sativa	41-1261
259	PHE0000160	627	E. coli fructose-1,6-bisphosphatase	Escherichia coli	208-1206
260	PHE0000161	628	Synechocystis fructose-1,6-bisphosphatase F-I	Synechocystis sp. PCC 6803	1-1164
261	PHE0000162	629	Synechocystis fructose-1,6-bisphosphatase F-II	Synechocystis sp. PCC 6803	480-1523
262	PHE0000163	630	Rice TBP1	Oryza sativa	26-1315
263	PHE0000164	631	Yeast RPT5	Saccharomyces cerevisiae	883-2187
264	PHE0000165	632	Yeast RRP5	Saccharomyces cerevisiae	331-5520
265	PHE0000166	633	Rice CBP-like gene	Oryza sativa	277-436,479-1524,1790-2065,2150-2425,3134-3262,3380-3580,3683-3825,3905-4190,4294-4433,4711-4789,4874-4929,5754-5946

NUCLEIC ACID SEQ ID NO	PHE_ID	AMINO_ACID SEQ ID NO	GENE_NAME	DONOR ORGANISM	CODING COORDINATES
266	PHE0000167	634	rice BAB09754	Oryza sativa	616-903,1848-1940,2046-2165,2254-2355,2443-2693,2849-2994,3165-3363,3475-4141,4438-4770,5028-5309
267	PHE0000168	635	LIB3061-001-H7_FLI	Zea mays	309-1037
268	PHE0000295	636	ubiquitin-conjugating enzyme-like protein	Zea mays	114-599
269	PHE0000296	637	unknown protein recognized by PF01169	Zea mays	90-785
270	PHE0000297	638	26S protease regulatory subunit 6A homolog	Oryza sativa	57-1343
271	PHE0000454	639	fC-zmhuLIB3062-044-Q1-K1-B8	Zea mays	113-853
272	PHE0000455	640	corn E4/E8 binding protein-like	Zea mays	253-2259
273	PHE0000009	641	F9L1.31/G975 transcription factor	Arabidopsis thaliana	58-654
274	PHE0000010	642	rice G975 transcription factor	Oryza sativa	201-283,516-1161
275	PHE0000011	643	glossy15	Zea mays	385-1722
276	PHE0000079	644	CUT1	Oryza sativa	372-1082,1176-1946
277	PHE0000278	645	corn G975 transcription factor	Zea mays	41-679
278	PHE0000024	646	700456686H1	Zea mays	441-2390
279	PHE0000025	647	ZmGRF1	Zea mays	55-1470
280	PHE0000026	648	OsGRF1	Oryza sativa	193-1380
281	PHE0000289	649	Zm-GRF1 (GA responsive factor)	Zea mays	96-1202
282	PHE0000290	650	ZmSE001-like	Zea mays	253-2115
283	PHE0000216	651	thylakoid carbonic anhydrase, ecaA	Nostoc PCC7120	49-843

NUCLEIC ACID SEQ ID NO	PHE_ID	AMINO_ACID SEQ ID NO	GENE_NAME	DONOR ORGANISM	CODING COORDINATES
284	PHE0000217	652	bicarbonate transporter, LIP-36G1	Chlamydomonas reinhardtii	156-1232
285	PHE0000218	653	bicarbonate transporter, cp stromal IctB	Synechococcus sp. PCC 7942	271-1674
286	PHE0000219	654	thylakoid carbonic anhydrase, cah3	Chlamydomonas reinhardtii	62-994
287	PHE0000251	655	corn TIR1-like	Zea mays	113-1906
288	PHE0000254	656	F-box protein	Glycine max	123-1304
289	PHE0000255	657	F-box protein	Glycine max	228-1916
290	PHE0000357	658	maize tubby-like	Zea mays	519-1958
291	PHE0000358	659	maize tubby-like	Zea mays	517-1269
292	PHE0000359	660	soy HMG CoA synthase	Glycine max	80-1441
293	PHE0000360	661	yeast HMGS	Saccharomyces cerevisiae	220-1695
294	PHE0000319	662	maize MnSOD	Zea mays	249-947
295	PHE0000320	663	sodB	Escherichia coli	94-675
296	PHE0000321	664	Synechococcus sp. PCC 7942 sodB	Synechococcus sp. PCC 7942	142-747
297	PHE0000322	665	maize catalase-1	Zea mays	208-1683
298	PHE0000323	666	maize catalase-3	Zea mays	30-1511
299	PHE0000324	667	ascorbate peroxidase	Zea mays	197-1063
300	PHE0000325	668	corn GDI	Zea mays	57-1397
301	PHE0000326	669	soy GDI	Glycine max	45-1418
302	PHE0000327	670	corn rho GDI	Zea mays	463-1203
303	PHE0000328	671	basic blue copper protein	Zea mays	13-408
304	PHE0000329	672	plantacyanin	Zea mays	109-489
305	PHE0000330	673	basic blue copper protein	Glycine max	83-463
306	PHE0000331	674	Similar to blue copper protein precursor	Zea mays	323-868
307	PHE0000332	675	lamin	Zea mays	62-646
308	PHE0000333	676	allyl alcohol dehydrogenase	Zea mays	56-1105
309	PHE0000334	677	allyl alcohol dehydrogenase	Glycine max	103-1128
310	PHE0000335	678	allyl alcohol dehydrogenase	Zea mays	6-1079
311	PHE0000336	679	quinone oxidoreductase	Zea mays	47-1051

NUCLEIC ACID SEQ ID NO	PHE_ID	AMINO_ACID SEQ ID NO	GENE_NAME	DONOR ORGANISM	CODING COORDINATES
312	PHE0000337	680	E. nidulans cysA	Emericella nidulans	384-1961
313	PHE0000338	681	serine acetyltransferase	Synechocystis sp. PCC 6803	801-1547
314	PHE0000339	682	Synechocystis thiol-specific antioxidant protein	Synechocystis sp. PCC 6803	36-638
315	PHE0000340	683	yeast TSA2	Saccharomyces cerevisiae	108-698
316	PHE0000341	684	yeast mTPx	Saccharomyces cerevisiae	730-1512
317	PHE0000342	685	yeast nTPx	Saccharomyces cerevisiae	103-750
318	PHE0000343	686	yeast TPx III	Saccharomyces cerevisiae	657-1187
319	PHE0000344	687	corn type 2 peroxiredoxin	Zea mays	37-522
320	PHE0000345	688	soy putative 2-cys peroxiredoxin	Glycine max	160-939
321	PHE0000346	689	soy peroxiredoxin	Glycine max	104-745
322	PHE0000347	690	heat shock protein 26, plastid-localized	Zea mays	117-836
323	PHE0000348	691	heat shock protein hsp22 precursor, mitochondrial	Zea mays	79-732
324	PHE0000349	692	heat shock protein	Zea mays	112-735
325	PHE0000350	693	low molecular weight heat shock protein	Zea mays	28-690
326	PHE0000351	694	18kDa heat shock protein	Zea mays	103-597
327	PHE0000352	695	heat shock protein 16.9	Zea mays	229-690
328	PHE0000353	696	HSP21-like protein	Zea mays	73-696
329	PHE0000354	697	Opt1p	Saccharomyces cerevisiae	508-2904
330	PHE0000355	698	SVCT2-like permease	Zea mays	220-1779
331	PHE0000356	699	SVCT2-like permease	Zea mays	34-1632
332	PHE0000385	700	H+ transporting ATPase	Zea mays	176-2836

NUCLEIC ACID SEQ ID NO	PHE_ID	AMINO_ACID SEQ ID NO	GENE_NAME	DONOR ORGANISM	CODING COORDINATES
333	PHE0000386	701	cation-transporting ATPase	Zea mays	222-2168
334	PHE0000387	702	yeast DRS2 (ALA1-like)	Saccharomyces cerevisiae	170-4237
335	PHE0000388	703	S. pombe ALA1-like	Schizosaccharo myces pombe	56-3832
336	PHE0000389	704	rice ALA1-like 1	Oryza sativa	47-1538,1619-1925,3116-3824,3920-4043,4143-4362,4590-5048,5937-6153
337	PHE0000469	705	yeast YKL091c	Saccharomyces cerevisiae	110-1042
338	PHE0000470	706	corn Ssh1-like protein 1	Zea mays	57-1037
339	PHE0000471	707	corn Ssh1-like protein 3	Zea mays	89-841
340	PHE0000472	708	corn Ssh1-like protein 4	Zea mays	309-1196
341	PHE0000473	709	soy Ssh1-like protein 2 [ssh2]	Glycine max	209-976
342	PHE0000017	710	Zm-AAA1 - AAA ATPase	Zea mays	184-2214
343	PHE0000018	711	Zm-AAA2 - AAA ATPase	Zea mays	104-2533
344	PHE0000395	712	soy phantastica	Glycine max	275-1345
345	PHE0000396	713	soy phantastica 2	Glycine max	178-1260
346	PHE0000397	714	maize rough sheath 1	Zea mays	92-1144
347	PHE0000398	715	soy lg3-like 1	Glycine max	103-1026
348	PHE0000399	716	soy rough sheath1-like 1	Glycine max	144-1076
349	PHE0000401	717	soy myb transcription factor - G1635-like 1	Glycine max	28-888
350	PHE0000411	718	corn monosaccharide transporter 2	Zea mays	331-2565
351	PHE0000412	719	corn monosaccharide transporter 1	Zea mays	75-1643
352	PHE0000413	720	soy monosaccharide transporter 3	Glycine max	132-1685
353	PHE0000414	721	corn monosaccharide transporter 3	Zea mays	141-1670

NUCLEIC ACID SEQ ID NO	PHE_ID	AMINO_ACID SEQ ID NO	GENE_NAME	DONOR ORGANISM	CODING COORDINATES
354	PHE0000415	722	soy monosaccharide transporter 1	Glycine max	160-1899
355	PHE0000416	723	corn monosaccharide transporter 6	Zea mays	74-1690
356	PHE0000418	724	corn monosaccharide transporter 4	Zea mays	146-1744
357	PHE0000419	725	soy monosaccharide transporter 2	Glycine max	63-1505
358	PHE0000420	726	soy sucrose transporter	Glycine max	63-1595
359	PHE0000421	727	corn sucrose transporter 2	Zea mays	76-1599
360	PHE0000422	728	corn monosaccharide transporter 8	Zea mays	201-1763
361	PHE0000423	729	corn monosaccharide transporter 7	Zea mays	93-1634
362	PHE0000390	730	rice chloroplastic sedoheptulose-1,7-bisphosphatase	Oryza sativa	136-1311
363	PHE0000391	731	rice cytosolic fructose-1,6-bisphosphatase	Oryza sativa	171-1187
364	PHE0000392	732	Wheat sedoheptulose-1,7-bisphosphatase	Triticum aestivum	14-1192
365	PHE0000393	733	dual function SBPase/FBPase	Ralstonia eutropha	80-1399
366	PHE0000394	734	sedoheptulose-1,7-bisphosphatase	Chlorella sorokiniana	90-1238
367	PHE0000425	735	soy isoflavone synthase	Glycine max	45-1607
368	PHE0000426	736	soy ttg1-like 2	Glycine max	52-1059

Plant Transformation Constructs

The construction of vectors which may be used in the invention will be known to those of skill of the art in light of the present disclosure. The techniques of the current invention are thus not limited to any particular DNA or method of plant transformation. In preparing populations of transgenic plants for phenotype screening the GATEWAY™ cloning technology (available

from Invitrogen Life Technologies, Carlsbad, California) is useful for construction of vectors for transgenic DNA constructs that can be used in transformation. GATEWAY™ vector construction technology uses the site specific recombinase LR cloning reaction of the Integrase/*att* system from bacterophage lambda vector construction, instead of restriction endonucleases and ligases. The LR cloning reaction is disclosed in U.S. Patents 5,888,732 and 6,277,608, U.S. published patent application 20020007051 and International Patent Publication WO 02/081711 A1, all of which are incorporated herein by reference, and in the GATEWAY™ Cloning Technology Instruction Manual. The GATEWAY™ technology produces a high frequency of inserts in a plasmid in the correct orientation relative to other elements in the plasmid such as promoters, enhancers, and the such. Routine cloning of any desired DNA sequence into a vector comprising operable plant expression elements is thereby facilitated. Using the GATEWAY™ cloning technology, a desired DNA sequence, such as a coding sequence, may be amplified by PCR with the phage lambda *att*B1 sequence added to the 5' primer and the *att*B2 sequence added to the 3' primer. Alternatively, nested primers comprising a set of *att*B1 and *att*B2 specific primers and a second set of primers specific for the selected DNA sequence can be used. Sequences, such as coding sequences, flanked by *att*B1 and *att*B2 sequences can be readily inserted into plant expression vectors using GATEWAY™ methods. In a more direct route a construct of interest flanked by *att*L1 and *att*L2 sequences can be incorporated by recombination into a plasmid destination vector comprising a bacterial negative marker flanked by *att*R1 and *att*R2 sites using LR clonase.

It is also contemplated that one may employ multiple genes on either the same or different vectors for transformation. In the latter case, the different vectors may be delivered concurrently to recipient cells if co-transformation into a single chromosomal location is desired.

Transgenic DNA constructs used for transforming plant cells will comprise the heterologous DNA which one desires to introduced into and a promoter to express the heterologous DNA in the host maize cells. As is well known in the art such constructs can further include elements such as regulatory elements, 3' untranslated regions (such as polyadenylation sites), transit or signal peptides and marker genes elements as desired.

1. Regulatory Elements

A number of promoters that are active in plant cells have been described in the literature both constitutive and tissue specific promoters and inducible promoters. See the background

section of U.S. Patent 6,437,217 for a description of a wide variety of promoters that are functional in plants. Such promoters include the nopaline synthase (NOS) and octopine synthase (OCS) promoters that are carried on tumor-inducing plasmids of *Agrobacterium tumefaciens*, the caulimovirus promoters such as the cauliflower mosaic virus (CaMV) 19S and 35S promoters and the figwort mosaic virus (FMV) 35S promoter, the enhanced CaMV35S promoter (e35S), the light-inducible promoter from the small subunit of ribulose bisphosphate carboxylase (ssRUBISCO, a very abundant plant polypeptide). For instance, see U.S. Patents 6,437,217 which discloses a maize RS81 promoter, 5,641,876 which discloses a rice actin promoter, 6,426,446 which discloses a maize RS324 promoter, 6,429,362 which discloses a maize PR-1 promoter, 6,232,526 which discloses a maize A3 promoter and 6,177,611 which discloses constitutive maize promoters, all of which are incorporated herein by reference. The rice actin 1 promoter with a rice actin intron is especially useful in the practice of the present invention.

It is preferred that the particular promoter selected should be capable of causing sufficient expression to result in the production of an effective amount of the heterologous DNA of interest. The promoters used in the transgenic DNA constructs of the present invention may be modified, if desired, to affect their control characteristics. Promoters can be derived by means of ligation with operator regions, random or controlled mutagenesis, etc. Furthermore, the promoters may be altered to contain multiple "enhancer sequences" to assist in elevating gene expression. Such enhancers are known in the art. By including an enhancer sequence with such constructs, the expression of the selected protein may be enhanced. These enhancers often are found 5' to the start of transcription in a promoter that functions in eukaryotic cells, but can often be inserted in the forward or reverse orientation 5' or 3' to the coding sequence. In some instances, these 5' enhancing elements are introns. Deemed to be particularly useful as enhancers are the 5' introns of the rice actin 1 and rice actin 2 genes. Examples of other enhancers which could be used in accordance with the invention include elements from the CaMV 35S promoter, octopine synthase genes, the maize alcohol dehydrogenase gene, the maize shrunken 1 gene and promoters from non-plant eukaryotes.

Where an enhancer is used in conjunction with a promoter for the expression of a selected protein, it is believed that it will be preferred to place the enhancer between the promoter and the start codon of the selected coding region. However, one also could use a different arrangement of the enhancer relative to other sequences and still realize the beneficial

properties conferred by the enhancer. For example, the enhancer could be placed 5' of the promoter region, within the promoter region, within the coding sequence (including within any other intron sequences which may be present), or 3' of the coding region.

In addition to introns with enhancing activity, other types of elements can influence gene expression. For example, untranslated leader sequences predicted to enhance gene expression as well as "consensus" and preferred leader sequences have been identified. Preferred leader sequences are contemplated to include those which have sequences predicted to direct optimum expression of the attached coding region, *i.e.*, to include a preferred consensus leader sequence which may increase or maintain mRNA stability and prevent inappropriate initiation of translation. The choice of such sequences will be known to those of skill in the art in light of the present disclosure. Sequences that are derived from genes that are highly expressed in plants, and in maize in particular, will be most preferred, for example, sequences derived from the small subunit of ribulose bisphosphate carboxylase (RUBISCO).

In general it is preferred to introduce heterologous DNA randomly, i.e. at a non-specific location, in the genome of a parental maize line. In special cases it may be useful to target heterologous DNA insertion in order to achieve site specific integration, e.g. to replace an existing gene in the genome. In some other cases it may be useful to target a heterologous DNA integration into the genome at a predetermined site from which it is known that gene expression occurs. Several site specific recombination systems exist which are known to function implants include cre-lox as disclosed in U.S. Patent 4,959,317 and FLP-FRT as disclosed in U.S. Patent 5,527,695, both incorporated herein by reference.

2. 3' Untranslated Regions (3' UTR)

Transformation constructs prepared in accordance with the invention will typically include a 3' end untranslated sequence DNA sequence that follows the coding sequence and typically contains a polyadenylation sequence. One type of 3' untranslated sequence which may be used is a 3' UTR from the nopaline synthase gene of *Agrobacterium tumefaciens* (*nos* 3' end). Where a 3' end other than a *nos* 3' UTR is used in accordance with the invention, the most preferred 3' ends are contemplated to be those from a gene encoding the small subunit of a ribulose-1,5-bisphosphate carboxylase-oxygenase (rbcS), and more specifically, from a rice rbcS gene (see PCT Publication WO 00/70066), the 3' UTR for the T7 transcript of *Agrobacterium tumefaciens*, the 3' end of the protease inhibitor I or II genes from potato or tomato, and the 3'

region isolated from Cauliflower Mosaic Virus. Alternatively, one also could use a gamma coixin, oleosin 3 or other 3' UTRs from the genus *Coix* (see PCT Publication WO 99/58659).

3. Transit or Signal Peptides

Sequences that are joined to the coding sequence of an expressed gene, which are removed post-translationally from the initial translation product and which facilitate the transport of the protein into or through intracellular or extracellular membranes, are termed transit sequences (usually into vacuoles, vesicles, plastids and other intracellular organelles) and signal sequences (usually to the endoplasmic reticulum, golgi apparatus, peroxisomes or glyoxysomes, and outside of the cellular membrane). By facilitating the transport of the protein into compartments inside and outside the cell, these sequences may increase the accumulation of a gene product protecting the protein from intracellular proteolytic degradation. These sequences also allow for additional mRNA sequences from highly expressed genes to be attached to the coding sequence of the genes. Since mRNA being translated by ribosomes is more stable than naked mRNA, the presence of translatable mRNA 5' of the gene of interest may increase the overall stability of the mRNA transcript from the gene and thereby increase synthesis of the gene product. Since transit and signal sequences are usually post-translationally removed from the initial translation product, the use of these sequences allows for the addition of extra translated sequences that may not appear on the final polypeptide. It further is contemplated that targeting of certain proteins may be desirable in order to enhance the efficacy or stability of the protein as disclosed in U.S. Patent 5,545,818, incorporated herein by reference.

A particular example of such a use concerns the direction of a protein conferring herbicide resistance, such as a glyphosate resistant EPSPS protein, to a particular organelle such as the chloroplast, rather than to the cytoplasm. This is exemplified by the use of the rbcS transit peptide, the chloroplast transit peptide described in U.S. Patent 5,728,925, or the optimized transit peptide described in U.S. Patent 5,510,471, which confer plastid-specific targeting of proteins, both of which are incorporated herein by reference. In addition, it may be desirable to target certain genes responsible for male sterility to the mitochondria, or to target certain genes for resistance to phytopathogenic organisms to the extracellular spaces, or to target proteins to the vacuole. A further use concerns the direction of enzymes involved in amino acid biosynthesis or oil synthesis to the plastid.

Additionally, vectors may be constructed and employed in the intracellular targeting of a specific gene product within the cells of a transgenic plant or in directing a protein to the extracellular environment. This generally will be achieved by joining a DNA sequence encoding a transit or signal peptide sequence to the coding sequence of a particular gene. An intracellular targeting DNA sequence may be operably linked 5' or 3' to the coding sequence depending on the particular targeting sequence. The resultant transit, or signal, peptide will transport the protein to a particular intracellular, or extracellular destination, respectively, and will then be post-translationally removed.

4. Marker Genes

In practice DNA is introduced into only a small percentage of target cells in any one experiment. Marker genes are used to provide an efficient system for identification of those cells that are stably transformed by receiving and integrating a transgenic DNA construct into their genomes. Preferred marker genes provide selective markers which confer resistance to a selective agent, such as an antibiotic or herbicide. Potentially transformed cells are exposed to the selective agent. In the population of surviving cells will be those cells where, generally, the resistance-conferring gene has been integrated and expressed at sufficient levels to permit cell survival. Cells may be tested further to confirm stable integration of the exogenous DNA. Useful selective marker genes include those conferring resistance to antibiotics such as kanamycin (*nptII*), hygromycin B (*aph IV*) and gentamycin (*aac3* and *aac*C4) or resistance to herbicides such as glufosinate (*bar* or *pat*) and glyphosate (EPSPS). Examples of such selectable are illustrated in U.S. Patents 5,550,318; 5,633,435; 5,780,708 and 6,118,047, all of which are incorporated herein by reference. Screenable markers which provide an ability to visually identify transformants can also be employed, *e.g.*, a gene expressing a colored or fluorescent protein such as a luciferase or green fluorescent protein (GFP) or a gene expressing a *beta*-glucuronidase or *uid*A gene (GUS) for which various chromogenic substrates are known. It is also contemplated that combinations of screenable and selectable markers will be useful for identification of transformed cells. See PCT publication WO 99/61129 which discloses use of a gene fusion between a selectable marker gene and a screenable marker gene, e.g. an NPTII gene and a GFP gene.

Culturing Transgenic Cells

Cells that survive the exposure to the selective agent, or cells that have been scored positive in a screening assay, may be cultured in media that supports regeneration of plants. Well known cell culture media, e.g. designated as MS and N6, may be modified by including further substances such as growth regulators. Preferred growth regulators for plant regeneration include cytokins such as 6-benzylamino pierine, zeahin or the like, and abscisic acid which facilitate the growth of cells at specific developmental stages. Tissue may be maintained on a media with auxin type growth regulators until sufficient tissue is available to begin plant regeneration efforts, or following repeated rounds of manual selection, until the morphology of the tissue is suitable for regeneration, then transferred to media conducive to maturation of embryoids. Cultures are transferred every 1-4 weeks, preferably every 2-3 weeks on this medium. Shoot development will signal the time to transfer to medium lacking growth regulators.

The transformed cells, identified by selection or screening and cultured in an appropriate medium that supports regeneration, are allowed to mature into plants. Developing plantlets can be transferred to soil less plant growth mix, and hardened off, *e.g.*, in an environmentally controlled chamber at about 85% relative humidity, 600 ppm CO_2, and 25-250 microeinsteins $m^{-2} s^{-1}$ of light, prior to transfer to a greenhouse or growth chamber for maturation. Plants are preferably matured either in a growth chamber or greenhouse. Plants are regenerated from about 6 wk to 10 months after a transformant is identified, depending on the initial tissue. During regeneration, cells are grown to plants on solid media at about 19 to 28 °C. After the regenerating plants have reached the stage of shoot and root development, they may be transferred to a greenhouse for further growth and testing. Plants may be pollinated using conventional plant breeding methods known to those of skill in the art and seed produced.

Progeny may be recovered from transformed plants and tested for expression of the exogenous expressible gene. Note however, that seeds on transformed plants may occasionally require embryo rescue due to cessation of seed development and premature senescence of plants. To rescue developing embryos, they are excised from surface-disinfected seeds 10-20 days post-pollination and cultured. An embodiment of media used for culture at this stage comprises MS salts, 2% sucrose, and 5.5 g/l agarose. In embryo rescue, large embryos (defined as greater than 3 mm in length) are germinated directly on an appropriate media. Embryos smaller than that

may be cultured for 1 wk on media containing the above ingredients along with 10^{-5}M abscisic acid and then transferred to growth regulator-free medium for germination.

Characterization

To confirm the presence of heterologous exogenous DNA or other exogenous "transgene(s)" in the regenerating plants or transformed callus a variety of assays may be performed. Such assays include, for example, "molecular biological" assays, such as Southern and Northern blotting and PCR; "biochemical" assays, such as detecting the presence of RNA, e.g. double stranded RNA, or a protein product, *e.g.*, by immunological means (ELISAs and Western blots) or by enzymatic function; plant part assays, such as leaf or root assays; and also, by analyzing the phenotype of the whole regenerated plant.

1. DNA Integration, RNA Expression and Inheritance

The presence of DNA elements introduced through the methods of this invention may be determined by polymerase chain reaction (PCR). Using this technique discreet fragments of DNA are amplified and detected by gel electrophoresis. This type of analysis permits one to determine whether a gene is present in a stable transformant, but does not necessarily prove integration of the introduced gene into the host cell genome. Typically, DNA has been integrated into the genome of all transformants that demonstrate the presence of the DNA through PCR analysis. In addition, it is possible using PCR techniques to determine whether transformants have exogenous genes introduced into different sites in the genome, *i.e.*, whether transformants are of independent origin by using PCR techniques to clone fragments of the host genomic DNA adjacent to an introduced DNA.

Positive proof of DNA integration into the host genome and the independent identities of transformants may be determined using the technique of Southern hybridization. Using this technique specific DNA sequences that were introduced into the host genome and flanking host DNA sequences can be identified. Hence the Southern hybridization pattern of a given transformant serves as an identifying characteristic of that transformant. In addition, it is possible through Southern hybridization to demonstrate the presence of introduced genes in high molecular weight DNA, *i.e.*, confirm that the introduced gene has been integrated into the host cell genome. The technique of Southern hybridization provides information that is obtained

using PCR, *e.g.*, the presence of a gene, but also demonstrates integration into the genome and characterizes each individual transformant.

While DNA analysis techniques may be conducted using DNA isolated from any part of a plant, RNA will only be expressed in particular cells or tissue types and hence it will be necessary to prepare RNA for analysis from these tissues. PCR techniques, referred to as RT-PCR, also may be used for detection and quantitation of RNA produced from introduced genes. In this RT-PCR it is first necessary to reverse transcribe RNA into DNA, using enzymes such as reverse transcriptase, and then through the use of conventional PCR techniques amplify the DNA. In most instances PCR techniques will not demonstrate integrity of the RNA product. Northern blotting will demonstrate the presence of an RNA species and give information about the integrity of that RNA. It is further contemplated that TAQMAN® technology (available from Applied Biosystems, Foster City, CA) may be used to quantitate both DNA and RNA in a transgenic cell.

Methods of Evaluating Phenotype

Expression, and in some cases suppression, of the various genes embodied by heterologous DNA used in the present invention leads to improved phenotypes in transformed plants. Phenotypic data is collected during the transformation process in callus as well as during plant regeneration, as well as in plant tissue. Phenotypic data can also be collected in transformed callus relating to the morphological appearance as well as growth of the callus, e.g., shooty, rooty, starchy, mucoid, non-embryogenic, increased growth rate, decreased growth rate, dead. It is expected that one of skill in the art may recognize other phenotypic characteristics in transformed callus and plants and select transformed plants having enhanced traits with minimal drag on other key traits, e.g. yield. Phenotypic data is also collected during the process of plant regeneration as well as in regenerated plants transferred to soil. It is expected that one of skill in the art may recognize other phenotypic characteristics in transformed plants.

Although a wide variety of phenotypes are monitored during the process of plant breeding and testing in both inbred and hybrid plants. For example, in R0 plants (plants directly regenerated from callus) and R1 plants (the direct progeny of R0 plants), plant characteristic phenotypes and plant seed characteristic phenotypes can be observed. In R2 and R3 plants, days to pollen shed, days to silking, and plant type can be observed. Metabolite profiling of R2 plants

can be conducted. A variety of phenotypes can also be assayed in hybrids of transgenic maize of this invention. For example, yield, moisture, test weight, nutritional composition, chlorophyll content, leaf temperature, stand, seedling vigor, plant height, leaf number, tillering, brace roots, stay green, stalk lodging, root lodging, plant health, barreness/prolificacy, green snap, pest resistance (including diseases, viruses and insects) and metabolic profiles can be recorded. In addition, phenotypic characteristics of grain harvested from hybrids will be recorded, including number of kernels per row on the ear, number of rows of kernels on the ear, kernel abortion, kernel weight, kernel size, kernel density and physical grain quality. Furthermore, characteristics such as photosynthesis, leaf area, husk structure, kernel dry down rate and internode length may be measured in hybrids or inbreds. It is expected that transcriptional profiling may be performed on transgenic plants expressing genes of the present invention.

In a further embodiment of the method of the invention, the transformation and selection steps may be followed by conventional plant improvement techniques thus leading to seeds having an even further improvement in the enhanced phenotype. In still another embodiment the seeds of the invention may be subjected to one or more further transformation treatments.

The maize plants with enhanced phenotype may be used in breeding programs for the development of elite maize lines or hybrids, which programs are aimed at the production of varieties meeting the requirements of farming practice regarding yield, disease resistance and other agronomically important traits in major maize growing areas in the world. Seeds resulting from these programs may be used in the growing of commercial maize crops.

To confirm hybrid yield in transgenic plants expressing genes of the present invention, it may be desirable that hybrids be tested over multiple years at multiple locations in a geographical location where maize is conventionally grown, e.g. in Iowa, Illinois or other locations in the midwestern United States, under "normal" field conditions as well as under stress conditions, e.g. under drought or population density stress. One of skill in the art knows how to design a yield trial such that a statistically significant yield difference can be detected between two hybrids at the desired rate of precision.

Plant Breeding

Backcrossing can be used to improve a starting plant. Backcrossing transfers a specific desirable trait from one source to an inbred or other plant that lacks that trait. This can be

accomplished, for example, by first crossing a superior inbred (A) (recurrent parent) to a donor inbred (non-recurrent parent), which carries the appropriate gene(s) for the trait in question, for example, a construct prepared in accordance with the current invention. The progeny of this cross first are selected in the resultant progeny for the desired trait to be transferred from the non-recurrent parent, then the selected progeny are mated back to the superior recurrent parent (A). After five or more backcross generations with selection for the desired trait, the progeny are hemizygous for loci controlling the characteristic being transferred, but are like the superior parent for most or almost all other genes. The last backcross generation would be selfed to give progeny which are pure breeding for the gene(s) being transferred, *i.e.* one or more transformation events.

Therefore, through a series a breeding manipulations, a selected transgene may be moved from one line into an entirely different line without the need for further recombinant manipulation. Transgenes are valuable in that they typically behave genetically as any other gene and can be manipulated by breeding techniques in a manner identical to any other corn gene. Therefore, one may produce inbred plants which are true breeding for one or more transgenes. By crossing different inbred plants, one may produce a large number of different hybrids with different combinations of transgenes. In this way, plants may be produced which have the desirable agronomic properties frequently associated with hybrids ("hybrid vigor"), as well as the desirable characteristics imparted by one or more transgene(s).

It is desirable to introgress the genes of the present invention into maize hybrids for characterization of the phenotype conferred by each gene in a transformed plant. The host genotype into which the transgene was introduced, preferably LH59, is an elite inbred and therefore only limited breeding is necessary in order to produce high yielding maize hybrids. The transformed plant, regenerated from callus is crossed, to the same genotype, e.g., LH59. The progeny are self pollinated twice and plants homozygous for the transgene are identified. Homozygous transgenic plants are crossed to a testcross parent in order to produce hybrids. The test cross parent is an inbred belonging to a heterotic group which is different from that of the transgenic parent and for which it is known that high yielding hybrids can be generated, for example hybrids are produced from crosses of LH59 to either LH195 or LH200.

The present invention will be further illustrated by means of the following examples which are given for illustration purposes only and are in no way intended to limit the scope of the

invention.

Materials used in the Examples

DNA constructs for use in this invention can be fabricated using Gateway® technology as described above. Figure 1 shows the elements of a plasmid, designated as pMON72472, which is useful as a destination vector into which the transgenic DNA construct can be cloned to provide a transformation vector for use in an Agrobacterium-mediated transformation. Figure 1 further illustrates restriction sites on plasmid pMON72472 which are useful for modification of the plasmid. The elements of the plasmid are summarized in Table 2. The plasmid comprises left and right T-DNA border sequences from *Agrobacterium*. The right border sequence is located 5' to the rice actin 1 promoter and the left border sequence is located 3' to the *pin*II 3' sequence situated 3' to the *npt*II gene. Furthermore the original pSK- backbone of pMON65164 is replaced by a plasmid backbone to facilitate replication of the plasmid in both *E. coli* and *Agrobacterium tumefaciens*. The backbone comprises an *ori*V wide host range origin of DNA replication functional in *Agrobacterium*, the *rop* sequence, a pBR322 origin of DNA replication functional in *E. coli* and a spectinomycin/streptomycin resistance gene for selection for the presence of the plasmid in both *E. coli* and *Agrobacterium*.

Table 2

Genetic Elements of Plasmid Vector pMON72472

CASSETTE	FUNCTION	ELEMENT	LOCATION	REFERENCE
Plant gene of interest expression	Promoter	Rice actin 1	5610-6452	Wang *et al.*, Molecular and Cellular Biology, Aug. 1992, p. 3399-3406
	Enhancer	Rice actin 1 exon 1, intron 1	6453-6984	Wang *et al.*, 1992
GATEWAY™ cloning	Recombination	*Att*R1	7002-7126	GATEWAY™Cloning Technology Instruction Manual (Invitrogen Life Technologies, Carlsbad, CA)

CASSETTE	FUNCTION	ELEMENT	LOCATION	REFERENCE
	Bacterial chloramphenical resistance gene	CmR gene	7235-7894	GATEWAY™Cloning Technology Instruction Manual (Invitrogen Life Technologies, Carlsbad, CA)
	Bacterial negative selectable markers	*Ccd*A, *ccd*B genes	8014-8541	GATEWAY™Cloning Technology Instruction Manual (Invitrogen Life Technologies, Carlsbad, CA)
	GATEWAY™ recombination site	*att*R2	8582-8706	GATEWAY™Cloning Technology Instruction Manual (Invitrogen Life Technologies, Carlsbad, CA)
Plant gene of interest expression cassette	3' region	Potato pinII	8721-9660	An *et al.*, The Plant Cell, Vol. 1, 115-122, January 1989
Plant selectable marker gene expression cassette	Promoter	Cauliflower Mosaic Virus 35S	1-324	Odell *et al.*, Nature, Vol. 313, 28 Feb 1985, p. 810
	Selectable marker gene	NptII	358-1152	Beck *et al.*, Gene, 19 (1982), 327-336
	3' region	Nos	1178-1433	Bevan *et al.*, Nucleic Acids Research, vol. 11, no. 2, 1983, p. 369
	3' region	PinII	1445-2191	An *et al.*, 1989
Agrobacterium mediated transformation	DNA transfer	Left border	2493-2516	Zambryski *et al.*, J. Mol. Appl. Genet., 1, 361-370 (1982); GenBank Accession AJ237588
Maintenance of plasmid in *E. coli* or *Agrobacterium*	Origin of replication	Ori-V	2755-3147	Honda *et al.*, Gene, 1988 Sep 7; 68(2) :221-8
Maintenance of plasmid in *E. coli*	Origin of replication	*ColE*1	3545-4199	Oka *et al.*, Mol Gen Genet, 1979 May 4, 172(2) :151-9

CASSETTE	FUNCTION	ELEMENT	LOCATION	REFERENCE
Maintenance of plasmid in *E. coli* or *Agrobacterium*	Spectinomycin/streptomycin resistance	Spc/Str	4242-5030	Fling *et al.* , Nucleic Acids Res., 1985 Oct 11; 13(19): 7095-7106
Agrobacterium mediated transformation	DNA transfer	Right border	5514-5538	Zambryski *et al.*, 1982; GenBank Accession AJ237588

Protein coding segments are amplified by PCR prior to insertion in a destination vector such as pMON72472. Primers for PCR amplification can be designed at or near the start and stop codons of the coding sequence, in order to eliminate most of the 5' and 3' untranslated regions. PCR products are tailed with *att*B1 and *att*B2 sequences, purified then recombined into a destination vectors to produce an expression vector which can be used in transformation.

All PCR amplification products are sequenced prior to introduction into a plant. PCR inserts in destination vectors are sequenced to confirm that the inserted sequenced encoded the expected amino acid sequence. See Table 1 for identification of protein coding sequence which was placed in transgenic DNA constructs in expression vectors.

Example 1

This example illustrates the preparation of recipient cells from a parental maize line. Parental maize plants of line LH59 were grown in the greenhouse. Ears are harvested from plants when the embryos were 1.5 to 2.0 mm in length, usually 10 to 15 days after pollination, and most frequently 11 to 12 days after pollination. Ears were surface sterilized by spraying or soaking the ears in 80% ethanol, followed by air drying. Alternatively, ears were surface sterilized by immersion in 50% CLOROX™ containing 10% SDS for 20 minutes, followed by three rinses with sterile water.

Immature embryos were isolated from individual kernels using methods known to those of skill in the art. Immature embryos were cultured on medium 211 (N6 salts, 2% sucrose, 1 mg/L 2,4-D, 0.5 mg/L niacin, 1.0 mg/L thiamine-HCl, 0.91 g/L L-asparagine, 100 mg/L myo-inositiol, 0.5 g/L MES, 100 mg/L casein hydrolysate, 1.6 g/L $MgCl_2$, 0.69 g/L L-proline, 2 g/L GELGRO™, pH 5.8) containing 16.9 mg/L $AgNO_3$ (designated medium 211V) for 3-6 days prior to *Agrobacterium*-mediated transformation, preferably 3-4 days prior to microprojectile bombardment.

Example 2

This example illustrates the transformation of maize immature embryos using *Agrobacterium tumefaciens,* strain ABI. The ABI strain of *Agrobacterium* is derived from strain A208, a C58 nopaline type strain. The ABI strain of *Agrobacterium* is derived from strain A208, a C58 nopaline type strain, from which the Ti plasmid was eliminated by culture at 37^{o}C, and further containing the modified Ti plasmid pMP90RK. An *Agrobacterium tumefaciens* binary vector system is preferably used to transform maize. See Klee *et al., Agrobacterium*-mediated plant transformation and its further applications to plant biology. Annu. Rev. Plant Physiol. Plant Mol Biol. 1987;38:467-486.

Prior to co-culture of maize cells, *Agrobacterium* cells may be grown at 28^{o}C in LB (DIFCO) liquid medium comprising appropriate antibiotics to select for maintenance of the modified Ti plasmid and binary vector. It is well known to those skilled in the art to use appropriate selection agents to maintain plasmids in the host *Agrobacterium* strain. For example, ABI/expression vector may be grown in LB medium containing 50 ug/ml kanamycin to select for maintenance of the pMP90RK modified Ti plasmid and 100 ug/ml spectinomycin to select for maintenance of the expression vector. Prior to inoculation of maize cells, *Agrobacterium* cells are grown overnight at room temperature in AB medium comprising appropriate antibiotics for plasmid maintenance and 200 uM acetosyringone. Immediately prior to inoculation of maize cells, *Agrobacterium* are preferably pelleted by centrifugation, washed in ½ MSVI medium (2.2 g/L GIBCO (Carlsbad, CA) MS salts, 2 mg/L glycine, 0.5 g/L niacin, 0.5 g/L L-pyridoxine-HCl, 0.1 mg/L thiamine, 115 g/L L-proline, 10 g/L D-glucose, and 10 g/L sucrose, pH 5.4) containing 200 uM acetosyringone, and resuspended at 0.1 to 1.0 x 10^{9} cells/ml in ½ MSPL medium (2.2 g/L GIBCO MS salts, 2 mg/L glycine, 0.5 g/L niacin, 0.5 g/L L-pyridoxine-HCl, 0.1 mg/L thiamine, 115 g/L L-proline, 26 g/L D-glucose, 68.5 g/L sucrose, pH 5.4) containing 200 uM acetosyringone. One of skill in the art may substitute other media for ½ MSVI or ½ MSPL.

Immature maize embryos are isolated as described previously. Embryos are inoculated with *Agrobacterium* 0-7 days after excision, preferably immediately after excision. Alternatively, immature embryos may be cultured for more than 7 days. For example, embryogenic callus may be initiated as described above and co-cultured with *Agrobacterium.* Preferably, immature maize embryos are excised, immersed in an *Agrobacterium* suspension in

½ MSPL medium prepared as described above and incubated at room temperature with *Agrobacterium* for 5-20 minutes.

Following inoculation embryos are transferred to one-half strength MS medium containing 3.0 mg/L 2,4-dichlorophenyoxyacetic acid (2,4-D), 1% D-glucose, 2% sucrose, 0.115 g/L L-proline, 0.5 mg/L thiamine-HCl, 200 uM acetosyringone, and 20 uM silver nitrate or silver thiosulfate. Immature embryos are co-cultured with *Agrobacterium* for 1 to 3 days at 23°C in the dark. One of skill in the art may substitute other media for the described media.

Co-cultured embryos are transferred to medium 15AA (462 mg/L (NH4)SO4, 400 mg/L KH2PO4, 186 mg/L MgSO4-7H20, 166 mg/L CaCl2-2H20, 10 mg/L MnSO4-H2O, 3 mg/L H3B03, 2 mg/L ZnSO4-7H20, 0.25 mg/L NaMoO4-2H20, 0.025 mg/L CuSO4-5H20, 0.025 mg/L CoCl2-6H20, 0.75 mg/L KI, 2.83 g/L KNO3, 0.2 mg/L niacin, 0.1 mg/L thiamine-HCl, 0.2 mg/L pyridoxine-HCl, 0.1 mg/L D-biotin, 0.1 mg/L choline chloride, 0.1 mg/L calcium pantothenate, 0.05 mg/L folic acid, 0.05 mg/L p-aminobenzoic acid, 0.05 mg/L riboflavin, 0.015 mg/L vitamin B12, 0.5 g/L casamino acids, 33.5 mg/L Na2EDTA, 1.38 g/L L-proline, 20 g/L sucrose, 10 g/L D-glucose), or MS medium containing 1.5 mg/L 2,4-D, 500 mg/L carbenicillin, 3% sucrose, 1.38 g/L L-proline and 20 uM silver nitrate or silver thiosulfate and cultured for 0 to 8 days in the dark at 27°C without selection. Culture media used for selection of transformants and regeneration of plants preferably contains 500 mg/L carbenicillin. One of skill in the art may substitute other antibiotics that control growth of *Agrobacterium*. Other culture media that support cell culture may be used alternatively. In the absence of a delay of selection (0 day culture), selection may be initiated on 25 mg/L paromomycin. Selection medium may comprise medium 211 (described above) or a variant of medium 211 in which N6 salts are replaced by MS salts. After two weeks, embryogenic callus are transferred to culture medium containing 100 mg/L paromomycin and subcultured at about two week intervals. When selection is delayed following co-culture, embryos are initially cultured on medium containing 50 mg/L paromomycin followed by subsequent culture of embryogenic callus on medium containing 100-200 mg/L paromomycin. One of skill in the art will culture tissue on concentrations of paromomycin which inhibit growth of cells lacking the selectable marker gene, but a concentration on which transformed callus will proliferate. Alternatively, one may use other selectable markers to identify transformed cells. It is believed that initial culture on 25 to 50 mg/L paromocyin for about two weeks, followed by culture on 50-200 mg/L paromoycin will

result in recovery of transformed callus. Transformants are recovered 6 to 8 weeks after initiation of selection. Plants are regenerated from transformed embryogenic callus, e.g. as described in Example 5.

Example 3

Agrobacterium Mediated Transformation of Maize Callus

This example describes methods for transformation of maize callus using *Agrobacterium*. The method is exemplified using an *npt*II selectable marker gene and paromomycin selective agent. One of skill in the art will be aware of other selectable marker and selective agent combinations that could be used alternatively.

Callus was initiated from immature embryos using methods known to those of skill in the art. For example, 1.5 mm to 2.0 mm immature embryos were excised from developing maize seed of a genotype such as LH59 and cultured with the embryonic axis side down on medium 211V (described in Example 1 above), usually for 8-21 days after excision. Alternatively, established an established callus culture may be initiated and maintained by methods known to those of skill in the art.

Agrobacterium was prepared for inoculation of plant tissue according to the methods described in Example 10. Fifty to 100 pieces of callus was transferred to a 60 mm X 20 mm petri dish containing about 15 ml of *Agrobacterium* suspension at 0.1 to 1.0 x 10^9 cfu/ml. A piece of callus was usually all of the callus produced by an immature embryo in up to 21 days of culture or a piece of established callus of 2 mm to 8 mm in diameter. Callus was incubated for about 30 minutes at room temperature with the *Agrobacterium* suspension, followed by removal of the liquid by aspiration.

About 50 mL of sterile distilled water was added to a Whatman #1 filter paper in a 60 mm x 20 mm petri dish. After 1-5 minutes, 15 to 20 pieces of callus were transferred to each filter paper and the plate sealed with PARAFILM®, for example. The callus and *Agrobacterium* were co-cultured for about 3 days at 23°C in the dark.

Calli were transferred from filter paper to medium 211 with 20 mM silver nitrate and 500 mg/L carbenicillin and cultured in the dark at 27°C to 28°C for 2-5 days, preferably 3 days. Selection was initiated by transferring callus to medium 211 containing 20 mM silver nitrate, 500 mg/L carbenicillin and 25 mg/L paromomycin. After 2 weeks culture in the dark at 27°C to 28°C, callus was transferred to medium 211 with 20 mM silver nitrate, 500 mg/L carbenicillin

and 50 mg/L paromomycin (medium 211QRG). Callus was subcultured after two weeks to fresh medium 211 QRG and further cultured for two weeks in the dark at 27°C to 28°C. Callus was then transferred to medium 211 with 20mM silver nitrate, 500 mg/L carbenicillin and 75 mg/L paromomycin. After 2-3 weeks culture in the dark at 27°C to 28°C, paromomycin resistant callus was identified. One of skill in the art would recognize that times between subcultures of callus are approximate and one may be able to accelerate the selection process by transferring tissue at more frequent intervals, e.g., weekly rather than biweekly.

Plants were regenerated from transformed callus, transferred to soil and grown in the greenhouse. Following *Agrobacterium* mediated transformation, medium 217 further contained 500 mg/L carbenicillin and medium 127T further contained 250 mg/L carbenicillin.

Example 4

Methods of microprojectile bombardment

Approximately four hours prior to microprojectile bombardment, immature embryos were transferred to medium 211SV (medium 211V with the addition of sucrose to 12%). Twenty five immature embryos were preferably placed in a 60 x 15 mm petri dish, arranged in a 5 x 5 grid with the coleoptilar end of the scutellum pressed slightly into the culture medium at a 20 degree angle. Tissue was maintained in the dark prior to bombardment.

Prior to microprojectile bombardment, a suspension of gold particles was prepared onto which the desired transgenic DNA construct was precipitated. Ten milligrams of 0.6 mm gold particles (BioRad) were suspended in 50 mL buffer (150 mM NaCl, 10 mM Tris-HCl, pH 8.0). Twenty five mL of a 2.4 nM solution of the desired DNA was added to the suspension of gold particles and gently vortexed for about five seconds. Seventy five mL of 0.1M spermidine was added and the solution vortexed gently for about 5 seconds. Seventy five mL of a 25% solution of polyethylene glycol (3000-4000 molecular weight, American Type Culture Collection) was added and the solution was gently vortexed for five seconds. Seventy five mL of 2.5 M $CaCl_2$ was added and the solution vortexed for five seconds. Following the addition of $CaCl_2$, the solution was incubated at room temperature for 10 to 15 minutes. The suspension was subsequently centrifuged for 20 seconds at 12,000 rpm (Sorval MC-12V centrifuge) and the supernatant discarded. The gold particle/DNA pellet was washed twice with 100% ethanol and

resuspended in 10 mL 100% ethanol. The gold particle/DNA preparation was stored at -20°C for up to two weeks.

The transgenic DNA construct was introduced into maize cells using the electric discharge particle acceleration gene delivery device (US Patent No. 5,015,580). The gold particle/DNA suspension was coated on Mylar® polyester sheets (Du Pont Mylar® polyester film type SMMC2, aluminum coated on one side, over coated with PVDC co-polymer on both sides, cut to 18 mm square) by dispersion of 310 to 320 mL of the gold particle/DNA suspension on a sheet. After the gold particle suspension settled for one to three minutes, excess ethanol was removed and the sheets were air dried. Microprojectile bombardment of maize tissue was conducted as described in U.S. Patent No. 5,015,580. AC voltage may be varied in the electric discharge particle delivery device. For microprojectile bombardment of LH59 pre-cultured immature embryos, 35% to 45% of maximum voltage was preferably used. Following microprojectile bombardment, tissue was cultured in the dark at 27°C.

Example 5

Selection of transformed cells

Transformants were selected on culture medium comprising paromomycin, based on expression of a transgenic neomycin phosphotransferase II (*npt*II) gene. Twenty four hours after DNA delivery, tissue was transferred to 211V medium containing 25 mg/L paromomycin (medium 211HV). After three weeks incubation in the dark at 27°C, tissue was transferred to medium 211 containing 50 mg/L paromomycin (medium 211G). Tissue was transferred to medium 211 containing 75 mg/L paromomycin (medium 211XX) after three weeks. Transformants were isolated following 9 weeks of selection.

Example 6

Regeneration of fertile transgenic plants

Fertile transgenic plants are produced from transformed maize cells. Transformed callus was transferred to medium 217 (N6 salts, 1 mg/L thiamine-HCl, 0.5 mg/L niacin, 3.52 mg/L benzylaminopurine, 0.91 mg/L L-asparagine monohydrate, 100 mg/L myo-inositol, 0.5 g/L MES, 1.6 g/L $MgCl_2$-$6H_2O$, 100 mg/L casein hydrolysate, 0.69 g/L L-proline, 20 g/L sucrose, 2 g/L GELGRO™, pH 5.8) for five to seven days in the dark at 27°C. Somatic embryos mature and shoot regeneration began on medium 217. Tissue was transferred to medium 127T (MS salts, 0.65 mg/L niacin, 0.125 mg/L pyridoxine-HCl, 0.125 mg/L thiamine-HCl, 0.125 mg/L Ca

pantothenate, 150 mg/L L-asparagine, 100 mg/L myo-inositol, 10 g/L glucose, 20 g/L L-maltose, 100 mg/L paromomycin, 5.5 g PHYTAGAR™, pH 5.8) for shoot development. Tissue on medium 127T was cultured in the light at 400-600 lux at 26°C. Plantlets are transferred to soil, preferable 3 inch pots, about four to 6 weeks after transfer to 127T medium when the plantlets are about 3 inches tall and have roots. Plants were maintained for two weeks in a growth chamber at 26°C, followed by two weeks on a mist bench in a greenhouse before transplanting to 5 gallon pots for greenhouse growth. R0 plants were grown in the greenhouse to maturity and reciprocal pollinations were made with the inbred LH59. Seed was collected from the R0 plants and used for further breeding activities. For each plant representing a transgenic event, F1 seed was planted in a field producing plants which were assayed for phenotype and for the selectable kanamycin resistant marker. Each of the plants were self pollinated to produce F2 seed. Seed from nptII-positive plants, e.g. a few ears from each transgenic event, was planted and grown to produce F2 plants which were assayed for phenotype and kanamycin resistance. Kanamycin-resistant F2 plants were self pollinated to produce F3 seed. F3 seed was screened for complete resistance to kanamycin indicating a homozygous transgene. Seeds from homozygous F3 ears were planted in the field to produce F3 plants which were self pollinated to produce F4 seed. F3 plants were also crossed to tester inbred lines to produce F1 hybrid transgenic seed. Phenotypes such as yield are determined from F1 hybrid transgenic seed; other phenotypes can be determined from either F1 hybrid transgenic lines or F1, F2, F3 or F4 inbred transgenic lines.

A variety of transgenic plants were grown in field conditions allowing observation of multiple events of the unexpected phenotypes listed in Table 3.

Table 3

Gene Seq ID	Gene Name	No. of events	Event	observed tissue type	observation results
241	corn SVP-like	6	ZM_M17107	Tassel, central axis	Elongated
241	corn SVP-like	6	ZM_M17109	Tassel, central axis	Elongated
241	corn SVP-like	6	ZM_M17113	Tassel, central axis	elongated
241	corn SVP-like	6	ZM_M17114	Tassel, central axis	elongated
241	corn SVP-like	6	ZM_M18338	Tassel, floret, anther	no extrusion
241	corn SVP-like	6	ZM_M18350	Stem, internode	increased length
66	Receiver domain (ARR2-like)	5	ZM_M15362	Tassel, floret, anther	no extrusion
66	Receiver domain (ARR2-like)	5	ZM_M15363	Tassel, floret, anther	no extrusion
66	Receiver domain (ARR2-like)	5	ZM_M15364	Tassel, floret, anther	no extrusion

Gene Seq ID	Gene Name	No. of events	Event	observed tissue type	observation results
241	corn SVP-like	6	ZM_M17107	Tassel, central axis	Elongated
241	corn SVP-like	6	ZM_M17109	Tassel, central axis	Elongated
241	corn SVP-like	6	ZM_M17113	Tassel, central axis	elongated
241	corn SVP-like	6	ZM_M17114	Tassel, central axis	elongated
241	corn SVP-like	6	ZM_M18338	Tassel, floret, anther	no extrusion
241	corn SVP-like	6	ZM_M18350	Stem, internode	increased length
66	Receiver domain (ARR2-like)	5	ZM_M15362	Tassel, floret, anther	no extrusion
66	Receiver domain (ARR2-like)	5	ZM_M15363	Tassel, floret, anther	no extrusion
66	Receiver domain (ARR2-like)	5	ZM_M15365	Tassel, floret, anther	no extrusion
66	Receiver domain (ARR2-like)	5	ZM_M16223	Tassel, floret, anther	no extrusion
68	Receiver domain (TOC1-like) 3	4	ZM_M12575	Stem, internode	decreased length
68	Receiver domain (TOC1-like) 3	4	ZM_M12576	Stem, internode	decreased length
68	Receiver domain (TOC1-like) 3	4	ZM_M12583	Leaf, blade	interveinal chlorosis
68	Receiver domain (TOC1-like) 3	4	ZM_M13665	Stem, internode	Other - see notes
71	Receiver domain (RR3-like) 6	4	ZM_M24601	Tassel, floret, anther	no extrusion
71	Receiver domain (RR3-like) 6	4	ZM_M24603	Tassel, floret, anther	no extrusion
71	Receiver domain (RR3-like) 6	4	ZM_M24623	Tassel, floret, anther	no extrusion
71	Receiver domain (RR3-like) 6	4	ZM_M24624	Tassel, floret, anther	no extrusion
197	corn HY5-like	2	ZM_M16307	Stem, internode	decreased length
197	corn HY5-like	2	ZM_M17106	Stem, internode	decreased length
165	RNAse S	2	ZM_M24554	Stem, internode	thin
165	RNAse S	2	ZM_M24557	Leaf, complete	light green
119	soy HSF	2	ZM_M20417	Stem, internode	decreased length
119	soy HSF	2	ZM_M20418	Tassel, floret, anther	no extrusion
14	cytochrome P450	2	ZM_M23446	Leaf, blade	early senescence
14	cytochrome P450	2	ZM_M23450	Leaf, blade	early senescence
3	sorghum proline permease	2	ZM_M18655	Stem, internode	decreased length
3	sorghum proline permease	2	ZM_M19446	Leaf, complete	light green
4	rice AA transporter	2	ZM_M18625	Plant, complete	delayed growth
4	rice AA transporter	2	ZM_M18626	Tassel, floret	feminized-silk/seed formation
203	helix-loop-helix protein (PIF3	2	ZM_M18452	Tassel, central axis	shortened, increased girth
203	helix-loop-helix protein (PIF3	2	ZM_M20549	Tassel, central axis	shortened, increased girth
349	soy myb transcription factor	7	ZM_M24248	Stem, internode	increased length
349	soy myb transcription factor	7	ZM_M24251	Stem, internode	increased length
349	soy myb transcription factor	7	ZM_M24349	Stem, internode	increased length
349	soy myb transcription factor	7	ZM_M24350	Stem, internode	increased length
349	soy myb transcription factor	7	ZM_M24808	Stem, internode	increased length
349	soy myb transcription factor	7	ZM_M24810	Stem, internode	increased length
349	soy myb transcription factor	7	ZM_M24811	Stem, internode	increased length

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and methods and in the steps or in the sequence of steps of the methods described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

All publications and patent applications cited herein are incorporated by reference in their entirely to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

What is claimed is:

1. Transgenic maize seed characterized by enhanced yield (as measured in weight of crop per area) as compared to a corresponding yield of its parental maize line, obtained by introduction into the genome of said parental line of a transgenic DNA construct comprising a promoter operably linked to heterologous DNA, wherein said heterologous DNA encodes a protein having an amino acid sequence which is at least 60% identical to a sequence selected from the group consisting of SEQ ID NO:368 through SEQ ID NO:736.

2. Transgenic maize seed according to claim 1wherein said heterologous DNA comprises a protein coding segment of DNA having at least 60% identity with a sequence selected from the group consisting of SEQ ID NO:1 through SEQ ID NO: 368.

3. Transgenic maize seed according to claim 1wherein said enhanced yield is the result of improved plant growth under one or more stress conditions in the group consisting of drought, shade, fungal disease, viral disease, bacterial disease, insect infestation, nematode infestation, cold temperature exposure, heat exposure, osmotic stress, reduced nitrogen nutrient availability, reduced phosphorus nutrient availability and high plant density.

4. Transgenic maize seed according to claim 1wherein said enhanced yield is also manifested by increase in number of kernels per ear, number of ears per unit planted area, or average weight of kernels.

5. Transgenic maize seed according to claim 1wherein said enhanced yield is also manifested by increase in biomass per unit planted area or an increase in the root/shoot ratio.

6. Transgenic maize seed according to claim 1wherein said enhanced yield is also manifested by increased efficiency by said transgenic plant in water use, nitrogen use or phosphorus use.

7. Transgenic maize seed characterized by enhanced quality in the plant morphology, physiology or seed as compared to a corresponding phenotype of a parental maize line, obtained by introduction into the genome of said parental line a transgenic DNA construct comprising a promoter operably linked to heterologous DNA, wherein said heterologous DNA encodes a protein having an amino acid sequence which is at least 80% identical to a sequence selected from the group consisting of SEQ ID NO:369 through SEQ ID NO:738.

8. Transgenic maize seed according to claim 7 wherein said heterologous DNA comprises a protein coding segment of DNA having at least 70% identity with a sequence selected from the group consisting of SEQ ID NO:1 through SEQ ID NO: 368.

9. Transgenic maize seed according to claim 7 wherein said enhanced quality is shortened internode length, increased internode length, early leaf senescence, sterility, elongated tassel central axis, setting a second ear at high planting density, earlier time of germination, increased production of kernel oil or increased production of kernel protein.

10. Transgenic maize seed according to claim 9 having an enhanced phenotype of decreased internode length resulting from introduction of heterologous DNA coding for:

(d) a TOC1-like receiver domain 3 having an amino acid sequence which is at least 60% identical to SEQ ID NO:436,

(e) a HY5-like protein having an amino acid sequence which is at least 60% identical to SEQ ID NO:565, or

(f) a proline permease having an amino acid sequence which is at least 60% identical to SEQ ID NO:371.

11. Transgenic maize seed according to claim 9 having an enhanced phenotype of increased internode length resulting from introduction of heterologous DNA coding for:

(c) a myb transcription factor having an amino acid sequence which is at least 60% identical to SEQ ID NO:717, or

(d) an SVP-like protein having an amino acid sequence which is at least 60% identical to SEQ ID NO: 609.

12. Transgenic maize seed according to claim 9 having an enhanced phenotype of early leaf senescence resulting from insertion of heterologous DNA coding for a Cytochrome P450 having an amino acid sequence which is at least 60% identical to SEQ ID NO:382.

13. Transgenic maize seed according to claim 9 having an enhanced phenotype of sterility resulting from insertion of heterologous DNA coding for:

(e) an RR3-like receiver domain 8 having an amino acid sequence which is at least 60% identical to SEQ ID NO:439,

(f) an ARR2-like receiver domain having an amino acid sequence which is at least 60% identical to SEQ ID NO:434,

(g) an HSF protein having an amino acid sequence which is at least 60% identical to SEQ ID NO:487, or

(h) an SVP-like protein having an amino acid sequence which is at least 60% identical to SEQ ID NO:609.

14. Transgenic maize seed according to claim 9 having an enhanced phenotype of elongated tassel central axis resulting from insertion of heterologous DNA coding for an SVP-like protein having an amino acid sequence which is at least 60% identical to SEQ ID NO:609.

15. A method for introducing into a maize line an enhanced phenotype as compared to a phenotype in parental units of said maize line, said method comprising

(a) generating a population of transgenic plants comprising a variety of heterologous DNA for the transcription of which there is no known phenotype in corn,

(b) observing phenotypes for said transgenic plants,

(c) selecting seeds from transgenic plants having an unexpected enhanced phenotype, and

(d) optionally, repeating a cycle of germinating transgenic seed, growing subsequent generation plants from said transgenic seed, observing phenotypes of said subsequent generation plants and collecting seeds from subsequent generation plants having an enhanced phenotype.

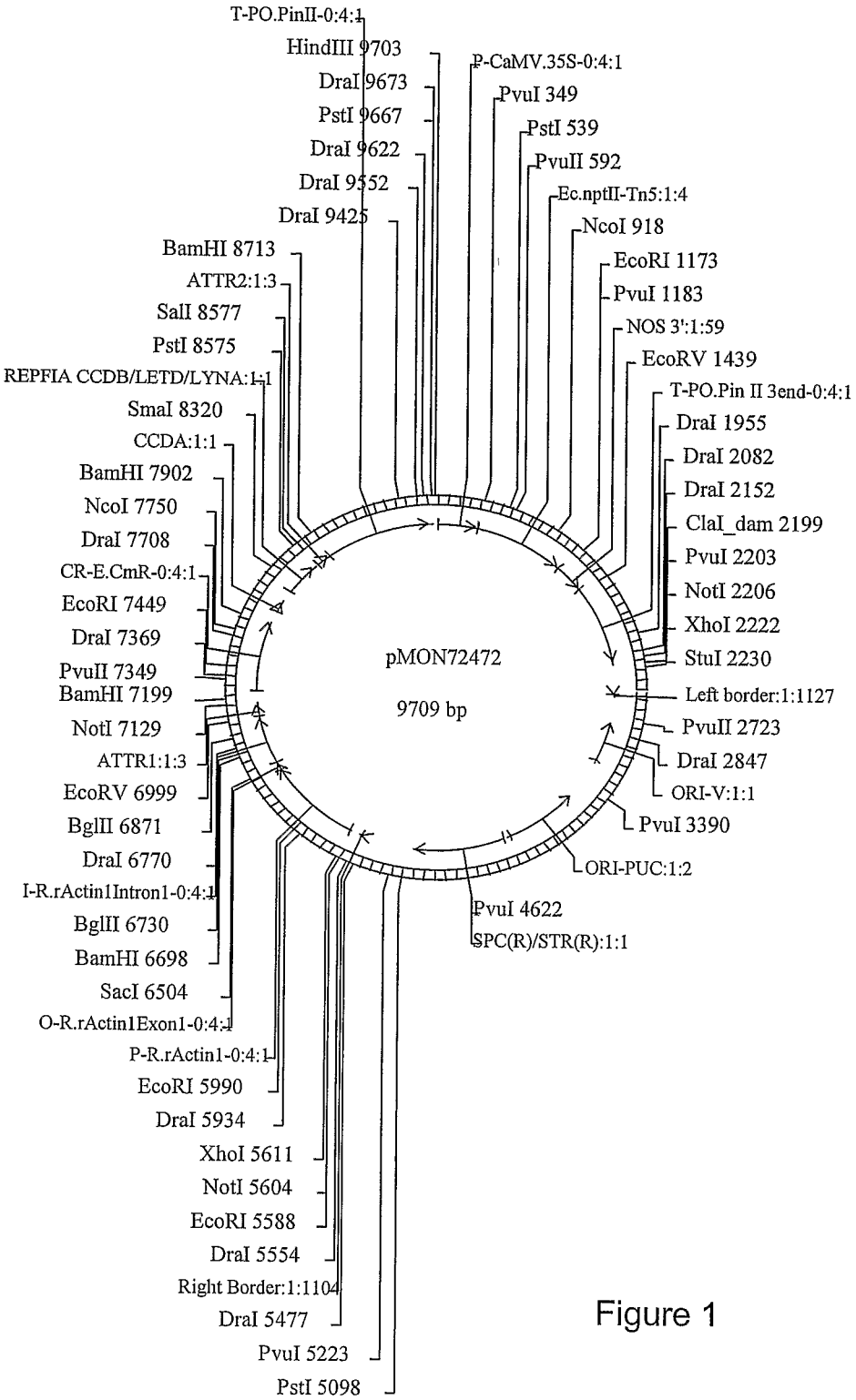
16. A method according to claim 15 wherein said population of transgenic plants is produced by generating a plurality of transgenic events for a plurality of unique transgenic DNA constructs wherein each of said transgenic events comprises introducing into the genome of a parental maize line a single transgenic DNA construct comprising a promoter operably linked to heterologous DNA, wherein said transgenic DNA construct is introduced into said genome in sufficient quantity to produce transgenic cells which can be cultured into plants of transgenic maize comprising said population. culturing said transgenic cells into a population of transgenic plants producing progeny transgenic seed,

17. A method according to claim 16 wherein said plurality of transgenic events is at least 2 and said plurality of unique transgenic DNA constructs is at least 20.

18. A method according to claim 16 wherein said plurality of transgenic events is at least 2 and said plurality of unique transgenic DNA constructs is at least 50.

19. A method according to claim 16 wherein said DNA construct comprises heterologous DNA operably linked to the 5' end of a promoter region comprising a rice actin promoter and rice actin intron.

20. A method according to claim 16 further comprising crossing transgenic plants from said population with at least one other maize line to produce a hybrid population, observing phenotypes in said hybrid population and selecting seed from plants having an unexpected phenotype.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PstI 8575
REPFIA CCDB/LETD/LYNA:1:1
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DraI 7708
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DraI 7369
PvuII 7349
BamHI 7199
NotI 7129
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BamHI 6698
SacI 6504
O-R.rActin1Exon1-0:4:1
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XhoI 5611
NotI 5604
EcoRI 5588
DraI 5554
Right Border:1:1104
DraI 5477
PvuI 5223
PstI 5098
P-CaMV.35S-0:4:1
PvuI 349
PstI 539
PvuII 592
Ec.nptII-Tn5:1:4
NcoI 918
EcoRI 1173
PvuI 1183
NOS 3':1:59
EcoRV 1439
T-PO.Pin II 3end-0:4:1
DraI 1955
DraI 2082
DraI 2152
ClaI_dam 2199
PvuI 2203
NotI 2206
XhoI 2222
StuI 2230
Left border:1:1127
PvuII 2723
DraI 2847
ORI-V:1:1
PvuI 3390
ORI-PUC:1:2
PvuI 4622
SPC(R)/STR(R):1:1
pMON72472
9709 bp

Figure 1