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[Continued on next page]

(54) Title: PLANTS WITH ALTERED ROOT ARCHITECTURE, INVOLVING THE RUM1 GENE, RELATED CONSTRUCTS AND METHODS

Figure 1

RUM1 Genetic Mapping with SSRs

Total SSRs analyzed: 258 F7/rum1 polymorphic SSRs: 95

Marker	Bin	IBM2	Results	Marker	Bin	IBM2	Results	Marker	Bin	IBM2	Results
umc1071	1.01S	85.2	No linkage	umc1750	4.01S	81	No linkage	umc1327	8.01S	55.0	No linkage
umc1403	1.03S	210.6	No linkage	umc2176	4.03S	174.6	No linkage	umc2004	8.02L	120.2	No linkage
bnlg1203	1.03S	259.3	No linkage	bnlg1265	4.05C	268.4	No linkage	umc1868	8.02L	200.3	No linkage
umc1590	1.08C	517.0	No linkage	umc2027	4.06L	349.8	No linkage	umc1735	8.03L	279.3	No linkage
umc1278	1.07L	631.9	No linkage	bnlg2291	4.06L	392.2	No linkage	umc1946	8.03L	357.9	No linkage
umc1833	1.07L	647.8	No linkage	umc1775	4.08L	443.2	No linkage	bnlg1031	8.03L	455.1	No linkage
umc1446	1.08L	791.6	No linkage	umc2188	4.08L	554.1	No linkage	umc1724	8.03L	457.3	No linkage
umc1512	1.08L	812.3	No linkage	umc1573	4.09L	644.3	No linkage	umc1058	8.03L	550.4	No linkage
umc1306	1.09L	891.2	No linkage	umc1260	5.00S	88.1	No linkage	umc1367	9.01S	24.3	No linkage
bnlg1671	1.10L	913.4	No linkage	bnlg585	5.02S	150.9	No linkage	umc1258	9.02S	155.7	No linkage
phi054	1.11L	1103.0	No linkage	umc1692	5.03S	271.5	No linkage	umc1191	9.03C	232.8	No linkage
umc1165	2.01S	47.4	No linkage	umc1349	5.04L	346.5	No linkage	bnlg1714	9.04L	266.4	No linkage
umc1823	2.02S	92.8	No linkage	umc1171	5.04L	376.4	No linkage	umc1657	9.04L	344.8	No linkage
umc1422	2.02S	156.6	No linkage	umc1155	5.05L	410.8	No linkage	umc1804	9.07L	538.5	No linkage
umc1448	2.04S	269.8	No linkage	umc1524	5.06L	493.5	No linkage	umc1137	9.07L	603.5	No linkage
umc1079	2.06L	386.3	No linkage	umc108	5.07L	536.6	No linkage	umc1380	10.00S	16.6	No linkage
bnlg2077	2.07L	474.8	No linkage	umc1225	5.09L	641.4	No linkage	umc1291	10.01S	50.8	No linkage
umc2065	2.08L	544.4	No linkage	umc1143	6.00S	17.3	No linkage	bnlg1451	10.02S	74.4	No linkage
mmc0381	2.08L	572.4	No linkage	umc2068	6.00S	46.1	No linkage	umc1319	10.02S	82.2	No linkage
bnlg1647	3.02S	103.3	No linkage	umc1133	6.01S	98.6	No linkage	umc1432	10.02S	120.09	No linkage
umc1644	3.06L	473.1	Linkage	umc1887	6.03L	166.8	No linkage	umc1179	10.03C	208.5	No linkage
umc1590	3.07L	544.6	Linkage	bnlg1154	6.05L	253	No linkage	bnlg640	10.03C	225.7	No linkage
umc1659	3.07L	544.6	Linkage	bnlg1702	6.05L	320.7	No linkage	umc1077	10.04L	253.5	No linkage
umc1489	3.07L	557.6	Linkage	phi299852	6.07L	450.7	No linkage	umc1115	10.04L	285.9	No linkage
umc1404	3.07L	593.3	Linkage	bnlg1136	6.07L	531.8	No linkage	umc1272	10.04L	298.4	No linkage
umc1644	3.08L	596.3	Linkage	umc1653	6.07L	539.6	No linkage	umc1477	10.05L	365.3	No linkage
umc2274	3.08L	608.6	Linkage	mmc0171	7.00S	64.3	No linkage	umc2122	10.06L	392.5	No linkage
phi046	3.08L	612.0	Linkage	umc2160	7.01S	118.5	No linkage	bnlg1450	10.07L	483.7	No linkage
umc1915	3.08L	617.5	Linkage	bnlg2203	7.01S	190.6	No linkage				
umc2152	3.09L	738.7	Linkage	umc2142	7.02L	246.3	No linkage				
mmc0001	3.09L	759.9	Linkage	umc1408	7.03L	365.4	No linkage				
umc2048	3.10L	824.7	Linkage	umc1029	7.04L	444.7	No linkage				
umc1639	3.09L	824.7	No linkage	umc1407	7.05L	600.2	No linkage				
umc1594	3.09L	828.9	No linkage								

(57) Abstract: Isolated polynucleotides and polypeptides and recombinant DNA constructs particularly useful for altering root structure of plants, compositions (such as plants or seeds) comprising these recombinant DNA constructs, and methods utilizing these recombinant DNA constructs. The recombinant DNA construct comprises a polynucleotide operably linked to a promoter functional in a plant, wherein said polynucleotide encodes a polypeptide useful for altering plant root architecture.



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- *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

TITLE

PLANTS WITH ALTERED ROOT ARCHITECTURE, INVOLVING THE *RUM1* GENE, RELATED CONSTRUCTS AND METHODS

FIELD OF THE INVENTION

5 This invention relates to compositions and methods useful in altering root architecture in plants. Additionally, the invention relates to plants that have been genetically transformed with the compositions of the invention.

BACKGROUND OF THE INVENTION

10 Relatively little is known about the genetic regulation of plant root development and function. Elucidation of the genetic regulation is important because roots serve important functions such as acquisition of water and nutrients and the anchorage of the plants in the soil.

Maize root architecture is composed of different root types formed at different plant developmental stages. A number of mutants affected in specific root types
15 during different developmental stages have been described in maize (e.g. *rtcs* (rootless concerning crown and seminal roots), *lrt1* (lateral rootless1)). The monogenic recessive *rum1* ((rootless with undetectable meristems 1) mutant was first reported by Woll et al. (2004) *Maize Genetics Cooperation Newsletter* 78: 59-60. A more detailed description of the mutant phenotype was published by Woll et
20 al. (2005) *Plant Physiology* 139 (3): 1255-1267. The maize mutant was shown to be impaired in the formation of seminal and lateral roots on the primary root. No obvious differences were detectable in aboveground development between *rum1* and wild-type plants. Genetic analysis of the *rum1* mutation indicated that it is inherited as a monogenic recessive trait. However, introduction of the *rum1*
25 mutation into different genetic backgrounds resulted in segregation ratios that suggested the presence of a recessive suppressor of the *rum1* mutation in those backgrounds.

The plant hormone auxin plays a crucial role during embryogenesis and is involved in various aspects of root development. In the *rum1* mutant, auxin
30 transport toward the root tip is severely reduced. Mutations in members of the auxin-inducible *Aux/IAA* and *ARF* gene families of *Arabidopsis* result in phenotypes that resemble the maize *rum1* phenotype in regard to the absence of lateral roots on

the primary root. Several gain-of-function mutants lacking lateral roots or inhibited in lateral root formation have been described in *Arabidopsis* (*Solitary-Root/IAA14* gene (*SLR/IAA14*) described by Fukaki et al. (2002) *The Plant Journal* 29(2): 153-168; *Massugu2/IAA19* gene (*MSG2/IAA19*) described by Tatematsu et al. (2004) *Plant Cell* 16: 379-393. Okushima et al. (2005) *Plant Cell* 17: 444-463 described a *arf7arf19* double mutant, that shows a phenotype similar to the *slr/iaa14* and *msg/iaa19* mutants.

In vitro experiments indicate that IAA14 interacts with both ARF7 and ARF19, and that IAA19 interacts with ARF7. Aux/IAA and ARFs are therefore considered major components of the auxin signaling pathway that controls plant growth responses to the hormone auxin.

Despite the extensive genetic and morphological characterization of the *rum1* mutant, there has been no molecular analysis of the nucleic acid encoding the protein associated with the *rum1* phenotype. Indeed, the identity of the protein encoded by *rum1* has not been reported.

SUMMARY OF THE INVENTION

The present invention includes:

In one embodiment, a plant comprising in its genome a recombinant DNA construct comprising a polynucleotide operably linked to at least one regulatory element, wherein said polynucleotide encodes a polypeptide having an amino acid sequence of at least 85% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 73 and wherein said plant exhibits altered root architecture when compared to a control plant not comprising said recombinant DNA construct.

In one embodiment, a plant comprising in its genome a recombinant DNA construct comprising a polynucleotide operably linked to at least one regulatory element, wherein said polynucleotide encodes a polypeptide having an amino acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73, and wherein said plant exhibits altered root architecture when compared to a control plant not comprising said recombinant DNA construct.

In another embodiment, a plant comprising in its genome a recombinant DNA construct comprising:

(a) a polynucleotide operably linked to at least one regulatory element, wherein said polynucleotide encodes a polypeptide having an amino acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73 or

5 (b) a suppression DNA construct comprising at least one regulatory element operably linked to: (i) all or part of: (A) a nucleic acid sequence encoding a polypeptide having an amino acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73 or (B) a full complement of the nucleic acid sequence of
10 (b)(i)(A); or (ii) a region derived from all or part of a sense strand or antisense strand of a target gene of interest, said region having a nucleic acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to said all or part of a sense strand or antisense strand from which said region is derived, and wherein said target gene of interest encodes a RUM1 or
15 RUM1-like polypeptide, and wherein said plant exhibits an alteration of at least one agronomic characteristic when compared to a control plant not comprising said recombinant DNA construct.

In another embodiment, a method of altering root architecture in a plant, comprising (a) introducing into a regenerable plant cell a recombinant DNA construct
20 comprising a polynucleotide operably linked to at least one regulatory sequence, wherein the polynucleotide encodes a polypeptide having an amino acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73; and (b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic
25 plant comprises in its genome the recombinant DNA construct and exhibits altered root architecture when compared to a control plant not comprising the recombinant DNA construct; and optionally, (c) obtaining a progeny plant derived from the transgenic plant, wherein said progeny plant comprises in its genome the recombinant DNA construct and exhibits altered root architecture when compared to
30 a control plant not comprising the recombinant DNA construct.

In another embodiment, a method of evaluating root architecture in a plant, comprising (a) introducing into a regenerable plant cell a recombinant DNA construct comprising a polynucleotide operably linked to at least one regulatory sequence,

wherein the polynucleotide encodes a polypeptide having an amino acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73; (b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the recombinant DNA construct; and (c) evaluating root architecture of the transgenic plant compared to a control plant not comprising the recombinant DNA construct; and optionally, (d) obtaining a progeny plant derived from the transgenic plant, wherein the progeny plant comprises in its genome the recombinant DNA construct; and optionally, (e) evaluating root architecture of the progeny plant compared to a control plant not comprising the recombinant DNA construct.

In another embodiment, a method of evaluating root architecture in a plant, comprising (a) introducing into a regenerable plant cell a recombinant DNA construct comprising a polynucleotide operably linked to at least one regulatory sequence, wherein the polynucleotide encodes a polypeptide having an amino acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73; (b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the recombinant DNA construct; (c) obtaining a progeny plant derived from the transgenic plant, wherein the progeny plant comprises in its genome the recombinant DNA construct; and (d) evaluating root architecture of the progeny plant compared to a control plant not comprising the recombinant DNA construct.

In another embodiment, a method of determining an alteration of an agronomic characteristic in a plant, comprising (a) introducing into a regenerable plant cell a recombinant DNA construct comprising a polynucleotide operably linked to at least one regulatory sequence, wherein the polynucleotide encodes a polypeptide having an amino acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73; (b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the recombinant DNA construct; and (c) determining whether the transgenic plant exhibits an alteration of at least one agronomic characteristic when compared to a

control plant not comprising the recombinant DNA construct; and optionally, (d) obtaining a progeny plant derived from the transgenic plant, wherein the progeny plant comprises in its genome the recombinant DNA construct; and optionally, (e) determining whether the progeny plant exhibits an alteration of at least one agronomic characteristic when compared to a control plant not comprising the recombinant DNA construct.

In another embodiment, a method of determining an alteration of an agronomic characteristic in a plant, comprising (a) introducing into a regenerable plant cell a recombinant DNA construct comprising a polynucleotide operably linked to at least one regulatory sequence, wherein the polynucleotide encodes a polypeptide having an amino acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73; (b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the recombinant DNA construct; (c) obtaining a progeny plant derived from the transgenic plant, wherein the progeny plant comprises in its genome the recombinant DNA construct; and (d) determining whether the progeny plant exhibits an alteration of at least one agronomic characteristic when compared to a control plant not comprising the recombinant DNA construct.

In another embodiment, a method of determining an alteration of an agronomic characteristic in a plant, comprising:

(a) introducing into a regenerable plant cell a suppression DNA construct comprising at least one regulatory element operably linked to:

(i) all or part of: (A) a nucleic acid sequence encoding a polypeptide having an amino acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73, or (B) a full complement of the nucleic acid sequence of (b)(i)(A); or

(ii) a region derived from all or part of a sense strand or antisense strand of a target gene of interest, said region having a nucleic acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to said all or part of a sense strand or antisense strand from which said

region is derived, and wherein said target gene of interest encodes a RUM1 or RUM1-like polypeptide;

(b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the suppression DNA construct; and

(c) determining whether the transgenic plant exhibits an alteration of at least one agronomic characteristic when compared to a control plant not comprising the suppression DNA construct;

and optionally, (d) obtaining a progeny plant derived from the transgenic plant, wherein the progeny plant comprises in its genome the suppression DNA construct; and optionally, (e) determining whether the progeny plant exhibits an alteration of at least one agronomic characteristic when compared to a control plant not comprising the suppression DNA construct.

In another embodiment, a method of determining an alteration of an agronomic characteristic in a plant, comprising:

(a) introducing into a regenerable plant cell a suppression DNA construct comprising at least one regulatory element operably linked to:

(i) all or part of: (A) a nucleic acid sequence encoding a polypeptide having an amino acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73, or (B) a full complement of the nucleic acid sequence of (a)(i)(A); or

(ii) a region derived from all or part of a sense strand or antisense strand of a target gene of interest, said region having a nucleic acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to said all or part of a sense strand or antisense strand from which said region is derived, and wherein said target gene of interest encodes a RUM1 or RUM1-like polypeptide;

(b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the suppression DNA construct and exhibits altered root architecture when compared to a control plant not comprising the suppression DNA construct;

(c) obtaining a progeny plant derived from the transgenic plant, wherein the progeny plant comprises in its genome the suppression DNA construct; and

(d) determining whether the progeny plant exhibits an alteration of at least one agronomic characteristic when compared to a control plant not comprising the suppression DNA construct.

In another embodiment, a method of altering root architecture in a plant, comprising:

(a) introducing into a regenerable plant cell a suppression DNA construct comprising at least one regulatory element operably linked to:

(i) all or part of: (A) a nucleic acid sequence encoding a polypeptide having an amino acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73; or (B) a full complement of the nucleic acid sequence of (a)(i)(A); or

(ii) a region derived from all or part of a sense strand or antisense strand of a target gene of interest, said region having a nucleic acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to said all or part of a sense strand or antisense strand from which said region is derived, and wherein said target gene of interest encodes a RUM1 or RUM1-like polypeptide; and

(b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the suppression DNA construct and wherein the transgenic plant exhibits altered root architecture when compared to a control plant not comprising the suppression DNA construct; and optionally, (c) obtaining a progeny plant derived from the transgenic plant, wherein said progeny plant comprises in its genome the recombinant DNA construct and wherein the progeny plant exhibits altered root architecture when compared to a control plant not comprising the suppression DNA construct.

In another embodiment, a method of evaluating root architecture in a plant, comprising:

(a) introducing into a regenerable plant cell a suppression DNA construct comprising at least one regulatory element operably linked to:

(i) all or part of: (A) a nucleic acid sequence encoding a polypeptide having an amino acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73, or (B) a full complement of the nucleic acid sequence of

5 (a)(i)(A); or

(ii) a region derived from all or part of a sense strand or antisense strand of a target gene of interest, said region having a nucleic acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to said all or part of a sense strand or antisense strand from which said region is derived, and wherein said target gene of interest encodes a RUM1 or RUM1-like polypeptide;

(b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the suppression DNA construct; and

15 (c) evaluating root architecture of the transgenic plant compared to a control plant not comprising the suppression DNA construct;

and optionally, (d) obtaining a progeny plant derived from the transgenic plant, wherein the progeny plant comprises in its genome the suppression DNA construct; and optionally, (e) evaluating root architecture of the progeny plant compared to a control plant not comprising the suppression DNA construct.

20 In another embodiment, a method of evaluating root architecture in a plant, comprising:

(a) introducing into a regenerable plant cell a suppression DNA construct comprising at least one regulatory element operably linked to:

25 (i) all or part of: (A) a nucleic acid sequence encoding a polypeptide having an amino acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73, or (B) a full complement of the nucleic acid sequence of (a)(i)(A); or

30 (ii) a region derived from all or part of a sense strand or antisense strand of a target gene of interest, said region having a nucleic acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to said all or part of a sense strand or antisense strand from which said

region is derived, and wherein said target gene of interest encodes a RUM1 or RUM1-like polypeptide;

(b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the suppression DNA construct;

(c) obtaining a progeny plant derived from the transgenic plant, wherein the progeny plant comprises in its genome the suppression DNA construct; and

(d) evaluating root architecture of the progeny plant compared to a control plant not comprising the suppression DNA construct.

Also included in the present invention is any progeny of the above plants, any seeds of the above plants, and cells from any of the above plants and progeny.

A method of producing seed that can be sold as a product offering with altered root architecture comprising any of the preceding preferred methods, and further comprising obtaining seeds from said progeny plant, wherein said seeds comprise in their genome said recombinant DNA construct.

BRIEF DESCRIPTION OF THE FIGURES AND SEQUENCE LISTINGS

The invention can be more fully understood from the following detailed description and the accompanying drawings and Sequence Listing which form a part of this application.

Fig.1 shows a map of the *RUM1* genomic sequence.

Fig.2 shows the *RUM1* physical map and its synteny with rice.

Fig.3 depicts the vector pDONORTM/Zeo.

Fig.4 depicts the vector pDONORTM221.

Fig.5 depicts the vector PHP27840.

Fig.6 depicts the vector PHP23236.

Fig.7 depicts the vector PHP10523.

Fig.8 depicts the vector PHP28408.

Fig.9 depicts the vector PHP20234.

Fig.10 depicts the vector PHP28529.

Fig.11 depicts the vector PHP22020.

Fig.12 depicts the vector PHP23112.

Fig.13 depicts the vector PHP23235.

Fig.14 depicts the vector PHP29635.

Fig.15 depicts the vector pIIOXS2a-FRT87(ni)m.

Fig.16 is the growth medium used for semi-hydroponics maize growth in Example 19.

Fig.17 is a chart setting forth data relating to the effect of different nitrate concentrations on the growth and development of Gaspe Bay Flint derived maize lines in Example 19.

Fig.18 shows the multiple alignment of the full length amino acid sequences of B73-Mu-wt RUM1 (SEQ ID NO:24), B73 RUM1 (SEQ ID NO:29), B73 RUL (SEQ ID NO:39), the mutant rum1 (SEQ ID NO:25) and the rice protein identified as belonging to the AUX-IAA family (NCBI General identifier No. 34911088, SEQ ID NO:65). Amino acids conserved among all sequences are indicated with an asterisk (*) on the top row; dashes are used by the program to maximize alignment of the sequences. The LxLxL motif described in Example 24 is shown in bold letters. The method parameters used to produce the multiple alignment of the sequences below was performed using the Clustal method of alignment (Higgins and Sharp (1989) CABIOS. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10)..

Fig.19 shows a chart of the percent sequence identity for each pair of amino acid sequences displayed in Fig.18.

The sequence descriptions and Sequence Listing attached hereto comply with the rules governing nucleotide and/or amino acid sequence disclosures in patent applications as set forth in 37 C.F.R. §1.821-1.825.

The Sequence Listing contains the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IUBMB standards described in *Nucleic Acids Res.* 13:3021-3030 (1985) and in the *Biochemical J.* 219 (No. 2):345-373 (1984) which are herein incorporated by reference. The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. §1.822.

SEQ ID NO:1 is the forward primer for SSR marker UMC1690 used in Example 1.

SEQ ID NO:2 is the reverse primer for SSR marker UMC1690 used in Example 1.

SEQ ID NO:3 is the forward primer for SSR marker BNLG 1108 used in Example 1.

SEQ ID NO:4 is the reverse primer for SSR marker BNLG 1108 used in Example 1.

5 SEQ ID NO:5 is the forward primer for marker UMC1844 used in Example 1.
SEQ ID NO:6 is the reverse primer for marker UMC1844 used in Example 1.
SEQ ID NO:7 is the forward primer for marker UMC1915 used in Example 1.
SEQ ID NO:8 is the reverse primer for marker UMC1915 used in Example 1.
SEQ ID NO:9 is the forward primer for marker PHP9257A used in

10 Example 1.

SEQ ID NO:10 is the reverse primer for marker PHP9257A used in Example 1.

SEQ ID NO:11 is the forward primer for marker UMC2274 used in Example 1.

15 SEQ ID NO:12 is the reverse primer for marker UMC2274 used in Example 1.

SEQ ID NO:13 is the forward primer for CAP marker MZA8411 used in Example 1.

20 SEQ ID NO:14 is the reverse primer for CAP marker MZA8411 used in Example 1.

SEQ ID NO:15 is the forward primer for CAP marker b0568n15 used in Example 1.

SEQ ID NO:16 is the reverse primer for CAP marker b0568n15 used in Example 1.

25 SEQ ID NO:17 is the forward primer for CAP marker MZA8828 used in Example 1.

SEQ ID NO:18 is the reverse primer for CAP marker MZA8828 used in Example 1.

30 SEQ ID NO:19 is the 4098 bp genomic fragment of b0568n15 containing the *RUM1* gene.

SEQ ID NO:20 is the sequence of the forward primer RUM1-70F as described in Example 3.

SEQ ID NO:21 is the sequence of the reverse primer RUM1+40R as described in Example 3.

SEQ ID NO:22 is the wild type *RUM1* cDNA sequence obtained from the mutant line (B73-Mu) described in Example 3.

5 SEQ ID NO:23 is the mutant *rum1* cDNA sequence obtained from the mutant line (B73-Mu) described in Example 3.

SEQ ID NO:24 is the amino acid sequence encoded by SEQ ID NO:22.

SEQ ID NO:25 is the amino acid sequence encoded by SEQ ID NO:23.

10 SEQ ID NO:26 is the partial EST corresponding to accession number CD439449 described in Example 4.

SEQ ID NO:27 is the amino acid sequence encoded by SEQ ID NO:26.

SEQ ID NO:28 is the full length *RUM1* cDNA from B73 described in Example 4.

SEQ ID NO:29 is the amino acid sequence encoded by SEQ ID NO:28.

15 SEQ ID NO:30 is the amino acid sequence of the *Arabidopsis* IAA8 protein (gi:15227275).

SEQ ID NO:31 is the amino acid sequence of the *Arabidopsis* protein SLRIAA14 (gi:22328628).

20 SEQ ID NO:32 is the amino acid sequence of the *Arabidopsis* protein MSG2/IAA19 (gi:1532612 or 17365900).

SEQ ID NO:33 is the forward primer RUM1 -354F used in Example 6.

SEQ ID NO:34 is the reverse RUM1 exon1-R1 used in Example 6.

SEQ ID NO:35 is the forward primer -132F used in Example 6.

SEQ ID NO:36 is the reverse primer RUM1 exon1-R2 used in Example 6.

25 SEQ ID NO:37 is the MuTIR primer used in Example 6.

SEQ ID NO:38 is the sequence of the *RUM1*-like (*RUL*) cDNA described in Example 7.

SEQ ID NO:39 is the amino acid sequence of the RUL protein encoded by SEQ ID NO:38.

30 SEQ ID NO:40 is the forward primer RUL -43F described in Example 8.

SEQ ID NO:41 is the reverse primer RUL +181R described in Example 8.

SEQ ID NO:42 is the attB1 sequence described in Example 9.

SEQ ID NO:43 is the attB2 sequence described in Example 9.

SEQ ID NO:44 is the sequence of the forward primer VC062 described in Example 9.

SEQ ID NO:45 is the sequence of the reverse primer VC063 described in Example 9.

5 SEQ ID NO:46 is the sequence of vector pDONORTM/Zeo described in Example 9.

SEQ ID NO:47 is the sequence of vector pDONORTM/221 described in Example 9.

SEQ ID NO:48 is the sequence of PHP27840 described in Example 9.

10 SEQ ID NO:49 is the sequence of PHP23236 described in Example 9.

SEQ ID NO:50 is the sequence of PHP10523.

SEQ ID NO:51 is the sequence of the NAS2 promoter.

SEQ ID NO:52 is the sequence of the GOS2 promoter.

SEQ ID NO:53 is the sequence of the ubiquitin promoter.

15 SEQ ID NO:54 is the sequence of the PINII terminator.

SEQ ID NO:55 is the sequence of PHP28408.

SEQ ID NO:56 is the sequence of PHP20234.

SEQ ID NO:57 is the sequence of PHP28529.

SEQ ID NO:58 is the sequence of PHP22020.

20 SEQ ID NO:59 is the sequence of PHP23112.

SEQ ID NO:60 is the sequence of PHP23235.

SEQ ID NO:61 is the sequence of PHP29635.

SEQ ID NO:62 is the sequence of pLIOXS2a-FRT87(ni)m.

SEQ ID NO:63 is the sequence of the S2A promoter.

25 SEQ ID NO:64 is the GAL4 DNA binding sequence.

SEQ ID NO:65 is the sequence corresponding to NCBI General identifier No. 34911088.

SEQ ID NO:66 is the cDNA corresponding to nucleotides 155 through 865 (Stop) of the RUM1 homolog ebb1c.pk008.p9:fis.

30 SEQ ID NO:67 is the amino acid sequence encoded by SEQ ID NO:66.

SEQ ID NO:68 is the cDNA corresponding to nucleotides 154 through 1218 (Stop) of the RUM1 homolog smj1c.pk013.h7.f:fis.

SEQ ID NO:69 is the amino acid sequence encoded by SEQ ID NO:68.

SEQ ID NO:70 is the cDNA corresponding to nucleotides 225 through 1304 (Stop) of the RUM1 homolog smj1c.pk007.k12.f: fis.

SEQ ID NO:71 is the amino acid sequence encoded by SEQ ID NO:70.

SEQ ID NO:72 is the cDNA corresponding to nucleotides 155 through 865

5 (Stop) of the RUM1 homolog wdk1c.pk023.b8: fis.

SEQ ID NO:73 is the amino acid sequence encoded by SEQ ID NO:72.

SEQ ID NO:74 is the sequence corresponding to NCBI General identifier No. 15229343.

10 SEQ ID NO:75 is the sequence corresponding to NCBI General identifier No. 2388689.

SEQ ID NO:76 is the sequence corresponding to NCBI General identifier No. 125553286.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

15 The disclosure of each reference set forth herein is hereby incorporated by reference in its entirety.

As used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to "a plant" includes a plurality of such plants, reference to "a cell" includes one or more cells and equivalents thereof known to those skilled in the art, and so forth.

20 The term "root architecture" refers to the arrangement of the different parts that comprise the root. The terms "root architecture", "root structure", "root system" or "root system architecture" are used interchangeably herewithin.

25 In general, the first root of a plant that develops from the embryo is called the primary root. In most dicots, the primary root is called the taproot. This main root grows downward and gives rise to branch (lateral) roots. In monocots the primary root of the plant branches, giving rise to a fibrous root system.

30 The term "altered root architecture" refers to aspects of alterations of the different parts that make up the root system at different stages of its development compared to a reference or control plant. It is understood that altered root architecture encompasses alterations in one or more measurable parameters, including but not limited to, the diameter, length, number, angle or surface of one or more of the root system parts, including but not limited to, the primary root, lateral or

branch root, crown roots, adventitious root, and root hairs, all of which fall within the scope of this invention. These changes can lead to an overall alteration in the area or volume occupied by the root. The reference or control plant does not comprise in its genome the recombinant DNA construct or heterologous construct.

5 “Agronomic characteristics” is a measurable parameter including but not limited to greenness, yield, growth rate, biomass, fresh weight at maturation, dry weight at maturation, fruit yield, seed yield, total plant nitrogen content, fruit nitrogen content, seed nitrogen content, nitrogen content in a vegetative tissue, total plant free amino acid content, fruit free amino acid content, seed free amino acid content, 10 free amino acid content in a vegetative tissue, total plant protein content, fruit protein content, seed protein content, protein content in a vegetative tissue, drought tolerance, nitrogen uptake, root lodging, stalk lodging, plant height, ear length, and harvest index.

 “Harvest index” refers to the grain weight divided by the total plant weight.

15 “*RUM1*-mu-wt” and “*RUM1*” refer to the *Zea Mays RUM1* wild type gene and includes without limitation SEQ ID NO:22 and SEQ ID NO:28, respectively).

 “*RUM1*-mu-wt ” and “*RUM1*” and refer to the *Zea Mays RUM1* wild type protein encoded by SEQ ID NO:24 and SEQ ID NO:29, respectively.

20 “*RUM1*-like” or *RUL* are used interchangeable herewithin and refer to the nucleotide homolog of the maize *RUM1* and *RUM1*-mu-wt sequences and includes without limitation the nucleotide sequence of SEQ ID NO:38.

 “*RUM1*-like” or *RUL* are used interchangeable herewithin and refer to the polypeptide homolog of the maize *RUM1* and *RUM1*-mu-wt proteins and includes without limitation the amino acid sequence of SEQ ID NO:39.

25 “*rum1*” refers to the nucleotide sequence of the *Zea Mays* “rootless with undetectable meristems 1” mutant and includes without limitation SEQ ID NO:23.

 “*rum1*” refers to the polypeptide of the *Zea Mays* “rootless with undetectable meristems 1” mutant and includes without limitation SEQ ID NO:25.

30 “Environmental conditions” refer to conditions under which the plant is grown, such as the availability of water, availability of nutrients (for example nitrogen or phosphate), or the presence of insects or disease.

 “Root lodging” refers to stalks leaning from the center. Root lodging can occur as early as the late vegetative stages and as late as harvest maturity. Root

lodging can be affected by hybrid susceptibility, environmental stress (drought, flooding), insect and disease injury. Root lodging can be attributed to corn rootworm injury in some cases.

"Transgenic" refers to any cell, cell line, callus, tissue, plant part or plant, the genome of which has been altered by the presence of a heterologous nucleic acid, such as a recombinant DNA construct, including those initial transgenic events as well as those created by sexual crosses or asexual propagation from the initial transgenic event. The term "transgenic" as used herein does not encompass the alteration of the genome (chromosomal or extra-chromosomal) by conventional plant breeding methods or by naturally occurring events such as random cross-fertilization, non-recombinant viral infection, non-recombinant bacterial transformation, non-recombinant transposition, or spontaneous mutation.

"Genome" as it applies to plant cells encompasses not only chromosomal DNA found within the nucleus, but organelle DNA found within subcellular components (e.g., mitochondrial, plastid) of the cell.

"Plant" includes reference to whole plants, plant organs, plant tissues, seeds and plant cells and progeny of same. Plant cells include, without limitation, cells from seeds, suspension cultures, embryos, meristematic regions, callus tissue, leaves, roots, shoots, gametophytes, sporophytes, pollen, and microspores.

"Progeny" comprises any subsequent generation of a plant.

"Transgenic" refers to any cell, cell line, callus, tissue, plant part or plant, the genome of which has been altered by the presence of a heterologous nucleic acid, such as a recombinant DNA construct, including those initial transgenic events as well as those created by sexual crosses or asexual propagation from the initial transgenic event. The term "transgenic" as used herein does not encompass the alteration of the genome (chromosomal or extra-chromosomal) by conventional plant breeding methods or by naturally occurring events such as random cross-fertilization, non-recombinant viral infection, non-recombinant bacterial transformation, non-recombinant transposition, or spontaneous mutation.

"Transgenic plant" includes reference to a plant which comprises within its genome a heterologous polynucleotide. Preferably, the heterologous polynucleotide is stably integrated within the genome such that the polynucleotide is passed on to

successive generations. The heterologous polynucleotide may be integrated into the genome alone or as part of a recombinant DNA construct.

"Heterologous" with respect to sequence means a sequence that originates from a foreign species, or, if from the same species, is substantially modified from its native form in composition and/or genomic locus by deliberate human intervention.

"Polynucleotide", "nucleic acid sequence", "nucleotide sequence", or "nucleic acid fragment" are used interchangeably and is a polymer of RNA or DNA that is single- or double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases. Nucleotides (usually found in their 5'-monophosphate form) are referred to by their single letter designation as follows: "A" for adenylate or deoxyadenylate (for RNA or DNA, respectively), "C" for cytidylate or deoxycytidylate, "G" for guanylate or deoxyguanylate, "U" for uridylate, "T" for deoxythymidylate, "R" for purines (A or G), "Y" for pyrimidines (C or T), "K" for G or T, "H" for A or C or T, "I" for inosine, and "N" for any nucleotide.

"Polypeptide", "peptide", "amino acid sequence" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers. The terms "polypeptide", "peptide", "amino acid sequence", and "protein" are also inclusive of modifications including, but not limited to, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation.

"Messenger RNA (mRNA)" refers to the RNA that is without introns and that can be translated into protein by the cell.

"cDNA" refers to a DNA that is complementary to and synthesized from a mRNA template using the enzyme reverse transcriptase. The cDNA can be single-stranded or converted into the double-stranded form using the Klenow fragment of DNA polymerase I.

"Mature" protein refers to a post-translationally processed polypeptide; i.e., one from which any pre- or pro-peptides present in the primary translation product have been removed.

"Precursor" protein refers to the primary product of translation of mRNA; i.e., with pre- and pro-peptides still present. Pre- and pro-peptides may be and are not limited to intracellular localization signals.

5 "Isolated" refers to materials, such as nucleic acid molecules and/or proteins, which are substantially free or otherwise removed from components that normally accompany or interact with the materials in a naturally occurring environment. Isolated polynucleotides may be purified from a host cell in which they naturally occur. Conventional nucleic acid purification methods known to skilled artisans may be used to obtain isolated polynucleotides. The term also embraces recombinant
10 polynucleotides and chemically synthesized polynucleotides.

"Recombinant" refers to an artificial combination of two otherwise separated segments of sequence, e.g., by chemical synthesis or by the manipulation of isolated segments of nucleic acids by genetic engineering techniques.

"Recombinant" also includes reference to a cell or vector, that has been modified by
15 the introduction of a heterologous nucleic acid or a cell derived from a cell so modified, but does not encompass the alteration of the cell or vector by naturally occurring events (e.g., spontaneous mutation, natural transformation/transduction/transposition) such as those occurring without deliberate human intervention.

20 "Recombinant DNA construct" refers to a combination of nucleic acid fragments that are not normally found together in nature. Accordingly, a recombinant DNA construct may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different
25 than that normally found in nature.

"Regulatory sequences" refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may
30 include, but are not limited to, promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

"Promoter" refers to a nucleic acid fragment capable of controlling transcription of another nucleic acid fragment.

"Promoter functional in a plant" is a promoter capable of controlling transcription in plant cells whether or not its origin is from a plant cell.

"Tissue-specific promoter" and "tissue-preferred promoter" are used interchangeably, and refer to a promoter that is expressed predominantly but not necessarily exclusively in one tissue or organ, but that may also be expressed in one specific cell.

"Developmentally regulated promoter" refers to a promoter whose activity is determined by developmental events.

"Operably linked" refers to the association of nucleic acid fragments in a single fragment so that the function of one is regulated by the other. For example, a promoter is operably linked with a nucleic acid fragment when it is capable of regulating the transcription of that nucleic acid fragment.

"Expression" refers to the production of a functional product. For example, expression of a nucleic acid fragment may refer to transcription of the nucleic acid fragment (e.g., transcription resulting in mRNA or functional RNA) and/or translation of mRNA into a precursor or mature protein.

"Phenotype" means the detectable characteristics of a cell or organism.

"Introduced" in the context of inserting a nucleic acid fragment (e.g., a recombinant DNA construct) into a cell, means "transfection" or "transformation" or "transduction" and includes reference to the incorporation of a nucleic acid fragment into a eukaryotic or prokaryotic cell where the nucleic acid fragment may be incorporated into the genome of the cell (e.g., chromosome, plasmid, plastid or mitochondrial DNA), converted into an autonomous replicon, or transiently expressed (e.g., transfected mRNA).

A "transformed cell" is any cell into which a nucleic acid fragment (e.g., a recombinant DNA construct) has been introduced.

"Transformation" as used herein refers to both stable transformation and transient transformation.

"Stable transformation" refers to the introduction of a nucleic acid fragment into a genome of a host organism resulting in genetically stable inheritance. Once stably transformed, the nucleic acid fragment is stably integrated in the genome of the host organism and any subsequent generation.

“Transient transformation” refers to the introduction of a nucleic acid fragment into the nucleus, or DNA-containing organelle, of a host organism resulting in gene expression without genetically stable inheritance.

“Allele” is one of several alternative forms of a gene occupying a given locus on a chromosome. When the alleles present at a given locus on a pair of homologous chromosomes in a diploid plant are the same that plant is homozygous at that locus. If the alleles present at a given locus on a pair of homologous chromosomes in a diploid plant differ that plant is heterozygous at that locus. If a transgene is present on one of a pair of homologous chromosomes in a diploid plant that plant is hemizygous at that locus.

Sequence alignments and percent identity calculations may be determined using a variety of comparison methods designed to detect homologous sequences including, but not limited to, the Megalign® program of the LASARGENE® bioinformatics computing suite (DNASTAR® Inc., Madison, WI). Unless stated otherwise, multiple alignment of the sequences provided herein were performed using the Clustal V method of alignment (Higgins and Sharp (1989) *CABIOS* 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments and calculation of percent identity of protein sequences using the Clustal V method are KTUPLE=1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. For nucleic acids these parameters are KTUPLE=2, GAP PENALTY=5, WINDOW=4 and DIAGONALS SAVED=4. After alignment of the sequences, using the Clustal V program, it is possible to obtain “percent identity” and “divergence” values by viewing the “sequence distances” table on the same program; unless stated otherwise, percent identities and divergences provided and claimed herein were calculated in this manner.

Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook, J., Fritsch, E.F. and Maniatis, T. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1989 (hereinafter “Sambrook”).

Turning now to preferred embodiments:

Preferred embodiments include isolated polynucleotides and polypeptides, recombinant DNA constructs, compositions (such as plants or seeds) comprising these recombinant DNA constructs, and methods utilizing these recombinant DNA constructs.

5 Preferred Isolated Polynucleotides and Polypeptides

The present invention includes the following preferred isolated polynucleotides and polypeptides:

An isolated polynucleotide comprising: (i) a nucleic acid sequence encoding a polypeptide having an amino acid sequence of at least 50%, 51%, 52%, 53%, 54%,
10 55%, 56%, 57%, 58%, 59%, 60%, 56%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73 and wherein
15 expression of said polypeptide in a plant results in an altered root architecture when compared to a control plant not comprising said recombinant DNA construct, or (ii) a full complement of the nucleic acid sequence of (i), wherein the full complement and the nucleic acid sequence of (i) consist of the same number of nucleotides and are 100%complementary.

20 Any of the foregoing isolated polynucleotides may be utilized in any recombinant DNA constructs (including suppression DNA constructs) of the present invention.

An isolated polypeptide having an amino acid sequence of at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 56%, 62%, 63%, 64%, 65%,
25 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73 and wherein expression of said polypeptide in a plant results in an altered plant root
30 architecture when compared to a control plant not comprising said recombinant DNA construct.

An isolated polynucleotide comprising (i) a nucleic acid sequence of at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 56%, 62%, 63%,

64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 22, 28, 38, 66, 68, 70 or 72 and wherein said polynucleotide encodes a polypeptide wherein expression of said polypeptide results in an altered root architecture when compared to a control plant not comprising said recombinant DNA construct or (ii) a full complement of the nucleic acid sequence of (i). Any of the foregoing isolated polynucleotides may be utilized in any recombinant DNA constructs (including suppression DNA constructs) of the present invention. The isolated polynucleotide encodes a RUM1 or RUM1-like protein.

Preferred Recombinant DNA Constructs and Suppression DNA Constructs

In one aspect, the present invention includes recombinant DNA constructs (including suppression DNA constructs).

In one preferred embodiment, a recombinant DNA construct comprises a polynucleotide operably linked to at least one regulatory sequence (e.g., a promoter functional in a plant), wherein the polynucleotide comprises (i) a nucleic acid sequence encoding an amino acid sequence of at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 56%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73 or (ii) a full complement of the nucleic acid sequence of (i).

In another preferred embodiment, a recombinant DNA construct comprises a polynucleotide operably linked to at least one regulatory sequence (e.g., a promoter functional in a plant), wherein said polynucleotide comprises (i) a nucleic acid sequence of at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 56%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID

NO: 22, 28, 38, 66, 68, 70 or 72 or (ii) a full complement of the nucleic acid sequence of (i).

Fig.18 shows the multiple alignment of the full length amino acid sequences of B73-Mu-wt RUM1 (SEQ ID NO:24), B73 RUM1 (SEQ ID NO:29), B73 RUL (SEQ ID NO:39), the mutant rum1 (SEQ ID NO:25) and the rice protein identified as belonging to the AUX-IAA family (NCBI General identifier No. 34911088, SEQ ID NO:65). Amino acids conserved among all sequences are indicated with an asterisk (*) on the top row; dashes are used by the program to maximize alignment of the sequences. The method parameters used to produce the multiple alignment of the sequences below was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS*. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10) , and the pairwise alignment default parameters of KTUPLE=1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5.

Fig.19 shows a chart of the percent sequence identity for each pair of amino acid sequences displayed in Fig.18.

In another preferred embodiment, a recombinant DNA construct comprises a polynucleotide operably linked to at least one regulatory sequence (e.g., a promoter functional in a plant), wherein said polynucleotide encodes a RUM1 or RUM1-like protein. Preferably, the RUM1 or RUM1-like protein is from *Arabidopsis thaliana*, *Zea mays*, *Glycine max*, *Glycine tabacina*, *Glycine soja* and *Glycine tomentella*.

In another aspect, the present invention includes suppression DNA constructs.

A suppression DNA construct preferably comprises at least one regulatory sequence (preferably a promoter functional in a plant) operably linked to (a) all or part of (i) a nucleic acid sequence encoding a polypeptide having an amino acid sequence of at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 56%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73 or (ii) a full complement of the nucleic acid sequence of (a)(i); or (b) a region derived from all or part of a sense strand or

antisense strand of a target gene of interest, said region having a nucleic acid sequence of at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 56%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity, based on the Clustal V method of alignment, when compared to said all or part of a sense strand or antisense strand from which said region is derived, and wherein said target gene of interest encodes a RUM1 protein; or (c) all or part of (i) a nucleic acid sequence of at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 56%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 22, 28, 38, 66, 68, 70 or 72 or (ii) a full complement of the nucleic acid sequence of (c)(i). The suppression DNA construct preferably comprises a cosuppression construct, antisense construct, viral-suppression construct, hairpin suppression construct, stem-loop suppression construct, double-stranded RNA-producing construct, RNAi construct, or small RNA construct (e.g., an siRNA construct or an miRNA construct).

It is understood, as those skilled in the art will appreciate, that the invention encompasses more than the specific exemplary sequences. Alterations in a nucleic acid fragment which result in the production of a chemically equivalent amino acid at a given site, but do not affect the functional properties of the encoded polypeptide, are well known in the art. For example, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also be expected to produce a functionally equivalent product. Nucleotide changes which result in alteration of the N-terminal and C-terminal portions of the polypeptide molecule would also not be expected to alter the activity of the polypeptide. Each of

the proposed modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products.

“Suppression DNA construct” is a recombinant DNA construct which when transformed or stably integrated into the genome of the plant, results in “silencing” of a target gene in the plant. The target gene may be endogenous or transgenic to the plant. “Silencing,” as used herein with respect to the target gene, refers generally to the suppression of levels of mRNA or protein/enzyme expressed by the target gene, and/or the level of the enzyme activity or protein functionality. The terms “suppression”, “suppressing” and “silencing”, used interchangeably herein, include lowering, reducing, declining, decreasing, inhibiting, eliminating or preventing. “Silencing” or “gene silencing” does not specify mechanism and is inclusive, and not limited to, anti-sense, cosuppression, viral-suppression, hairpin suppression, stem-loop suppression, RNAi-based approaches, and small RNA-based approaches.

A suppression DNA construct may comprise a region derived from a target gene of interest and may comprise all or part of the nucleic acid sequence of the sense strand (or antisense strand) of the target gene of interest. Depending upon the approach to be utilized, the region may be 100% identical or less than 100% identical (e.g., at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 56%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical) to all or part of the sense strand (or antisense strand) of the gene of interest.

Suppression DNA constructs are well-known in the art, are readily constructed once the target gene of interest is selected, and include, without limitation, cosuppression constructs, antisense constructs, viral-suppression constructs, hairpin suppression constructs, stem-loop suppression constructs, double-stranded RNA-producing constructs, and more generally, RNAi (RNA interference) constructs and small RNA constructs such as siRNA (short interfering RNA) constructs and miRNA (microRNA) constructs.

“Antisense inhibition” refers to the production of antisense RNA transcripts capable of suppressing the expression of the target protein.

“Antisense RNA” refers to an RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target isolated

nucleic acid fragment (U.S. Patent No. 5,107,065). The complementarity of an antisense RNA may be with any part of the specific gene transcript, i.e., at the 5' non-coding sequence, 3' non-coding sequence, introns, or the coding sequence.

“Cosuppression” refers to the production of sense RNA transcripts capable of suppressing the expression of the target protein. “Sense” RNA refers to RNA transcript that includes the mRNA and can be translated into protein within a cell or *in vitro*. Cosuppression constructs in plants have been previously designed by focusing on overexpression of a nucleic acid sequence having homology to a native mRNA, in the sense orientation, which results in the reduction of all RNA having homology to the overexpressed sequence (see Vaucheret et al. (1998) *Plant J.* 16:651-659; and Gura (2000) *Nature* 404:804-808).

Another variation describes the use of plant viral sequences to direct the suppression of proximal mRNA encoding sequences (PCT Publication WO 98/36083 published on August 20, 1998).

Previously described is the use of “hairpin” structures that incorporate all, or part, of an mRNA encoding sequence in a complementary orientation that results in a potential “stem-loop” structure for the expressed RNA (PCT Publication WO 99/53050 published on October 21, 1999). In this case the stem is formed by polynucleotides corresponding to the gene of interest inserted in either sense or anti-sense orientation with respect to the promoter and the loop is formed by some polynucleotides of the gene of interest, which do not have a complement in the construct. This increases the frequency of cosuppression or silencing in the recovered transgenic plants. For review of hairpin suppression see Wesley, S.V. et al. (2003) *Methods in Molecular Biology, Plant Functional Genomics: Methods and Protocols* 236:273-286.

A construct where the stem is formed by at least 30 nucleotides from a gene to be suppressed and the loop is formed by a random nucleotide sequence has also effectively been used for suppression (PCT Publication No. WO 99/61632 published on December 2, 1999).

The use of poly-T and poly-A sequences to generate the stem in the stem-loop structure has also been described (PCT Publication No. WO 02/00894 published January 3, 2002).

Yet another variation includes using synthetic repeats to promote formation of a stem in the stem-loop structure. Transgenic organisms prepared with such recombinant DNA fragments have been shown to have reduced levels of the protein encoded by the nucleotide fragment forming the loop as described in PCT

5 Publication No. WO 02/00904, published 03 January 2002.

RNA interference refers to the process of sequence-specific post-transcriptional gene silencing in animals mediated by short interfering RNAs (siRNAs) (Fire et al., Nature 391:806 1998). The corresponding process in plants is commonly referred to as post-transcriptional gene silencing (PTGS) or RNA
10 silencing and is also referred to as quelling in fungi. The process of post-transcriptional gene silencing is thought to be an evolutionarily-conserved cellular defense mechanism used to prevent the expression of foreign genes and is commonly shared by diverse flora and phyla (Fire et al., Trends Genet. 15:358 1999). Such protection from foreign gene expression may have evolved in response
15 to the production of double-stranded RNAs (dsRNAs) derived from viral infection or from the random integration of transposon elements into a host genome via a cellular response that specifically destroys homologous single-stranded RNA of viral genomic RNA. The presence of dsRNA in cells triggers the RNAi response through a mechanism that has yet to be fully characterized.

20 The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III enzyme referred to as dicer. Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNAs) (Berstein et al., Nature 409:363 2001). Short interfering RNAs derived from dicer activity are typically about 21 to about 23 nucleotides in length and comprise about 19 base pair
25 duplexes (Elbashir et al., Genes Dev. 15:188 2001). Dicer has also been implicated in the excision of 21- and 22-nucleotide small temporal RNAs (stRNAs) from precursor RNA of conserved structure that are implicated in translational control (Hutvagner et al., 2001, Science 293:834). The RNAi response also features an endonuclease complex, commonly referred to as an RNA-induced silencing complex
30 (RISC), which mediates cleavage of single-stranded RNA having sequence complementarity to the antisense strand of the siRNA duplex. Cleavage of the target RNA takes place in the middle of the region complementary to the antisense strand of the siRNA duplex (Elbashir et al., Genes Dev. 15:188 2001). In addition,

RNA interference can also involve small RNA (e.g., miRNA) mediated gene silencing, presumably through cellular mechanisms that regulate chromatin structure and thereby prevent transcription of target gene sequences (see, e.g., Allshire, Science 297:1818-1819 2002; Volpe et al., Science 297:1833-1837 2002; Jenuwein, Science 297:2215-2218 2002; and Hall et al., Science 297:2232-2237 2002). As such, miRNA molecules of the invention can be used to mediate gene silencing via interaction with RNA transcripts or alternately by interaction with particular gene sequences, wherein such interaction results in gene silencing either at the transcriptional or post-transcriptional level.

RNAi has been studied in a variety of systems. Fire et al. (Nature 391:806 1998) were the first to observe RNAi in *C. elegans*. Wianny and Goetz (Nature Cell Biol. 2:70 1999) describe RNAi mediated by dsRNA in mouse embryos. Hammond et al. (Nature 404:293 2000) describe RNAi in *Drosophila* cells transfected with dsRNA. Elbashir et al., (Nature 411:494 2001) describe RNAi induced by introduction of duplexes of synthetic 21-nucleotide RNAs in cultured mammalian cells including human embryonic kidney and HeLa cells.

Small RNAs play an important role in controlling gene expression. Regulation of many developmental processes, including flowering, is controlled by small RNAs. It is now possible to engineer changes in gene expression of plant genes by using transgenic constructs which produce small RNAs in the plant.

Small RNAs appear to function by base-pairing to complementary RNA or DNA target sequences. When bound to RNA, small RNAs trigger either RNA cleavage or translational inhibition of the target sequence. When bound to DNA target sequences, it is thought that small RNAs can mediate DNA methylation of the target sequence. The consequence of these events, regardless of the specific mechanism, is that gene expression is inhibited.

It is thought that sequence complementarity between small RNAs and their RNA targets helps to determine which mechanism, RNA cleavage or translational inhibition, is employed. It is believed that siRNAs, which are perfectly complementary with their targets, work by RNA cleavage. Some miRNAs have perfect or near-perfect complementarity with their targets, and RNA cleavage has been demonstrated for at least a few of these miRNAs. Other miRNAs have several mismatches with their targets, and apparently inhibit their targets at the translational

level. Again, without being held to a particular theory on the mechanism of action, a general rule is emerging that perfect or near-perfect complementarity causes RNA cleavage, whereas translational inhibition is favored when the miRNA/target duplex contains many mismatches. The apparent exception to this is microRNA 172 (miR172) in plants. One of the targets of miR172 is APETALA2 (AP2), and although miR172 shares near-perfect complementarity with AP2 it appears to cause translational inhibition of AP2 rather than RNA cleavage.

MicroRNAs (miRNAs) are noncoding RNAs of about 19 to about 24 nucleotides (nt) in length that have been identified in both animals and plants (Lagos-Quintana et al., Science 294:853-858 2001, Lagos-Quintana et al., Curr. Biol. 12:735-739 2002; Lau et al., Science 294:858-862 2001; Lee and Ambros, Science 294:862-864 2001; Llave et al., Plant Cell 14:1605-1619 2002; Mourelatos et al., Genes. Dev. 16:720-728 2002; Park et al., Curr. Biol. 12:1484-1495 2002; Reinhart et al., Genes. Dev. 16:1616-1626 2002). They are processed from longer precursor transcripts that range in size from approximately 70 to 200 nt, and these precursor transcripts have the ability to form stable hairpin structures. In animals, the enzyme involved in processing miRNA precursors is called Dicer, an RNase III-like protein (Grishok et al., Cell 106:23-34 2001; Hutvagner et al., Science 293:834-838 2001; Ketting et al., Genes. Dev. 15:2654-2659 2001). Plants also have a Dicer-like enzyme, DCL1 (previously named CARPEL FACTORY/SHORT INTEGUMENTS1/ SUSPENSOR1), and recent evidence indicates that it, like Dicer, is involved in processing the hairpin precursors to generate mature miRNAs (Park et al., Curr. Biol. 12:1484-1495 2002; Reinhart et al., Genes. Dev. 16:1616-1626 2002). Furthermore, it is becoming clear from recent work that at least some miRNA hairpin precursors originate as longer polyadenylated transcripts, and several different miRNAs and associated hairpins can be present in a single transcript (Lagos-Quintana et al., Science 294:853-858 2001; Lee et al., EMBO J 21:4663-4670 2002). Recent work has also examined the selection of the miRNA strand from the dsRNA product arising from processing of the hairpin by DICER (Schwartz, et al. 2003 Cell 115:199-208). It appears that the stability (i.e. G:C vs. A:U content, and/or mismatches) of the two ends of the processed dsRNA affects the strand selection, with the low stability end being easier to unwind by a helicase activity.

The 5' end strand at the low stability end is incorporated into the RISC complex, while the other strand is degraded.

MicroRNAs appear to regulate target genes by binding to complementary sequences located in the transcripts produced by these genes. In the case of lin-4 and let-7, the target sites are located in the 3' UTRs of the target mRNAs (Lee et al.,
5 Cell 75:843-854 1993; Wightman et al., Cell 75:855-862 1993; Reinhart et al., Nature 403:901-906 2000; Slack et al., Mol. Cell 5:659-669 2000), and there are several mismatches between the lin-4 and let-7 miRNAs and their target sites.

Binding of the lin-4 or let-7 miRNA appears to cause downregulation of steady-state
10 levels of the protein encoded by the target mRNA without affecting the transcript itself (Olsen and Ambros, Dev. Biol. 216:671-680 1999). On the other hand, recent evidence suggests that miRNAs can in some cases cause specific RNA cleavage of the target transcript within the target site, and this cleavage step appears to require 100% complementarity between the miRNA and the target transcript (Hutvagner and
15 Zamore, Science 297:2056-2060 2002; Llave et al., Plant Cell 14:1605-1619 2002).

It seems likely that miRNAs can enter at least two pathways of target gene regulation: Protein downregulation when target complementarity is <100%, and RNA cleavage when target complementarity is 100%. MicroRNAs entering the RNA cleavage pathway are analogous to the 21-25 nt short interfering RNAs (siRNAs)
20 generated during RNA interference (RNAi) in animals and posttranscriptional gene silencing (PTGS) in plants (Hamilton and Baulcombe 1999; Hammond et al., 2000; Zamore et al., 2000; Elbashir et al., 2001), and likely are incorporated into an RNA-induced silencing complex (RISC) that is similar or identical to that seen for RNAi.

Identifying the targets of miRNAs with bioinformatics has not been successful
25 in animals, and this is probably due to the fact that animal miRNAs have a low degree of complementarity with their targets. On the other hand, bioinformatic approaches have been successfully used to predict targets for plant miRNAs (Llave et al., Plant Cell 14:1605-1619 2002; Park et al., Curr. Biol. 12:1484-1495 2002; Rhoades et al., Cell 110:513-520 2002), and thus it appears that plant miRNAs have
30 higher overall complementarity with their putative targets than do animal miRNAs. Most of these predicted target transcripts of plant miRNAs encode members of transcription factor families implicated in plant developmental patterning or cell differentiation.

A recombinant DNA construct (including a suppression DNA construct) of the present invention preferably comprises at least one regulatory sequence.

A preferred regulatory sequence is a promoter.

A number of promoters can be used in recombinant DNA constructs (and suppression DNA constructs) of the present invention. The promoters can be selected based on the desired outcome, and may include constitutive, tissue-specific, inducible, or other promoters for expression in the host organism.

High level, constitutive expression of the candidate gene under control of the 35S promoter may have pleiotropic effects. Candidate gene efficacy may be tested when driven by different promoters.

Suitable constitutive promoters for use in a plant host cell include, for example, the core promoter of the Rsyn7 promoter and other constitutive promoters disclosed in WO 99/43838 and U.S. Patent No. 6,072,050; the core CaMV 35S promoter (Odell et al., Nature 313:810-812 (1985)); rice actin (McElroy et al., Plant Cell 2:163-171 (1990)); ubiquitin (Christensen et al., Plant Mol. Biol. 12:619-632 (1989) and Christensen et al., Plant Mol. Biol. 18:675-689 (1992)); pEMU (Last et al., Theor. Appl. Genet. 81:581-588 (1991)); MAS (Velten et al., EMBO J. 3:2723-2730 (1984)); ALS promoter (U.S. Patent No. 5,659,026), and the like. Other constitutive promoters include, for example, those discussed in U.S. Patent Nos. 5,608,149; 5,608,144; 5,604,121; 5,569,597; 5,466,785; 5,399,680; 5,268,463; 5,608,142; and 6,177,611 and maize GOS2 (WO0020571 A2).

In choosing a promoter to use in the methods of the invention, it may be desirable to use a tissue-specific or developmentally regulated promoter.

A preferred tissue-specific or developmentally regulated promoter is a DNA sequence which regulates the expression of a DNA sequence selectively in the cells/tissues of a plant critical to tassel development, seed set, or both, and limits the expression of such a DNA sequence to the period of tassel development or seed maturation in the plant. Any identifiable promoter may be used in the methods of the present invention which causes the desired temporal and spatial expression.

Promoters which are seed or embryo specific and may be useful in the invention include soybean Kunitz trypsin inhibitor (Kti3, Jofuku and Goldberg, Plant Cell 1:1079-1093 (1989)), patatin (potato tubers) (Rocha-Sosa, M., et al. (1989) EMBO J. 8:23-29), convicilin, vicilin, and legumin (pea cotyledons) (Rerie, W.G., et

al. (1991) Mol. Gen. Genet. 259:149-157; Newbigin, E.J., et al. (1990) Planta 180:461-470; Higgins, T.J.V., et al. (1988) Plant. Mol. Biol. 11:683-695), zein (maize endosperm) (Schemthaner, J.P., et al. (1988) EMBO J. 7:1249-1255), phaseolin (bean cotyledon) (Segupta-Gopalan, C., et al. (1985) Proc. Natl. Acad. Sci. U.S.A. 82:3320-3324), phytohemagglutinin (bean cotyledon) (Voelker, T. et al. (1987) EMBO J. 6:3571-3577), B-conglycinin and glycinin (soybean cotyledon) (Chen, Z-L, et al. (1988) EMBO J. 7:297- 302), glutelin (rice endosperm), hordein (barley endosperm) (Marris, C., et al. (1988) Plant Mol. Biol. 10:359-366), glutenin and gliadin (wheat endosperm) (Colot, V., et al. (1987) EMBO J. 6:3559-3564), and sporamin (sweet potato tuberous root) (Hattori, T., et al. (1990) Plant Mol. Biol. 14:595-604). Promoters of seed-specific genes operably linked to heterologous coding regions in chimeric gene constructions maintain their temporal and spatial expression pattern in transgenic plants. Such examples include *Arabidopsis thaliana* 2S seed storage protein gene promoter to express enkephalin peptides in *Arabidopsis* and *Brassica napus* seeds (Vanderkerckhove et al., Bio/Technology 7:L929-932 (1989)), bean lectin and bean beta-phaseolin promoters to express luciferase (Riggs et al., Plant Sci. 63:47-57 (1989)), and wheat glutenin promoters to express chloramphenicol acetyl transferase (Colot et al., EMBO J 6:3559- 3564 (1987)).

Inducible promoters selectively express an operably linked DNA sequence in response to the presence of an endogenous or exogenous stimulus, for example by chemical compounds (chemical inducers) or in response to environmental, hormonal, chemical, and/or developmental signals. Inducible or regulated promoters include, for example, promoters regulated by light, heat, stress, flooding or drought, phytohormones, wounding, or chemicals such as ethanol, jasmonate, salicylic acid, or safeners.

Preferred promoters include the following: 1) the stress-inducible RD29A promoter (Kasuga et al. (1999) Nature Biotechnol. 17:287-91); 2) the barley promoter, B22E; expression of B22E is specific to the pedicel in developing maize kernels ("Primary Structure of a Novel Barley Gene Differentially Expressed in Immature Aleurone Layers". Klemsdal, S.S. et al., Mol. Gen. Genet. 228(1/2):9-16 (1991)); and 3) maize promoter, Zag2 ("Identification and molecular characterization of ZAG1, the maize homolog of the *Arabidopsis* floral homeotic gene AGAMOUS",

Schmidt, R.J. et al., Plant Cell 5(7):729-737 (1993)). "Structural characterization, chromosomal localization and phylogenetic evaluation of two pairs of *AGAMOUS*-like *MADS*-box genes from maize", Theissen et al., *Gene* 156(2): 155-166 (1995); NCBI GenBank Accession No. X80206)). *Zag2* transcripts can be detected 5 days prior to pollination to 7 to 8 days after pollination (DAP), and directs expression in the carpel of developing female inflorescences and *Cim1* which is specific to the nucleus of developing maize kernels. *Cim1* transcript is detected 4 to 5 days before pollination to 6 to 8 DAP. Other useful promoters include any promoter which can be derived from a gene whose expression is maternally associated with developing female florets.

Additional preferred promoters for regulating the expression of the nucleotide sequences of the present invention in plants are stalk-specific promoters. Such stalk-specific promoters include the alfalfa S2A promoter (GenBank Accession No. EF030816; Abrahams et al., Plant Mol. Biol. 27:513-528 (1995)) and S2B promoter (GenBank Accession No. EF030817) and the like, herein incorporated by reference.

Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic DNA segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions. It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, DNA fragments of some variation may have identical promoter activity. Promoters that cause a gene to be expressed in most cell types at most times are commonly referred to as "constitutive promoters". New promoters of various types useful in plant cells are constantly being discovered; numerous examples may be found in the compilation by Okamuro, J. K., and Goldberg, R. B., *Biochemistry of Plants* 15:1-82 (1989).

Preferred promoters may include: *RIP2*, *mLIP15*, *ZmCOR1*, *Rab17*, *CaMV 35S*, *RD29A*, *B22E*, *Zag2*, *SAM synthetase*, *ubiquitin*, *CaMV 19S*, *nos*, *Adh*, *sucrose synthase*, *R-allele*, *root cell promoter*, the vascular tissue preferred promoters *S2A* (Genbank accession number EF030816; SEQ ID NO:76) and *S2B* (Genbank accession number EF030817) and the constitutive promoter *GOS2* from

Zea mays. Other preferred promoters include root preferred promoters, such as the maize NAS2 promoter, the maize Cyclo promoter (US 2006/0156439, published July 13, 2006), the maize ROOTMET2 promoter (WO05063998, published July 14, 2005), the CR1BIO promoter (WO06055487, published May 26, 2006), the
5 CRWAQ81 (WO05035770, published April 21, 2005) and the maize ZRP2.47 promoter (NCBI accession number: U38790, gi: 1063664),

Recombinant DNA constructs (and suppression DNA constructs) of the present invention may also include other regulatory sequences, including but not limited to, translation leader sequences, introns, and polyadenylation recognition
10 sequences. In another preferred embodiment of the present invention, a recombinant DNA construct of the present invention further comprises an enhancer or silencer.

An intron sequence can be added to the 5' untranslated region or the coding sequence of the partial coding sequence to increase the amount of the mature
15 message that accumulates in the cytosol. Inclusion of a spliceable intron in the transcription unit in both plant and animal expression constructs has been shown to increase gene expression at both the mRNA and protein levels up to 1000-fold. Buchman and Berg, *Mol. Cell Biol.* 8:4395-4405 (1988); Callis et al., *Genes Dev.* 1:1183-1200 (1987). Such intron enhancement of gene expression is typically
20 greatest when placed near the 5' end of the transcription unit. Use of maize introns Adh1-S intron 1, 2, and 6, the Bronze-1 intron are known in the art. See generally, *The Maize Handbook*, Chapter 116, Freeling and Walbot, Eds., Springer, New York (1994).

If polypeptide expression is desired, it is generally desirable to include a
25 polyadenylation region at the 3'-end of a polynucleotide coding region. The polyadenylation region can be derived from the natural gene, from a variety of other plant genes, or from T-DNA. The 3' end sequence to be added can be derived from, for example, the nopaline synthase or octopine synthase genes, or alternatively from another plant gene, or less preferably from any other eukaryotic gene.

30 A translation leader sequence is a DNA sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect processing of the primary

transcript to mRNA, mRNA stability or translation efficiency. Examples of translation leader sequences have been described (Turner, R. and Foster, G. D. Molecular Biotechnology 3:225 (1995)).

In another preferred embodiment of the present invention, a recombinant DNA construct of the present invention further comprises an enhancer or silencer.

Any plant can be selected for the identification of regulatory sequences and genes to be used in creating recombinant DNA constructs and suppression DNA constructs of the present invention. Examples of suitable plant targets for the isolation of genes and regulatory sequences would include but are not limited to alfalfa, apple, apricot, *Arabidopsis*, artichoke, arugula, asparagus, avocado, banana, barley, beans, beet, blackberry, blueberry, broccoli, brussels sprouts, cabbage, canola, cantaloupe, carrot, cassava, castorbean, cauliflower, celery, cherry, chicory, cilantro, citrus, clementines, clover, coconut, coffee, corn, cotton, cranberry, cucumber, Douglas fir, eggplant, endive, escarole, eucalyptus, fennel, figs, garlic, gourd, grape, grapefruit, honey dew, jicama, kiwifruit, lettuce, leeks, lemon, lime, Loblolly pine, linseed, mango, melon, mushroom, nectarine, nut, oat, oil palm, oil seed rape, okra, olive, onion, orange, an ornamental plant, palm, papaya, parsley, parsnip, pea, peach, peanut, pear, pepper, persimmon, pine, pineapple, plantain, plum, pomegranate, poplar, potato, pumpkin, quince, radiata pine, radicchio, radish, rapeseed, raspberry, rice, rye, sorghum, Southern pine, soybean, spinach, squash, strawberry, sugarbeet, sugarcane, sunflower, sweet potato, sweetgum, tangerine, tea, tobacco, tomato, triticale, turf, turnip, a vine, watermelon, wheat, yams, and zucchini. Particularly preferred plants for the identification of regulatory sequences are *Arabidopsis*, corn, wheat, soybean, and cotton.

Preferred Compositions

A preferred composition of the present invention is a plant comprising in its genome any of the recombinant DNA constructs (including any of the suppression DNA constructs) of the present invention (such as those preferred constructs discussed above). Preferred composition also includes any progeny of the plant, and any seed obtained from the plant or its progeny. Progeny includes subsequent generations obtained by self-pollination or out-crossing of a plant. Progeny also includes hybrids and inbreds.

Preferably, in hybrid seed propagated crops, mature transgenic plants can be self-pollinated to produce a homozygous inbred plant. The inbred plant produces seed containing the newly introduced recombinant DNA construct (or suppression DNA construct). These seeds can be grown to produce plants that would exhibit an altered agronomic characteristic (e.g. an increased agronomic characteristic under nitrogen or phosphate limiting conditions), or used in a breeding program to produce hybrid seed, which can be grown to produce plants that would exhibit altered root architecture. Preferably, the seeds are maize.

Preferably, the plant is a monocotyledonous or dicotyledonous plant, more preferably, a maize or soybean plant, even more preferably a maize plant, such as a maize hybrid plant or a maize inbred plant. The plant may also be sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley or millet.

Preferably, the recombinant DNA construct is stably integrated into the genome of the plant.

Particularly preferred embodiments include but are not limited to the following preferred embodiments:

1. A plant (preferably a maize or soybean plant) comprising in its genome a recombinant DNA construct comprising a polynucleotide operably linked to at least one regulatory sequence, wherein said polynucleotide encodes a polypeptide having an amino acid sequence of at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73, and wherein said plant exhibits an altered root architecture when compared to a control plant not comprising said recombinant DNA construct. Preferably, the plant further exhibits an alteration of at least one agronomic characteristic when compared to the control plant.

2. A plant (preferably a maize or soybean plant) comprising in its genome a recombinant DNA construct comprising a polynucleotide operably linked to at least one regulatory sequence, wherein said polynucleotide encodes a RUM1 or RUM1-like protein, and wherein said plant exhibits an altered root architecture when

compared to a control plant not comprising said recombinant DNA construct.

Preferably, the plant further exhibits an alteration of at least one agronomic characteristic when compared to the control plant. Preferably, the RUM1 or RUM1-like protein is from *Arabidopsis thaliana*, *Zea mays*, *Glycine max*, *Glycine tabacina*,

5 *Glycine soja* or *Glycine tomentella*.

3. A plant (preferably a maize or soybean plant) comprising in its genome a suppression DNA construct comprising at least one regulatory element operably linked to a region derived from all or part of a sense strand or antisense strand of a target gene of interest, said region having a nucleic acid sequence of at least 50%,
10 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 56%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity, based on the Clustal V method of alignment, when compared to said all or part of a sense strand
15 or antisense strand from which said region is derived, and wherein said target gene of interest encodes a RUM1 or RUM1-like protein, and wherein said plant exhibits an alteration of at least one agronomic characteristic when compared to a control plant not comprising said recombinant DNA construct.

4. A plant (preferably a maize or soybean plant) comprising in its genome
20 a suppression DNA construct comprising at least one regulatory element operably linked to all or part of (a) a nucleic acid sequence encoding a polypeptide having an amino acid sequence of at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 56%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%,
25 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73, or (b) a full complement of the nucleic acid sequence of (a), and wherein said plant exhibits an alteration of at least one agronomic characteristic when compared to a control plant not comprising said
30 recombinant DNA construct.

5. Any progeny of the above plants in preferred embodiments 1-4, any seeds of the above plants in preferred embodiments 1-4, any seeds of progeny of

the above plants in preferred embodiments 1-4, and cells from any of the above plants in preferred embodiments 1-4 and progeny thereof.

5 In any of the foregoing preferred embodiments 1-5 or any other embodiments of the present invention, the recombinant DNA construct (or suppression DNA construct) preferably comprises at least a promoter that is functional in a plant as a preferred regulatory sequence.

In any of the foregoing preferred embodiments 1-5 or any other embodiments of the present invention, the alteration of at least one agronomic characteristic is either an increase or decrease, preferably an increase.

10 In any of the foregoing preferred embodiments 1-5 or any other embodiments of the present invention, the at least one greenness, yield, growth rate, biomass, fresh weight at maturation, dry weight at maturation, fruit yield, seed yield, total plant nitrogen content, fruit nitrogen content, seed nitrogen content, nitrogen content in a vegetative tissue, total plant free amino acid content, fruit free amino acid content,
15 seed free amino acid content, free amino acid content in a vegetative tissue, total plant protein content, fruit protein content, seed protein content, protein content in a vegetative tissue, drought tolerance, nitrogen uptake, root lodging, and harvest index.

20 With greenness, harvest index, yield, biomass, resistance to root lodging being a particularly preferred agronomic characteristic for alteration (preferably an increase).

In any of the foregoing preferred embodiments 1-5 or any other embodiments of the present invention, the plant preferably exhibits the alteration of at least one agronomic characteristic irrespective of the for example water and nutrient
25 availability when compared to a control plant .

One of ordinary skill in the art is familiar with protocols for determining alteration in plant root architecture. For example, alterations in root architecture can be determined by counting the nodal root numbers of the top 3 or 4 nodes of the greenhouse grown plants or the width of the root band. Other measures of
30 alterations in root architecture include but are not limited to alterations in vigor, growth, size, yield, biomass, or resistance to root lodging when compared to a control or reference plant.

The Examples below describe some representative protocols and techniques for detecting alterations in root architecture.

One can also evaluate alterations in root architecture by the ability of the plant to maintain sufficient yield thresholds in field testing under various environmental conditions (e.g. nutrient over-abundance or limitation, water over-abundance or limitation, exposure to insects or disease) by measuring for substantially equivalent yield at those conditions compared to normal nutrient or water conditions, or by measuring for less yield drag under over-abundant or limiting nutrient and water conditions compared to a control or reference plant.

Alterations in root architecture can also be measured by determining the resistance to root lodging of the transgenic plants compared to reference or control plants.

One of ordinary skill in the art would readily recognize a suitable control or reference plant to be utilized when assessing or measuring an agronomic characteristic or phenotype of a transgenic plant in any embodiment of the present invention in which a control or reference plant is utilized (e.g., compositions or methods as described herein). For example, by way of non-limiting illustrations:

1. Progeny of a transformed plant which is hemizygous with respect to a recombinant DNA construct (or suppression DNA construct), such that the progeny are segregating into plants either comprising or not comprising the recombinant DNA construct (or suppression DNA construct): the progeny comprising the recombinant DNA construct (or suppression DNA construct) would be typically measured relative to the progeny not comprising the recombinant DNA construct (or suppression DNA construct) (i.e., the progeny not comprising the recombinant DNA construct (or suppression DNA construct) is the control or reference plant).

2. Introgression of a recombinant DNA construct (or suppression DNA construct) into an inbred line, such as in maize, or into a variety, such as in soybean: the introgressed line would typically be measured relative to the parent inbred or variety line (i.e., the parent inbred or variety line is the control or reference plant).

3. Two hybrid lines, where the first hybrid line is produced from two parent inbred lines, and the second hybrid line is produced from the same two parent inbred lines except that one of the parent inbred lines contains a recombinant DNA construct (or suppression DNA construct): the second hybrid line would

typically be measured relative to the first hybrid line (i.e., the parent inbred or variety line is the control or reference plant).

4. A plant comprising a recombinant DNA construct (or suppression DNA construct): the plant may be assessed or measured relative to a control plant not comprising the recombinant DNA construct (or suppression DNA construct) but otherwise having a comparable genetic background to the plant (e.g., sharing at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity of nuclear genetic material compared to the plant comprising the recombinant DNA construct (or suppression DNA construct)). There are many laboratory-based techniques available for the analysis, comparison and characterization of plant genetic backgrounds; among these are Isozyme Electrophoresis, Restriction Fragment Length Polymorphisms (RFLPs), Randomly Amplified Polymorphic DNAs (RAPDs), Arbitrarily Primed Polymerase Chain Reaction (AP-PCR), DNA Amplification Fingerprinting (DAF), Sequence Characterized Amplified Regions (SCARs), Amplified Fragment Length Polymorphisms (AFLP®s), and Simple Sequence Repeats (SSRs) which are also referred to as Microsatellites.

Furthermore, one of ordinary skill in the art would readily recognize that a suitable control or reference plant to be utilized when assessing or measuring an agronomic characteristic or phenotype of a transgenic plant would not include a plant that had been previously selected, via mutagenesis or transformation, for the desired agronomic characteristic or phenotype.

Preferred Methods

Preferred methods include but are not limited to methods for altering root architecture in a plant, methods for evaluating alteration of root architecture in a plant, methods for altering an agronomic characteristic in a plant, methods for evaluating an alteration of an agronomic characteristic in a plant, and methods for producing seed. Preferably, the plant is a monocotyledonous or dicotyledonous plant, more preferably, a maize or soybean plant, even more preferably a maize plant. The plant may also be sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley or millet. The seed is preferably a maize or soybean seed, more preferably a maize seed, and even more preferably, a maize hybrid seed or maize inbred seed.

Particularly preferred methods include but are not limited to the following:

A method of altering root architecture of a plant, comprising: (a) introducing into a regenerable plant cell a recombinant DNA construct comprising a polynucleotide operably linked to at least one regulatory sequence (preferably a promoter functional in a plant), wherein the polynucleotide encodes a polypeptide having an amino acid sequence of at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 56%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73, and (b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the recombinant DNA construct and exhibits in altered root architecture when compared to a control plant not comprising the recombinant DNA construct. The method may further comprise (c) obtaining a progeny plant derived from the transgenic plant.

A method of altering root architecture in a plant, comprising: (a) introducing into a regenerable plant cell a suppression DNA construct comprising at least one regulatory sequence (preferably a promoter functional in a plant) operably linked to all or part of (i) a nucleic acid sequence encoding a polypeptide having an amino acid sequence of at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 56%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73 or (ii) a full complement of the nucleic acid sequence of (a)(i); and (b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the recombinant DNA construct and exhibits an altered root architecture when compared to a control plant not comprising the recombinant DNA construct. The method may further comprise (c) obtaining a progeny plant derived from the transgenic plant.

A method of altering root architecture in a plant, comprising: (a) introducing into a regenerable plant cell a suppression DNA construct comprising at least one regulatory sequence (preferably a promoter functional in a plant) operably linked to a region derived from all or part of a sense strand or antisense strand of a target gene of interest, said region having a nucleic acid sequence of at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity, based on the Clustal V method of alignment, when compared to said all or part of a sense strand or antisense strand from which said region is derived, and wherein said target gene of interest encodes a RUM1 or RUM1-like protein; and (b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the recombinant DNA construct and exhibits an altered root architecture when compared to a control plant not comprising the recombinant DNA construct. The method may further comprise (c) obtaining a progeny plant derived from the transgenic plant.

A method of evaluating altered root architecture in a plant, comprising (a) introducing into a regenerable plant cell a recombinant DNA construct comprising a polynucleotide operably linked to at least one regulatory sequence (preferably a promoter functional in a plant), wherein the polynucleotide encodes a polypeptide having an amino acid sequence of at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73 (b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the recombinant DNA construct; and (c) evaluating the transgenic plant for altered root architecture compared to a control plant not comprising the recombinant DNA construct. The method may further comprise (d) obtaining a progeny plant derived from the transgenic plant, wherein the progeny plant comprises in its genome the recombinant DNA construct; and (e) evaluating

the progeny plant for altered root architecture compared to a control plant not comprising the recombinant DNA construct.

A method of evaluating altered root architecture in a plant, comprising (a) introducing into a regenerable plant cell a suppression DNA construct comprising at least one regulatory sequence (preferably a promoter functional in a plant) operably linked to all or part of (i) a nucleic acid sequence encoding a polypeptide having an amino acid sequence of at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 56%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73, or (ii) a full complement of the nucleic acid sequence of (a)(i); (b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the suppression DNA construct; and (c) evaluating the transgenic plant for altered root architecture compared to a control plant not comprising the suppression DNA construct. The method may further comprise (d) obtaining a progeny plant derived from the transgenic plant, wherein the progeny plant comprises in its genome the suppression DNA construct; and (e) evaluating the progeny plant for altered root architecture compared to a control plant not comprising the suppression DNA construct.

A method of evaluating altered root architecture in a plant, comprising (a) introducing into a regenerable plant cell a suppression DNA construct comprising at least one regulatory sequence (preferably a promoter functional in a plant) operably linked to a region derived from all or part of a sense strand or antisense strand of a target gene of interest, said region having a nucleic acid sequence of at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 56%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity, based on the Clustal V method of alignment, when compared to said all or part of a sense strand or antisense strand from which said region is derived, and wherein said target gene of interest encodes a RUM1 or RUM1-like protein; (b) regenerating a transgenic

plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the suppression DNA construct; and (c) evaluating the transgenic plant for altered root architecture compared to a control plant not comprising the suppression DNA construct. The method may further comprise (d) obtaining a progeny plant derived from the transgenic plant, wherein the progeny plant comprises in its genome the suppression DNA construct; and (e) evaluating the progeny plant for altered root architecture compared to a control plant not comprising the suppression DNA construct.

A method of evaluating altered root architecture in a plant, comprising (a) introducing into a regenerable plant cell a recombinant DNA construct comprising a polynucleotide operably linked to at least one regulatory sequence (preferably a promoter functional in a plant), wherein said polynucleotide encodes a polypeptide having an amino acid sequence of at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 56%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73 (b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the recombinant DNA construct; (c) obtaining a progeny plant derived from said transgenic plant, wherein the progeny plant comprises in its genome the recombinant DNA construct; and (d) evaluating the progeny plant for altered root architecture compared to a control plant not comprising the recombinant DNA construct.

A method of evaluating altered root architecture in a plant, comprising (a) introducing into a regenerable plant cell a suppression DNA construct comprising at least one regulatory sequence (preferably a promoter functional in a plant) operably linked to all or part of (i) a nucleic acid sequence encoding a polypeptide having an amino acid sequence of at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 56%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity, based on the Clustal V method of alignment, when compared to

SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73, or (ii) a full complement of the nucleic acid sequence of (a)(i); (b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the suppression DNA construct; (c) obtaining a progeny plant derived from said transgenic plant, wherein the progeny plant comprises in its genome the suppression DNA construct; and (e) evaluating the progeny plant for altered root architecture compared to a control plant not comprising the suppression DNA construct.

A method of evaluating altered root architecture in a plant, comprising (a) introducing into a regenerable plant cell a suppression DNA construct comprising at least one regulatory sequence (preferably a promoter functional in a plant) operably linked to a region derived from all or part of a sense strand or antisense strand of a target gene of interest, said region having a nucleic acid sequence of at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity, based on the Clustal V method of alignment, when compared to said all or part of a sense strand or antisense strand from which said region is derived, and wherein said target gene of interest encodes a RUM1 or RUM1-like protein; (b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the suppression DNA construct; (c) obtaining a progeny plant derived from the transgenic plant, wherein the progeny plant comprises in its genome the suppression DNA construct; and (d) evaluating the progeny plant for altered root architecture compared to a control plant not comprising the recombinant DNA construct.

A method of evaluating an alteration of an agronomic characteristic in a plant, comprising (a) introducing into a regenerable plant cell a recombinant DNA construct comprising a polynucleotide operably linked to at least one regulatory sequence (preferably a promoter functional in a plant), wherein said polynucleotide encodes a polypeptide having an amino acid sequence of at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%,

83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73 (b)

regenerating a transgenic plant from the regenerable plant cell after step (a),

5 wherein the transgenic plant comprises in its genome said recombinant DNA construct; and (c) determining whether the transgenic plant exhibits an alteration in at least one agronomic characteristic when compared to a control plant not comprising the recombinant DNA construct. The method may further comprise (d) obtaining a progeny plant derived from the transgenic plant, wherein the progeny
10 plant comprises in its genome the recombinant DNA construct; and (e) determining whether the progeny plant exhibits an alteration in at least one agronomic characteristic when compared to a control plant not comprising the recombinant DNA construct.

A method of evaluating an alteration of an agronomic characteristic in a plant,
15 comprising (a) introducing into a regenerable plant cell a suppression DNA construct comprising at least one regulatory sequence (preferably a promoter functional in a plant) operably linked to all or part of (i) a nucleic acid sequence encoding a polypeptide having an amino acid sequence of at least 50%, 51%, 52%, 53%, 54%,
20 55%, 56%, 57%, 58%, 59%, 60%, 56%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73, or (ii) a full complement of the nucleic acid sequence of (i); (b) regenerating a transgenic plant
25 from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the suppression DNA construct; and (c) determining whether the transgenic plant exhibits an alteration in at least one agronomic characteristic when compared to a control plant not comprising the suppression DNA construct. The method may further comprise (d) obtaining a progeny plant derived from the
30 transgenic plant, wherein the progeny plant comprises in its genome the suppression DNA construct; and (e) determining whether the progeny plant exhibits an alteration in at least one agronomic characteristic when compared to a control plant not comprising the suppression DNA construct.

A method of evaluating alteration of an agronomic characteristic in a plant, comprising (a) introducing into a regenerable plant cell a suppression DNA construct comprising at least one regulatory sequence (preferably a promoter functional in a plant) operably linked to a region derived from all or part of a sense strand or antisense strand of a target gene of interest, said region having a nucleic acid sequence of at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 56%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity, based on the Clustal V method of alignment, when compared to said all or part of a sense strand or antisense strand from which said region is derived, and wherein said target gene of interest encodes RUM1 protein; (b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the suppression DNA construct; and (c) determining whether the transgenic plant exhibits an alteration in at least one agronomic characteristic when compared to a control plant not comprising the suppression DNA construct. The method may further comprise (d) obtaining a progeny plant derived from the transgenic plant, wherein the progeny plant comprises in its genome the suppression DNA construct; and (e) determining whether the progeny plant exhibits an alteration in at least one agronomic characteristic when compared to a control plant not comprising the suppression DNA construct.

A method of evaluating an alteration of an agronomic characteristic in a plant, comprising (a) introducing into a regenerable plant cell a recombinant DNA construct comprising a polynucleotide operably linked to at least one regulatory sequence (preferably a promoter functional in a plant), wherein said polynucleotide encodes a polypeptide having an amino acid sequence of at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 56%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73 (b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome said recombinant DNA

construct; (c) obtaining a progeny plant derived from said transgenic plant, wherein the progeny plant comprises in its genome the recombinant DNA construct; and (d) determining whether the progeny plant exhibits an alteration in at least one agronomic characteristic when compared to a control plant not comprising the recombinant DNA construct.

A method of evaluating an alteration of an agronomic characteristic in a plant, comprising (a) introducing into a regenerable plant cell a suppression DNA construct comprising at least one regulatory sequence (preferably a promoter functional in a plant) operably linked to all or part of (i) a nucleic acid sequence encoding a polypeptide having an amino acid sequence of at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73 or (ii) a full complement of the nucleic acid sequence of (i); (b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the suppression DNA construct; (c) obtaining a progeny plant derived from said transgenic plant, wherein the progeny plant comprises in its genome the suppression DNA construct; and (d) determining whether the progeny plant exhibits an alteration in at least one agronomic characteristic when compared to a control plant not comprising the recombinant DNA construct.

A method of evaluating an alteration of an agronomic characteristic in a plant, comprising (a) introducing into a regenerable plant cell a suppression DNA construct comprising at least one regulatory sequence (preferably a promoter functional in a plant) operably linked to a region derived from all or part of a sense strand or antisense strand of a target gene of interest, said region having a nucleic acid sequence of at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity, based on the Clustal V method of alignment, when compared to said all or part of a sense strand or antisense strand from which said region is derived, and

wherein said target gene of interest encodes a RUM1 protein; (b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the suppression DNA construct; (c) obtaining a progeny plant derived from said transgenic plant, wherein the progeny plant
5 comprises in its genome the suppression DNA construct; and (d) determining whether the progeny plant exhibits an alteration in at least one agronomic characteristic when compared to a control plant not comprising the suppression DNA construct.

A method of producing seed (preferably seed that can be sold as a product
10 offering with altered root architecture) comprising any of the preceding preferred methods, and further comprising obtaining seeds from said progeny plant, wherein said seeds comprise in their genome said recombinant DNA construct (or suppression DNA construct).

In any of the preceding preferred methods, in said introducing step said
15 regenerable plant cell preferably comprises a callus cell (preferably embryogenic), a gametic cell, a meristematic cell, or a cell of an immature embryo. The regenerable plant cells are preferably from an inbred maize plant.

In any of the preceding preferred methods or any other embodiments of methods of the present invention, said regenerating step preferably comprises: (i)
20 culturing said transformed plant cells in a media comprising an embryogenic promoting hormone until callus organization is observed; (ii) transferring said transformed plant cells of step (i) to a first media which includes a tissue organization promoting hormone; and (iii) subculturing said transformed plant cells after step (ii) onto a second media, to allow for shoot elongation, root development
25 or both.

The introduction of recombinant DNA constructs of the present invention into plants may be carried out by any suitable technique, including but not limited to direct DNA uptake, chemical treatment, electroporation, microinjection, cell fusion, infection, vector mediated DNA transfer, bombardment, or *Agrobacterium* mediated
30 transformation.

In any of the preceding preferred methods or any other embodiments of methods of the present invention, the at least one agronomic characteristic is preferably selected from the group consisting of greenness, yield, growth rate,

biomass, fresh weight at maturation, dry weight at maturation, fruit yield, seed yield, total plant nitrogen content, fruit nitrogen content, seed nitrogen content, nitrogen content in a vegetative tissue, total plant free amino acid content, fruit free amino acid content, seed free amino acid content, free amino acid content in a vegetative tissue, total plant protein content, fruit protein content, seed protein content, protein content in a vegetative tissue, drought tolerance, nitrogen uptake, root lodging, stalk lodging, plant height, ear length, and harvest index; with greenness, yield, biomass or resistance to root lodging being a particularly preferred agronomic characteristic for alteration (preferably an increase).

In any of the preceding preferred methods or any other embodiments of methods of the present invention, the plant preferably exhibits the alteration of at least one agronomic characteristic irrespective of the environmental conditions when compared to a control plant (e.g., water, nutrient availability, insect or disease),

The introduction of recombinant DNA constructs of the present invention into plants may be carried out by any suitable technique, including but not limited to direct DNA uptake, chemical treatment, electroporation, microinjection, cell fusion, infection, vector mediated DNA transfer, bombardment, or *Agrobacterium* mediated transformation.

Preferred techniques are set forth below in the Examples.

Other preferred methods for transforming dicots, primarily by use of *Agrobacterium tumefaciens*, and obtaining transgenic plants include those published for cotton (U.S. Patent No. 5,004,863, U.S. Patent No. 5,159,135, U.S. Patent No. 5,518, 908); soybean (U.S. Patent No. 5,569,834, U.S. Patent No. 5,416,011, McCabe et. al., *Bio/Technology* 6:923 (1988), Christou et al., *Plant Physiol.* 87:671 674 (1988)); Brassica (U.S. Patent No. 5,463,174); peanut (Cheng et al., *Plant Cell Rep.* 15:653 657 (1996), McKently et al., *Plant Cell Rep.* 14:699 703 (1995)); papaya; and pea (Grant et al., *Plant Cell Rep.* 15:254 258, (1995)).

Transformation of monocotyledons using electroporation, particle bombardment, and *Agrobacterium* have also been reported and are included as preferred methods, for example, transformation and plant regeneration as achieved in asparagus (Bytebier et al., *Proc. Natl. Acad. Sci. U.S.A.* 84:5354, (1987)); barley (Wan and Lemaux, *Plant Physiol.* 104:37 (1994)); Zea mays (Rhodes et al., *Science* 240:204 (1988), Gordon-Kamm et al., *Plant Cell* 2:603 618 (1990), Fromm et al.,

Bio/Technology 8:833 (1990), Koziel et al., *Bio/Technology* 11:194, (1993), Armstrong et al., *Crop Science* 35:550-557

(1995)); oat (Somers et al., *Bio/Technology* 10:1589 (1992)); orchard grass (Horn et al., *Plant Cell Rep.* 7:469 (1988)); rice (Toriyama et al., *Theor. Appl. Genet.* 205:34, (1986); Part et al., *Plant Mol. Biol.* 32:1135 1148, (1996); Abedinia et al., *Aust. J. Plant Physiol.* 24:133 141 (1997); Zhang and Wu, *Theor. Appl. Genet.* 76:835 (1988); Zhang et al., *Plant Cell Rep.* 7:379, (1988); Battraw and Hall, *Plant Sci.* 86:191 202 (1992); Christou et al., *Bio/Technology* 9:957 (1991)); rye (De la Pena et al., *Nature* 325:274 (1987)); sugarcane (Bower and Birch, *Plant J.* 2:409 (1992)); tall fescue (Wang et al., *Bio/Technology* 10:691 (1992)), and wheat (Vasil et al., *Bio/Technology* 10:667 (1992); U.S. Patent No. 5,631,152).

There are a variety of methods for the regeneration of plants from plant tissue. The particular method of regeneration will depend on the starting plant tissue and the particular plant species to be regenerated.

The regeneration, development, and cultivation of plants from single plant protoplast transformants or from various transformed explants is well known in the art (Weissbach and Weissbach, In: *Methods for Plant Molecular Biology*, (Eds.), Academic Press, Inc. San Diego, CA, (1988)). This regeneration and growth process typically includes the steps of selection of transformed cells, culturing those individualized cells through the usual stages of embryonic development through the rooted plantlet stage. Transgenic embryos and seeds are similarly regenerated. The resulting transgenic rooted shoots are thereafter planted in an appropriate plant growth medium such as soil.

The development or regeneration of plants containing the foreign, exogenous isolated nucleic acid fragment that encodes a protein of interest is well known in the art. Preferably, the regenerated plants are self-pollinated to provide homozygous transgenic plants. Otherwise, pollen obtained from the regenerated plants is crossed to seed-grown plants of agronomically important lines. Conversely, pollen from plants of these important lines is used to pollinate regenerated plants. A transgenic plant of the present invention containing a desired polypeptide is cultivated using methods well known to one skilled in the art.

EXAMPLES

The present invention is further illustrated in the following Examples, in which parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these Examples, while indicating preferred
5 embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, various modifications of the invention in
10 addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

EXAMPLE 1

Map-based Cloning of *RUM1*

15 The *rum1* mutation was mapped using one mapping population and its corresponding corn seeds, segregating for the *rum1* mutation. The mapping populations consisted of 3886 corn plants derived from a F1 cross between the line carrying the *rum1* mutation, and the inbred line F7. The line carrying the *rum1* mutation was isolated from mutagenized F2 families generated from selfed F1
20 crosses between the inbred line B73 and active *Mutator* stocks. For convenience this line was named B73-Mu.

Homozygous *rum1/rum1* plants were scored twice at 7 and 10 days after germination as plants with no visible lateral roots on primary roots when grown on paper rolls. A total of 630 plants were retrieved from the mapping population.

25 These plants were selected for fine mapping of the *rum1* locus.

DNA was extracted from those plants using standard molecular biology procedures.

To obtain F2 plants that carry recombination near the *rum1* locus, public PCR-based DNA markers (SSRs) present in the Maize Genetics and Genomic
30 Database (MaizeGDB), were used. When these were not available, CAP (allele-specific PCR primers) markers were developed from the DuPont proprietary sequences of BAC (Bacterial Artificial Chromosome) clones of known map positions. Both CAP and SSR primers were used in a PCR reaction containing 10ng of DNA.

Flanking SSR marker UMC1690 [UMC1690 forward primer (SEQ ID NO:1), UMC1690 reverse primer (SEQ ID NO:2)] and BNLG1108 [BNLG1108 forward primer (SEQ ID NO:3), BNLG1108 reverse primer (SEQ ID NO:4)] were retrieved from the MaizeGDB. These markers are localized at 544.6 cM and 618.6 cM of Chromosome 3 respectively, based on the public map IBM2 2004 neighbors 3.

SSR markers amplifications were performed in a 10 ul PCR reaction using the Qiagen HotStart mix (Qiagen, Valencia, CA) and 10 ng DNA. The thermal cycle conditions were: 95°C 15min (1 cycle), 94°C 30 sec, 60°C 30 sec, 72°C 60 sec, (40 cycles) 72°C 5 min. Amplification products were examined for polymorphisms on 4% high resolution agarose (Sigma-Aldrich, Saint Louis, MO).

When using these 2 primer sets on an initial population of 213 *rum1* plants, a total of 16 out of 213 recombinants were obtained, 14 with marker UMC1690 and 2 from marker BNLG1108, indicating that *rum1* was closer to BNLG1108.

In order to obtain genetic markers closer to *rum1*, more primers were retrieved from the Maize GDB based on their position along chromosome 3 and tested on the above mentioned 213 *rum1* plants plus an additional 204 *rum1* plants, in a total of 417 *rum1* plants. In particular, markers UMC1844 [UMC1844 forward primer (SEQ ID NO:5), UMC1844 reverse primer (SEQ ID NO:6)] gave 15 out of 417 recombinants and marker UMC1915 [UMC1915 forward primer (SEQ ID NO:7), UMC1915 reverse primer (SEQ ID NO:8)] gave 14 out of 417 recombinants, indicating a distance of 1.8 cM and 1.7 cM from the *rum1* locus respectively. Marker UMC1844 and UMC1915 have been physically positioned by hybridization onto a single maize contig, named 320 (Dupont Genomix database).

Two more SSR markers reported to be localized between UMC1844 and UMC1915 on the public IBM2 2004 neighbors 3 map, but not physically positioned onto contig 320 were analyzed. Screening of the public BAC library using the marker PHP9257A [PHP9257A forward primer (SEQ ID NO:9), PHP9257A reverse primer (SEQ ID NO:10)] or marker UMC2274 [UMC2274 forward primer (SEQ ID NO:11), UMC2274 reverse primer (SEQ ID NO:12)] as probes, revealed that PHP9257A localizes immediately downstream of UMC1844 and UMC2274 localizes immediately upstream of UMC1915 on contig 320. Marker PHP9257A gave 11 recombinants while marker UMC2274 gave 6 recombinants, indicating a distance of

1.3 cM and 0.7 cM from the *rum1* locus respectively. The physical distance comprising the two markers encompasses approximately 10 BACs.

Based on this information, new CAP markers were designed using available BAC-end sequences of the BACs constituting the region of contig 320 surrounded by markers PHP9257A and UMC2274.

Cap marker MZA8411 [MZA8411 forward primer (SEQ ID NO:13), MZA8411 reverse primer (SEQ ID NO:14)] was designed based on the MZA8411 sequence, which is downstream of PHP9257A. This primer set amplifies a region of 544 bp, showing polymorphism between F7 and the mutant background line after restriction with the 5-cutter enzyme *TspRI* (New England Biolabs, Ipswich, MA).

CAP marker amplifications were performed in a 20 ul PCR reaction using the Qiagen HotStart mix (Qiagen, Valencia, CA) and 10 ng DNA. Thermal cycle conditions were the same as described previously. Fifteen microliters of the amplification product were used for a restriction digest (total volume of 100 ul) with the 5-cutter restriction enzyme *TspRI*. Restriction reaction was carried out at 65°C for one hour. Restricted amplification products were extracted one time in phenol/chlorophorm/isoamyl alcohol (25:24:1), precipitated in 100% ethanol/3M sodium acetate (2.5 vol:1/10 vol), rinsed in 70% ethanol and examined on 2% agarose gels. By screening the 17 previously obtained recombinants with this primers set, 7 recombination breakpoints were found, indicating that it is located at a distance of 0.8 cM from the *rum1* locus on the same side of the marker PHP9257A.

Cap marker b0568n15 [b0568n15 forward primer (SEQ ID NO:15), b0568n15 reverse primer (SEQ ID NO:16)] was designed based on the BAC-end sequence of clone BAC b0568n15, which is localized upstream of UMC2274. This primer set amplifies a region of 706 bp, showing polymorphism between F7 and the mutant background line after restriction with the 5-cutter enzyme *TspRI*. Two recombination breakpoints were found using this primer set, indicating that b0568n15 is located at a distance of 0.2 cM from the *rum1* locus on the same side of the marker UMC2274.

Cap marker MZA8828 [MZA8828 forward primer (SEQ ID NO:17), MZA8828 reverse primer (SEQ ID NO:18)] was designed based on the sequence of MZA8828, which is downstream of MZA8411. This primer set amplifies a region of 763 bp, showing polymorphism between F7 and the mutant background line after restriction with the 5-cutter enzyme *NciI* (New England Biolabs, Ipswich, MA) at 37 °C. One

recombination breakpoint was found using this primer set, indicating that MZA8828 is located at a distance of 0.1 cM from the *rum1* locus on the same side of MZA8411.

PCR amplification showed that the MZA8828 marker is also located on the BAC clone b0568n15. Therefore, the *RUM1* locus could be narrowed down to the genomic region on Bac clone b0568n15 between marker MZA8828 marker (at a distance of 0.1 cM, one recombinant) and the BAC-end marker b0568n15 (at a distance of 0.2 cM, two recombinants).

EXAMPLE 2

Identification of the *RUM1* Gene

BAC clone b0568n15, to which the *rum1* locus mapped, was sequenced. For this purpose, BAC DNA was nebulized using high-pressure nitrogen gas as described in Roe et al. 1996 (Roe et al. (1996) "DNA isolation and Sequencing" John Wiley and Sons, New York).

The region between the marker MZA8828 and BAC-end marker b0568n15 is about 69 kb long and comprises six genic regions according to BLAST searches of the BAC b0568n15 against maize EST databases (Public and DuPont proprietary EST databases). This region was also found to be syntenic with the rice chromosome 1 region: 27753126 to 27823073 bp by homology search of the markers against the rice genomic database. Among the six genic regions found in maize, four were also conserved in rice and annotated as: Os01g0676200 (Conserved hypothetical protein), Os01g675800 (NAC domain containing protein), Os01g675700 (Auxin-responsive Solitary-root/IAA14-like protein (SLR/IAA14-like)), Os01g0675500 (Glycoprotein-specific UDP-glucosyltransferase-like protein).

The gene homologous to the rice SLR/IAA14-like gene was selected as the strongest candidate to be the *RUM1* gene due to its location regarding the distance from the markers MZA8828 and b0568n15 (1/3 and 2/3, respectively), as well as for the phenotypic similarity of the *rum1* mutant to the *slr* from *Arabidopsis*, which is also defective in lateral root formation (Fukaki et al., 2002). The 4098 bp fragment of b0568n15 containing the *RUM1* gene is shown in SEQ ID NO:19 and Fig.1. Fig.2 shows the *RUM1* physical map and its synteny with Rice.

DNA extracted from B73-Mu, carrying a wild type allele for *RUM1* (B73-Mu-wt), or from *rum1* plants and digested with *Xho*I (Promega) was examined by

Southern hybridization using a fragment comprising exons 1 and 2 of the *RUM1* gene as probe. While a fragment of about 700 bp segregated with B73-Mu-wt DNA, a fragment of about 1.8 kb segregated with mutant *rum1* plants, indicating the insertion of an exogenous element in the mutants. The element was amplified by PCR and consisted of a fragment of 1719 bp with terminal inverted repeats (TIRs) of 212 bp that show about 85% of identity with the TIRs of the maize transposable element *Mu1*.

RT-PCR of *RUM1* with poly(A) RNA extracted from B73-Mu-wt and mutant *rum1* plants primary roots, revealed that the *rum1* transcript was shorter than the *RUM1* B73-Mu-wt transcript.

EXAMPLE 3

Cloning of the full length *RUM1* and *rum1* cDNAs

Primary roots B73-Mu-wt and *rum1* sibling seedlings obtained from the selfed progeny of a heterozygote plant were used to extract total RNA using TRIzol® (Invitrogen™), containing phenol and guanidine thiocyanate. Poly(A) mRNA was purified from total RNA with a mRNA Purification kit obtained from Amersham Biosciences/GE Healthcare, Piscataway, NJ, 08855, which consists of oligo (dT)-cellulose spin columns. To perform RT-PCR, 0.5 µg of poly(A) RNA was used for cDNA synthesis using the Thermoscript® RT-PCR system (Invitrogen™). The cDNA was then amplified by PCR using the Platinum® Taq DNA polymerase combined with PCR_x Enhancer System (Invitrogen™). Primers specific to the 5' and 3' UTR of *RUM1* [RUM1 -70F forward primer (SEQ ID NO:20), RUM1 +40R reverse primer (SEQ ID NO:21)] were used in the PCR reaction. PCR products were cloned into the pPCR®II-Topo® nt vector (Invitrogen™) and sequenced to confirm identity. The *RUM1* B73-Mu-wt and *rum1* mutant cDNAs are shown in SEQ ID NO:22 and 23, respectively. The corresponding amino acid sequences are shown in SEQ ID NO's: 24 and 25, respectively). The mutant has a deletion of 72 nucleotides. Therefore, the transposon insertion in *rum1* plants results in an alternative splicing of the *RUM1* transcript and consequently deletion of 24 amino acids from the protein sequence.

EXAMPLE 4

Identification of the full length B73 *RUM1* cDNA

Using BLAST N, the sequence of the full length *RUM1* cDNA (SEQ ID NO.:22), obtained as described in Example 3, was used to search for ESTs in the

public EST database, which is derived from the inbred line B73. The highest homology found was to a partial EST from B73 with the accession number CD439449 (SEQ ID NO:26). The protein encoded by CD439449 is shown in SEQ ID NO:27. The 5' terminus of the B73 *RUM1* cDNA (SEQ ID NO:26) was deduced from the sequence of the public BAC clone b0568n15 mentioned in Example 3 (SEQ ID NO:19). The full length coding sequence of B73 *RUM1* is shown in SEQ ID NO:28 and the corresponding amino acid sequence in SEQ ID NO:29. The *RUM1* amino acid sequence from B73 shares 99.3% identity with the wild type *RUM1* sequence from the background line of the mutant (B73-Mu-wt) and 39.8%, 38.6% and 33.5% sequence identity with the *Arabidopsis* proteins IAA8 (NCBI General Identifier No. 15227275, SEQ ID NO:30), SLR/ IAA14 (NCBI General Identifier No. 22328628, SEQ ID NO:31) and MSG2/IAA19 (NCBI General Identifier No. 1532612, SEQ ID NO:32), respectively. MSG2/IAA19 has been shown to be involved in the regulation of the differential growth responses of hypocotyl and formation of lateral roots in *Arabidopsis thaliana* (Tatematsu et al. Plant Cell. 2004 Feb;16(2):379-93).

Percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal V method of alignment (Higgins and Sharp (1989) *CABIOS*. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10).

EXAMPLE 5

Expression pattern of the *RUM1* gene

The expression pattern of *RUM1* was analyzed via Lynx MPSS (Brenner et al (2000) *Proc Natl Acad Sci U S A* 97:1665-70). MPSS tags in the B73 *RUM1* cDNA sequence were searched using the DuPont proprietary LynxMPSS database. *RUM1* expression was detected at high levels in several tissues as summarized in Table 1 below.

TABLE 1

MPSS tags in B73 *RUM1* cDNA sequence
(PPM: parts per million)

PPM	Tissue
229	meristem

221	embryo
164	seedling
154	tassel
144	ear
111	silk
110	root
99	leaf
86	cell culture
70	pericarp
55	kernel
51	endosperm
46	whorl
41	stem
41	pedicel
40	husk
26	vascular bundles
19	scutellum
19	stalk

EXAMPLE 6

Identification of new *rum1* mutant alleles

Four independent *Mutator* (*Mu*) insertion lines were identified by screening
5 the *Mu* active TUSC populations: PV04 47 E-04, PV03 103 E-03, PV03 128 B-04
and BT94 104 G-05. Twenty five seeds from each line were planted in the 2006
Summer field to generate homozygous insertions by selfing, and also to introgress
the insertion into the inbred line B73.

DNA was extracted from leaves of the seedlings that germinated in the field
10 and insertion was confirmed by PCR using two combinations of nested *RUM1*
primers [set 1: RUM1 -354F forward primer (SEQ ID NO:33), RUM1 exon1-R1
reverse primer (SEQ ID NO:34); set 2: RUM1 -132F forward primer (SEQ ID NO:35),
RUM1 exon1-R2 reverse primer (SEQ ID NO:36)], and two combinations of nested
primers for *RUM1* and for the *Mu* TIR [set 1: RUM1 -354F forward primer (SEQ ID

NO:33), MuTIR primer (SEQ ID NO:37); set 2: RUM1 -132F forward primer (SEQ ID NO:35), MuTIR primer (SEQ ID NO:37)].

The progeny of these plants will be used for analyses of the insertion lines phenotype.

EXAMPLE 7

Identification of the *RUM1* duplicate gene *RUL*.

The *RUM1* cDNA from B73 was used to search the public EST database for additional maize *RUM1* genes. An EST with accession number DR813588 was identified. The two sequences share 85.2% sequence identity. The DR813588 cDNA sequence was used to search homologous sequences in the public and proprietary DNA databases. The highest homology was obtained with AZM5_100875 from the TIGR Genomic Assembly Release 5.0. The predicted cDNA from AZM5_100875 shows around 70% of identity with the *RUM1* cDNA from B73 and B73-Mu-wt. On the protein level the B73 and B73-Mu-wt *RUM1* share 84.6% identity with the predicted protein encoded by the AZM5_100875 sequence.

Recently, a public BAC clone comprising the AZM5_100875 sequence has been released. The BAC clone c0491g17 (accession number AC187246) is physically mapped to chromosome 8 bin 5. Patterns of chromosome duplication between chromosomes 3 and 8 of maize have been reported [Gaut B.S. (2001) *Genomic Research* 11, 55-66.]. Therefore, AZM5_100875 appears to encode a duplicate gene of *RUM1*. The full length sequence of the *RUM1* duplicate sequence was assembled from the alignment of the cDNA sequences from DR813588 and AZM5_100875 and was named *Rum1-like* (*RUL*). The full length cDNA sequence encoding the *RUL* protein is shown in SEQ ID NO:38 and the corresponding protein sequence in SEQ ID NO:39. All sequence alignments and % identity calculations were done using the Clustal method of alignment.

EXAMPLE 8

Cloning of the full length *RUL* cDNA

Primers specific for the 5' and 3' UTR of *RUL* [*RUL* -43F forward primer (SEQ ID NO:40), *RUL* +181R reverse primer (SEQ ID NO:41)] were used for PCR amplification the *RUL* full length cDNA (SEQ ID NO:38) as described in Example 3. Primary roots of B73-Mu-wt and *rum1* sibling seedlings obtained from the selfed progeny of a heterozygote plant were used as template. PCR products were cloned

into the pPCRII-Topo vector obtained from Invitrogen, Carlsbad, CA, 92008 and sequenced to confirm identity. *RUL* transcripts derived from wild type (B73-Mu-wt) or *rum1* siblings were identical, indicating that the *RUL* gene is not altered in the *rum1* mutants.

EXAMPLE 9

Preparation of a Plant Expression Vector

Containing the *RUM1* or a *RUM1*-like gene

Sequences homologous to the *RUM1* gene can be identified using sequence comparison algorithms such as BLAST (Basic Local Alignment Search Tool; Altschul et al., J. Mol. Biol. 215:403-410 (1993); see also the explanation of the BLAST algorithm on the world wide web site for the National Center for Biotechnology Information at the National Library of Medicine of the National Institutes of Health). The *RUM1* gene (SEQ ID NO:22 or 28), or *RUM1*-like genes, such as the one disclosed in SEQ ID NO:38, can be PCR-amplified by either of the following methods.

Method 1 (RNA-based): Based on the 5' and 3' sequence information for the protein-coding region of *RUM1* (SEQ ID NO:22 or 28) or a *RUM1* homolog (for example *RUL*, SEQ ID NO:38, gene-specific primers can be designed. RT-PCR can be used with plant RNA to obtain a nucleic acid fragment containing the *RUM1* protein-coding region flanked by attB1 (SEQ ID NO:42) and attB2 (SEQ ID NO:43) sequences. The primer may contain a consensus Kozak sequence (CAACA) upstream of the start codon.

Method 2 (DNA-based): Alternatively, the entire cDNA insert (containing 5' and 3' non-coding regions) of a clone encoding *RUM1* or a polypeptide homolog (such as *RUL*, SEQ ID NO:38), can be PCR amplified. Forward and reverse primers can be designed that contain either the attB1 sequence and vector-specific sequence that precedes the cDNA insert or the attB2 sequence and vector-specific sequence that follows the cDNA insert, respectively. For a cDNA insert cloned into the vector pBluescript SK+, the forward primer VC062 (SEQ ID NO:44) and the reverse primer VC063 (SEQ ID NO:45) can be used.

Methods 1 and 2 can be modified according to procedures known by one skilled in the art. For example, the primers of method 1 may contain restriction sites instead of attB1 and attB2 sites, for subsequent cloning of the PCR product into a

vector containing attB1 and attB2 sites. Additionally, method 2 can involve amplification from a cDNA clone, a lambda clone, a BAC clone or genomic DNA.

A PCR product obtained by either method above can be combined with the Gateway® donor vector, such as pDONR™/Zeo (Invitrogen™, Fig.3; SEQ ID NO:46) or pDONR™221 (Invitrogen™, Figure 4; SEQ ID NO:47) using a BP Recombination Reaction. This process removes the bacteria lethal *ccdB* gene, as well as the chloramphenicol resistance gene (CAM) from the donor vectors and directionally clones the PCR product with flanking attB1 and attB2 sites to create an entry clone. Using the Invitrogen Gateway® Clonase™ technology, the *RUM1* or *RUM1-like* gene from the entry clone can then be transferred to a suitable destination vector to obtain a plant expression vector for use with soy and corn, such as PHP27840 (Fig.5; SEQ ID NO:48) or PHP23236 (Figure 6; SEQ ID NO:49), respectively.

Alternatively a MultiSite Gateway® LR recombination reaction between multiple entry clones and a suitable destination vector can be performed to create an expression vector. An Example of this type of reaction is outlined in Example 14, which describes the construction of maize expression vectors for transformation of maize lines.

EXAMPLE 10

Preparation of Soybean Expression Vectors and Transformation of Soybean with the *RUM1* gene

Soybean plants can be transformed to over-express the *RUM1* and *RUM1-like* sequences in order to examine the resulting phenotype.

The entry clones described in Example 9 can be used to directionally clone each gene into PHP27840 vector (Figure 5, SEQ ID NO:48) such that expression of the gene is under control of the SCP1 promoter.

Soybean embryos may then be transformed with the expression vector comprising sequences encoding the instant polypeptides.

To induce somatic embryos, cotyledons, 3-5 mm in length dissected from surface sterilized, immature seeds of the soybean cultivar A2872, can be cultured in the light or dark at 26 °C on an appropriate agar medium for 6-10 weeks. Somatic embryos, which produce secondary embryos, are then excised and placed into a suitable liquid medium. After repeated selection for clusters of somatic embryos

which multiply as early, globular staged embryos, the suspensions are maintained as described below.

Soybean embryogenic suspension cultures can be maintained in 35mL liquid media on a rotary shaker, 150 rpm, at 26 °C with florescent lights on a 16:8 hour day/night schedule. Cultures are subcultured every two weeks by inoculating approximately 35 mg of tissue into 35 mL of liquid medium.

Soybean embryogenic suspension cultures may then be transformed by the method of particle gun bombardment (Klein et al. (1987) *Nature* (London) 327:70-73, U.S. Patent No. 4,945,050). A DuPont Biolistic™ PDS1000/HE instrument (helium retrofit) can be used for these transformations.

A selectable marker gene which can be used to facilitate soybean transformation is a chimeric gene composed of the 35S promoter from cauliflower mosaic virus (Odell et al. (1985) *Nature* 313:810-812), the hygromycin phosphotransferase gene from plasmid pJR225 (from *E. coli*; Gritz et al. (1983) *Gene* 25:179-188) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*. Another selectable marker gene which can be used to facilitate soybean transformation is an herbicide-resistant acetolactate synthase (ALS) gene from soybean or *Arabidopsis*. ALS is the first common enzyme in the biosynthesis of the branched-chain amino acids valine, leucine and isoleucine. Mutations in ALS have been identified that convey resistance to some or all of three classes of inhibitors of ALS (US Patent No. 5,013,659; the entire contents of which are herein incorporated by reference). Expression of the herbicide-resistant ALS gene can be under the control of a SAM synthetase promoter (U.S. Patent Application No. US-2003-0226166-A1; the entire contents of which are herein incorporated by reference).

To 50 µL of a 60 mg/mL 1 µm gold particle suspension is added (in order): 5 µL DNA (1 µg/µL), 20 µL spermidine (0.1 M), and 50 µL CaCl₂ (2.5 M). The particle preparation is then agitated for three minutes, spun in a microfuge for 10 seconds and the supernatant removed. The DNA-coated particles are then washed once in 400 µL 70% ethanol and resuspended in 40 µL of anhydrous ethanol. The DNA/particle suspension can be sonicated three times for one second each. Five µL of the DNA-coated gold particles are then loaded on each macro carrier disk.

Approximately 300-400 mg of a two-week-old suspension culture is placed in an empty 60x15 mm petri dish and the residual liquid removed from the tissue with a pipette. For each transformation experiment, approximately 5-10 plates of tissue are normally bombarded. Membrane rupture pressure is set at 1100 psi and the chamber is evacuated to a vacuum of 28 inches mercury. The tissue is placed approximately 3.5 inches away from the retaining screen and bombarded three times. Following bombardment, the tissue can be divided in half and placed back into liquid and cultured as described above.

Five to seven days post bombardment, the liquid media may be exchanged with fresh media, and eleven to twelve days post bombardment with fresh media containing 50 mg/mL hygromycin. This selective media can be refreshed weekly. Seven to eight weeks post bombardment, green, transformed tissue may be observed growing from untransformed, necrotic embryogenic clusters. Isolated green tissue is removed and inoculated into individual flasks to generate new, clonally propagated, transformed embryogenic suspension cultures. Each new line may be treated as an independent transformation event. These suspensions can then be subcultured and maintained as clusters of immature embryos or regenerated into whole plants by maturation and germination of individual somatic embryos.

Enhanced root architecture can be measured in soybean by growing the plants in soil and wash the roots before analysis of the total root mass with the software WinRHIZO® (Regent Instruments Inc), an image analysis system specifically designed for root measurement. WinRHIZO® uses the contrast in pixels to distinguish the light root from the darker background.

Soybean plants transformed with the *RUM1* gene can then be assayed to study agronomic characteristics relative to control or reference plants. For example, nitrogen utilization efficacy, yield enhancement and/or stability under various environmental conditions (e.g. nitrogen limiting conditions, drought etc.).

EXAMPLE 11

Transformation of Maize with the *RUM1* and *RUM1-like* Gene Using Particle Bombardment

Maize plants can be transformed to overexpress *RUM1* and *RUM1-like* genes in order to examine the resulting phenotype.

The Gateway® entry clones described in Example 9 can be used to directionally clone each gene into a maize transformation vector. Expression of the gene in maize can be under control of a constitutive promoter such as the maize ubiquitin promoter (Christensen et al., *Plant Mol. Biol.* 12:619-632 (1989) and

Christensen et al., *Plant Mol. Biol.* 18:675-689 (1992))

The recombinant DNA construct described above can then be introduced into maize cells by the following procedure. Immature maize embryos can be dissected from developing caryopses derived from crosses of the inbred maize lines H99 and LH132. The embryos are isolated ten to eleven days after pollination when they are 1.0 to 1.5 mm long. The embryos are then placed with the axis-side facing down and in contact with agarose-solidified N6 medium (Chu et al., *Sci. Sin. Peking* 18:659-668 (1975)). The embryos are kept in the dark at 27 °C. Friable embryogenic callus consisting of undifferentiated masses of cells with somatic proembryoids and embryoids borne on suspensor structures proliferates from the scutellum of these immature embryos. The embryogenic callus isolated from the primary explant can be cultured on N6 medium and sub-cultured on this medium every two to three weeks.

The plasmid, p35S/Ac (obtained from Dr. Peter Eckes, Hoechst Ag, Frankfurt, Germany) may be used in transformation experiments in order to provide for a selectable marker. This plasmid contains the *pat* gene (see European Patent Publication 0 242 236) which encodes phosphinothricin acetyl transferase (PAT). The enzyme PAT confers resistance to herbicidal glutamine synthetase inhibitors such as phosphinothricin. The *pat* gene in p35S/Ac is under the control of the 35S promoter from cauliflower mosaic virus (Odell et al., *Nature* 313:810-812 (1985)) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*.

The particle bombardment method (Klein et al., *Nature* 327:70-73 (1987)) may be used to transfer genes to the callus culture cells. According to this method, gold particles (1 µm in diameter) are coated with DNA using the following technique. Ten µg of plasmid DNAs are added to 50 µL of a suspension of gold particles (60 mg per mL). Calcium chloride (50 µL of a 2.5 M solution) and spermidine free base (20 µL of a 1.0 M solution) are added to the particles. The suspension is vortexed during the addition of these solutions. After ten minutes, the tubes are briefly

centrifuged (5 sec at 15,000 rpm) and the supernatant removed. The particles are resuspended in 200 μ L of absolute ethanol, centrifuged again and the supernatant removed. The ethanol rinse is performed again and the particles resuspended in a final volume of 30 μ L of ethanol. An aliquot (5 μ L) of the DNA-coated gold particles
5 can be placed in the center of a KaptonTM flying disc (Bio-Rad Labs). The particles are then accelerated into the maize tissue with a Biolistic[®] PDS-1000/He (Bio-Rad Instruments, Hercules CA), using a helium pressure of 1000 psi, a gap distance of 0.5 cm and a flying distance of 1.0 cm.

For bombardment, the embryogenic tissue is placed on filter paper over
10 agarose-solidified N6 medium. The tissue is arranged as a thin lawn and covered a circular area of about 5 cm in diameter. The petri dish containing the tissue can be placed in the chamber of the PDS-1000/He approximately 8 cm from the stopping screen. The air in the chamber is then evacuated to a vacuum of 28 inches of Hg. The macrocarrier is accelerated with a helium shock wave using a rupture
15 membrane that bursts when the He pressure in the shock tube reaches 1000 psi.

Seven days after bombardment the tissue can be transferred to N6 medium that contains bialaphos (5 mg per liter) and lacks casein or proline. The tissue continues to grow slowly on this medium. After an additional two weeks the tissue can be transferred to fresh N6 medium containing bialaphos. After six weeks, areas
20 of about 1 cm in diameter of actively growing callus can be identified on some of the plates containing the bialaphos-supplemented medium. These calli may continue to grow when sub-cultured on the selective medium.

Plants can be regenerated from the transgenic callus by first transferring clusters of tissue to N6 medium supplemented with 0.2 mg per liter of 2,4-D. After
25 two weeks the tissue can be transferred to regeneration medium (Fromm et al., *Bio/Technology* 8:833-839 (1990)).

Transgenic T0 plants can be regenerated and their phenotype determined following HTP procedures. T1 seed can be collected.

T1 plants can be grown and analyzed for phenotypic changes. The following
30 parameters can be quantified using image analysis: plant area, volume, growth rate and color analysis can be collected and quantified. Expression constructs that result in an alteration of root architecture compared to suitable control plants, can be

considered evidence that the *RUM1* gene functions in maize to alter root architecture.

Furthermore, a recombinant DNA construct containing the *RUM1* gene can be introduced into an maize line either by direct transformation or introgression from a separately transformed line.

Transgenic plants, either inbred or hybrid, can undergo more vigorous field-based experiments to study yield enhancement and/or resistance to root lodging under various environmental conditions (e.g. variations in nutrient and water availability).

Subsequent yield analysis can also be done to determine whether plants that contain the *RUM1* gene have an improvement in yield performance, when compared to the control (or reference) plants that do not contain the *RUM1* gene. Plants containing the *RUM1* gene would have less yield loss relative to the control plants, preferably 50% less yield loss or would have increased yield relative to the control plants under varying environmental conditions.

EXAMPLE 12

Electroporation of *Agrobacterium* LBA4404

Electroporation competent cells (40 µl), such as *Agrobacterium tumefaciens* LBA4404 (containing PHP10523, Fig.7, SEQ ID NO:50), are thawed on ice (20-30 min). PHP10523 contains VIR genes for T-DNA transfer, an *Agrobacterium* low copy number plasmid origin of replication, a tetracycline resistance gene, and a cos site for in vivo DNA biomolecular recombination. Meanwhile the electroporation cuvette is chilled on ice. The electroporator settings are adjusted to 2.1 kV. A DNA aliquot (0.5 µL JT (US 7,087,812) parental DNA at a concentration of 0.2 µg - 1.0 µg in low salt buffer or twice distilled H₂O) is mixed with the thawed *Agrobacterium* cells while still on ice. The mix is transferred to the bottom of electroporation cuvette and kept at rest on ice for 1-2 min. The cells are electroporated (Eppendorf electroporator 2510) by pushing "Pulse" button twice (ideally achieving a 4.0 msec pulse). Subsequently 0.5 ml 2xYT medium (or SOC medium) are added to cuvette and transferred to a 15 ml Falcon tube. The cells are incubated at 28-30° C, 200-250 rpm for 3 h.

Aliquots of 250 µl are spread onto #30B (YM + 50µg/mL Spectinomycin) plates and incubated 3 days at 28-30° C. To increase the number of transformants one of two optional steps can be performed:

Option 1: overlay plates with 30 µl of 15 mg/ml Rifampicin. LBA4404 has a

- 5 chromosomal resistance gene for Rifampicin. This additional selection eliminates some contaminating colonies observed when using poorer preparations of LBA4404 competent cells.

Option 2: Perform two replicates of the electroporation to compensate for poorer electrocompetent cells.

10 Identification of transformants:

Four independent colonies are picked and streaked on AB minimal medium plus 50mg/mL Spectinomycin plates (#12S medium) for isolation of single colonies. The plated are incubate at 28° C for 2-3 days.

A single colony for each putative co-integrate is picked and inoculated with 4 ml

- 15 #60A with 50 mg/l Spectinomycin. The mix is incubated for 24 h at 28° C with shaking. Plasmid DNA from 4 ml of culture is isolated using Qiagen Miniprep + optional PB wash. The DNA is eluted in 30 µl . Aliquots of 2 µl are used to electroporate 20 µl of DH10b + 20 µl of ddH₂O as per above.

Optionally a 15 µl aliquot can be used to transform 75-100 µl of Invitrogen Library

- 20 Efficiency DH5α. The cells are spread on LB medium plus 50mg/mL Spectinomycin plates (#34T medium) and incubated at 37° C overnight.

Three to four independent colonies are picked for each putative co-integrate and inoculated 4 ml of 2xYT (#60A) with 50 µg/ml Spectinomycin. The cells are incubated at 37° C overnight with shaking.

- 25 Isolate plasmid DNA from 4 ml of culture using QIAprep® Miniprep with optional PB wash (elute in 50 µl). Use 8 µl for digestion with Sall (using JT parent and PHP10523 as controls).

Three more digestions using restriction enzymes BamHI, EcoRI, and HindIII are performed for 4 plasmids that represent 2 putative co-integrates with correct Sall

- 30 digestion pattern (using parental DNA and PHP10523 as controls). Electronic gels are recommended for comparison.

Alternatively, for high throughput applications, such as described for Gaspe Bay Flint Derived Maize Lines (Examples 16-18), instead of evaluating the resulting co-

integrate vectors by restriction analysis, three colonies can be simultaneously used for the infection step as described in Example 13.

EXAMPLE 13

Agrobacterium mediated Transformation into Maize

Maize plants can be transformed to overexpress *RUM1* and *RUL* in order to examine the resulting phenotype.

Agrobacterium-mediated transformation of maize is performed essentially as described by Zhao et al., in *Meth. Mol. Biol.* 318:315-323 (2006) (see also Zhao et al., *Mol. Breed.* 8:323-333 (2001) and U.S. Patent No. 5,981,840 issued November 9, 1999, incorporated herein by reference). The transformation process involves bacterium inoculation, co-cultivation, resting, selection and plant regeneration.

1. Immature Embryo Preparation

Immature embryos are dissected from caryopses and placed in a 2mL microtube containing 2 mL PHI-A medium.

2. *Agrobacterium* Infection and Co-Cultivation of Embryos

2.1 Infection Step

PHI-A medium is removed with 1 mL micropipettor and 1 mL *Agrobacterium* suspension is added. Tube is gently inverted to mix. The mixture is incubated for 5 min at room temperature.

2.2 Co-Culture Step

The *Agrobacterium* suspension is removed from the infection step with a 1 mL micropipettor. Using a sterile spatula the embryos are scraped from the tube and transferred to a plate of PHI-B medium in a 100x15 mm Petri dish. The embryos are oriented with the embryonic axis down on the surface of the medium. Plates with the embryos are cultured at 20°C, in darkness, for 3 days. L-Cysteine can be used in the co-cultivation phase. With the standard binary vector, the co-cultivation medium supplied with 100-400 mg/L L-cysteine is critical for recovering stable transgenic events.

3. Selection of Putative Transgenic Events

To each plate of PHI-D medium in a 100x15 mm Petri dish, 10 embryos are transferred, maintaining orientation and the dishes are sealed with Parafilm. The plated are incubated in darkness at 28 °C. Actively growing putative events, as pale yellow embryonic tissue are expected to be visible in 6-8 weeks. Embryos that

produce no events may be brown and necrotic, and little friable tissue growth is evident. Putative transgenic embryonic tissue is subcultured to fresh PHI-D plates at 2-3 week intervals, depending on growth rate. The events are recorded.

4. Regeneration of T0 plants

- 5 Embryonic tissue propagated on PHI-D medium is subcultured to PHI-E medium (somatic embryo maturation medium); in 100x25 mm Petri dishes and incubated at 28 °C, in darkness, until somatic embryos mature, for about 10-18 days. Individual, matured somatic embryos with well-defined scutellum and coleoptile are transferred to PHI-F embryo germination medium and incubated at 28 °C in the light (about 80
10 µE from cool white or equivalent fluorescent lamps). In 7-10 days, regenerated plants, about 10 cm tall, are potted in horticultural mix and hardened-off using standard horticultural methods.

Media for Plant Transformation

- 15 1. PHI-A: 4g/L CHU basal salts, 1.0 mL/L 1000X Eriksson's vitamin mix, 0.5mg/L thiamin HCL, 1.5 mg/L 2,4-D, 0.69 g/L L-proline, 68.5 g/L sucrose, 36g/L glucose, pH 5.2. Add 100µM acetosyringone, filter-sterilized before using.
- 20 2. PHI-B: PHI-A without glucose, increased 2,4-D to 2mg/L, reduced sucrose to 30 g/L and supplemented with 0.85 mg/L silver nitrate (filter-sterilized), 3.0 g/L gelrite, 100µM acetosyringone (filter-sterilized), 5.8.
- 25 3. PHI-C: PHI-B without gelrite and acetosyringonee, reduced 2,4-D to 1.5 mg/L and supplemented with 8.0 g/L agar, 0.5 g/L Ms-morpholino ethane sulfonic acid (MES) buffer, 100mg/L carbenicillin (filter-sterilized).
4. PHI-D: PHI-C supplemented with 3mg/L bialaphos (filter-sterilized).
- 30 5. PHI-E: 4.3 g/L of Murashige and Skoog (MS) salts, (Gibco, BRL 11117-074), 0.5 mg/L nicotinic acid, 0.1 mg/L thiamine HCl, 0.5mg/L pyridoxine HCl, 2.0 mg/L glycine, 0.1 g/L myo-inositol, 0.5 mg/L zeatin (Sigma, cat.no. Z-0164), 1 mg/L indole acetic acid (IAA), 26.4 µg/L abscisic acid (ABA), 60 g/L sucrose, 3 mg/L bialaphos (filter-sterilized), 100 mg/L carbenicillin (fileter-sterilized), 8g/L agar, pH 5.6.

6. PHI-F: PHI-E without zeatin, IAA, ABA; sucrose reduced to 40 g/L; replacing agar with 1.5 g/L gelrite; pH 5.6.

Plants can be regenerated from the transgenic callus by first transferring clusters of tissue to N6 medium supplemented with 0.2 mg per liter of 2,4-D. After two weeks the tissue can be transferred to regeneration medium (Fromm et al. (1990) *Bio/Technology* 8:833-839).

Phenotypic analysis of transgenic T0 plants and T1 plants can be performed.

T1 plants can be analyzed for phenotypic changes. Using image analysis T1 plants can be analyzed for phenotypical changes in plant area, volume, growth rate and color analysis can be taken at multiple times during growth of the plants. Alteration in root architecture can be assayed as described In Example 21.

Subsequent analysis of alterations in agronomic characteristics can be done to determine whether plants containing the *RUM1* or the *RUL* gene have an improvement of at least one agronomic characteristic, when compared to the control (or reference) plants that do not contain *RUM1* or the *RUL* gene. The alterations may also be studied under various environmental conditions.

EXAMPLE 14

Construction of Maize expression vectors with the *RUM1* and *RUL* Gene using

Agrobacterium mediated Transformation

Maize expression vectors can be prepared with the *RUM1* (SEQ ID NO:22 or 28 and *RUM1-like* genes (such as *RUL*, SEQ ID NO:38) under the control of the NAS2 (SEQ ID NO:51), GOS 2 (SEQ ID NO:52) or Ubiquitin (UBI1ZM; SEQ ID NO:53) promoter. PINII is the terminator (SEQ ID NO:54)

Using Invitrogen's™ Gateway® technology the entry clone, created as described in Example 9, containing the maize *RUM1* gene or maize *RUL* gene can be used in separate Gateway® LR reactions with:

1) the constitutive maize GOS2 promoter entry clone PHP28408 (Fig.8, SEQ ID NO:55) and the PinII Terminator entry clone PHP20234 (Fig.9, SEQ ID NO:56), into the destination vector PHP28529 (Fig.10 , SEQ ID NO:57).

2) the root maize NAS2 promoter entry clone PHP22020 (Fig.11,SEQ ID NO:58) and the PinII Terminator entry clone PHP20234 (Fig.9, SEQ ID NO:56) into the destination vector PHP28529 (Fig.10, SEQ ID NO:57).

3) the constitutive maize UBI1ZM promoter entry clone PHP23112 (Fig.12, SEQ ID NO:59) and the PinII Terminator entry clone PHP20234 (Fig.9, SEQ ID NO:56) into the destination vector PHP28529 (Fig.10, SEQ ID NO:57).

The destination vector PHP28529 adds to each of the final vectors also an:

- 1) RD29A promoter::yellow fluorescent protein::PinII terminator cassette for Arabidopsis seed sorting.
- 2) a Ubiquitin promoter::moPAT/red fluorescent protein fusion::PinII terminator cassette for transformation selection and Z.mays seed sorting.

In addition to the GOS2 or NAS2 promoter, other promoters such as, but not limited to the S2A and S2B promoter, the maize ROOTMET2 promoter, the maize Cyclo, the CR1BIO, the CRWAQ81 and the maize ZRP2.4447 are useful for directing expression of *RUM1* and *RUM1-like* genes in maize. Furthermore, a variety of terminators, such as, but not limited to the PINII terminator, could be used to achieve expression of the gene of interest in maize.

EXAMPLE 15

Transformation of Maize Lines with *RUM1* and *RUM1-like* genes using *Agrobacterium* mediated Transformation

The final vectors (Example 14) can then electroporated separately into LBA4404 *Agrobacterium* containing PHP10523 (Figure 7; SEQ ID NO:50, Komari *et al.* Plant J 10:165-174 (1996), NCBI GI: 59797027) to create the co-integrate vectors for maize transformation. The co-integrate vectors are formed by recombination of the final vectors (maize expression vectors) with PHP10523, through the COS recombination sites contained on each vector. The co-integrate vectors contain in addition to the expression cassettes described in Example 14, also genes needed for the *Agrobacterium* strain and the *Agrobacterium* mediated transformation, (TET, TET, TRFA, ORI terminator, CTL, ORI V, VIR C1, VIR C2, VIR G, VIR B). Transformation into a maize line can be performed as described in Example 13.

EXAMPLE 16

Preparation of the destination vectors PHP23236 and PHP29635 for Transformation of Gaspe Bay Flint derived Maize Lines

Destination vector PHP23236 (Fig.6, SEQ ID NO:49) was obtained by transformation of *Agrobacterium* strain LBA4404 containing plasmid PHP10523

(Fig.7, SEQ ID NO:50) with plasmid PHP23235 (Fig.13, SEQ ID NO:60) and isolation of the resulting co-integration product. Destination vector PHP23236, can be used in a recombination reaction with an entry clone as described in Example 9 to create a maize expression vector for transformation of Gaspe Bay Flint derived maize lines. Expression of the gene of interest is under control of the ubiquitin promoter (SEQ ID NO:53).

PHP29635 (Fig.14, SEQ ID NO:61) was obtained by transformation of *Agrobacterium* strain LBA4404 containing plasmid PHP10523 with plasmid PIIOS2a-FRT87(ni)m (Fig.15, SEQ ID NO:62) and isolation of the resulting co-integration product. Destination vector PHP29635 can be used in a recombination reaction with an entry clone as described in Example 9 to create a maize expression vector for transformation of Gaspe Bay Flint derived maize lines. Expression of the gene of interest is under control of the S2A promoter (SEQ ID NO:63).

EXAMPLE 17

Preparation of plasmids containing *RUM1* or *RUL*

genes for transformation of Gaspe Bay Flint Derived Maize Lines

Using Invitrogen's Gateway® Recombination technology, entry clones containing the *RUM1* or *RUM1*-like genes can be created, as described in Example 9 and used to directionally clone each gene into destination vector PHP23236 (Example 16) for expression under the ubiquitin promoter or into destination vector PHP29635 (Example 16) for expression under the S2A promoter. Each of the expression vectors are T-DNA binary vectors for *Agrobacterium*-mediated transformation into corn.

Gaspe Bay Flint Derived Maize Lines can be transformed with the expression vectors as described in Example 18.

EXAMPLE 18

Transformation of Gaspe Bay Flint Derived Maize Lines with *RUM1* and *RUM1-like* Genes

Maize plants can be transformed to over-express the *RUM1* and *RUM1*-like genes, in order to examine the resulting phenotype.

Recipient Plants

Recipient plant cells can be from a uniform maize line having a short life cycle ("fast cycling"), a reduced size, and high transformation potential. Typical of these

plant cells for maize are plant cells from any of the publicly available Gaspe Bay Flint (GBF) line varieties. One possible candidate plant line variety is the F1 hybrid of GBF x QTM (Quick Turnaround Maize, a publicly available form of Gaspe Bay Flint selected for growth under greenhouse conditions) disclosed in Tomes et al.

U.S. Patent Application Publication No. 2003/0221212. Transgenic plants obtained from this line are of such a reduced size that they can be grown in four inch pots (1/4 the space needed for a normal sized maize plant) and mature in less than 2.5 months. (Traditionally 3.5 months is required to obtain transgenic T0 seed once the transgenic plants are acclimated to the greenhouse.) Another suitable line is a double haploid line of GS3 (a highly transformable line) X Gaspe Flint. Yet another suitable line is a transformable elite inbred line carrying a transgene which causes early flowering, reduced stature, or both.

Transformation Protocol

Any suitable method may be used to introduce the transgenes into the maize cells, including but not limited to inoculation type procedures using *Agrobacterium* based vectors as described in Example 17. Transformation may be performed on immature embryos of the recipient (target) plant.

Precision Growth and Plant Tracking

The event population of transgenic (T0) plants resulting from the transformed maize embryos is grown in a controlled greenhouse environment using a modified randomized block design to reduce or eliminate environmental error. A randomized block design is a plant layout in which the experimental plants are divided into groups (e.g., thirty plants per group), referred to as blocks, and each plant is randomly assigned a location with the block.

For a group of thirty plants, twenty-four transformed, experimental plants and six control plants (plants with a set phenotype) (collectively, a "replicate group") are placed in pots which are arranged in an array (a.k.a. a replicate group or block) on a table located inside a greenhouse. Each plant, control or experimental, is randomly assigned to a location with the block which is mapped to a unique, physical greenhouse location as well as to the replicate group. Multiple replicate groups of thirty plants each may be grown in the same greenhouse in a single experiment. The layout (arrangement) of the replicate groups should be determined to minimize

space requirements as well as environmental effects within the greenhouse. Such a layout may be referred to as a compressed greenhouse layout.

An alternative to the addition of a specific control group is to identify those transgenic plants that do not express the gene of interest. A variety of techniques
5 such as RT-PCR can be applied to quantitatively assess the expression level of the introduced gene. T0 plants that do not express the transgene can be compared to those which do.

Each plant in the event population is identified and tracked throughout the evaluation process, and the data gathered from that plant is automatically
10 associated with that plant so that the gathered data can be associated with the transgene carried by the plant. For example, each plant container can have a machine readable label (such as a Universal Product Code (UPC) bar code) which includes information about the plant identity, which in turn is correlated to a greenhouse location so that data obtained from the plant can be automatically
15 associated with that plant.

Alternatively any efficient, machine readable, plant identification system can be used, such as two-dimensional matrix codes or even radio frequency identification tags (RFID) in which the data is received and interpreted by a radio frequency receiver/processor. See U.S. Published Patent Application No.
20 2004/0122592, incorporated herein by reference.

Phenotypic Analysis Using Three-Dimensional Imaging

Each greenhouse plant in the T0 event population, including any control plants, is analyzed for agronomic characteristics of interest, and the agronomic data for each plant is recorded or stored in a manner so that it is associated with the
25 identifying data (see above) for that plant. Confirmation of a phenotype (gene effect) can be accomplished in the T1 generation with a similar experimental design to that described above.

The T0 plants are analyzed at the phenotypic level using quantitative, non-destructive imaging technology throughout the plant's entire greenhouse life cycle to
30 assess the traits of interest. Preferably, a digital imaging analyzer is used for automatic multi-dimensional analyzing of total plants. The imaging may be done inside the greenhouse. Two camera systems, located at the top and side, and an apparatus to rotate the plant, are used to view and image plants from all sides.

Images are acquired from the top, front and side of each plant. All three images together provide sufficient information to evaluate the biomass, size and morphology of each plant.

Due to the change in size of the plants from the time the first leaf appears from the soil to the time the plants are at the end of their development, the early stages of plant development are best documented with a higher magnification from the top. This may be accomplished by using a motorized zoom lens system that is fully controlled by the imaging software.

In a single imaging analysis operation, the following events occur: (1) the plant is conveyed inside the analyzer area, rotated 360 degrees so its machine readable label can be read, and left at rest until its leaves stop moving; (2) the side image is taken and entered into a database; (3) the plant is rotated 90 degrees and again left at rest until its leaves stop moving, and (4) the plant is transported out of the analyzer.

Plants are allowed at least six hours of darkness per twenty four hour period in order to have a normal day/night cycle.

Imaging Instrumentation

Any suitable imaging instrumentation may be used, including but not limited to light spectrum digital imaging instrumentation commercially available from LemnaTec GmbH of Wurselen, Germany. The images are taken and analyzed with a LemnaTec Scanalyzer HTS LT-0001-2 having a 1/2" IT Progressive Scan IEE CCD imaging device. The imaging cameras may be equipped with a motor zoom, motor aperture and motor focus. All camera settings may be made using LemnaTec software. Preferably, the instrumental variance of the imaging analyzer is less than about 5% for major components and less than about 10% for minor components.

Software

The imaging analysis system comprises a LemnaTec HTS Bonit software program for color and architecture analysis and a server database for storing data from about 500,000 analyses, including the analysis dates. The original images and the analyzed images are stored together to allow the user to do as much reanalyzing as desired. The database can be connected to the imaging hardware for automatic data collection and storage. A variety of commercially available software systems

(e.g. Matlab, others) can be used for quantitative interpretation of the imaging data, and any of these software systems can be applied to the image data set.

Conveyor System

A conveyor system with a plant rotating device may be used to transport the plants to the imaging area and rotate them during imaging. For example, up to four plants, each with a maximum height of 1.5 m, are loaded onto cars that travel over the circulating conveyor system and through the imaging measurement area. In this case the total footprint of the unit (imaging analyzer and conveyor loop) is about 5 m x 5 m.

The conveyor system can be enlarged to accommodate more plants at a time. The plants are transported along the conveyor loop to the imaging area and are analyzed for up to 50 seconds per plant. Three views of the plant are taken. The conveyor system, as well as the imaging equipment, should be capable of being used in greenhouse environmental conditions.

Illumination

Any suitable mode of illumination may be used for the image acquisition. For example, a top light above a black background can be used. Alternatively, a combination of top- and backlight using a white background can be used. The illuminated area should be housed to ensure constant illumination conditions. The housing should be longer than the measurement area so that constant light conditions prevail without requiring the opening and closing of doors. Alternatively, the illumination can be varied to cause excitation of either transgene (e.g., green fluorescent protein (GFP), red fluorescent protein (RFP)) or endogenous (e.g. Chlorophyll) fluorophores.

Biomass Estimation Based on Three-Dimensional Imaging

For best estimation of biomass the plant images should be taken from at least three axes, preferably the top and two side (sides 1 and 2) views. These images are then analyzed to separate the plant from the background, pot and pollen control bag (if applicable). The volume of the plant can be estimated by the calculation:

$$Volume(voxels) = \sqrt{TopArea(pixels)} \times \sqrt{Side1Area(pixels)} \times \sqrt{Side2Area(pixels)}$$

In the equation above the units of volume and area are "arbitrary units". Arbitrary units are entirely sufficient to detect gene effects on plant size and growth in this system because what is desired is to detect differences (both positive-larger and negative-smaller) from the experimental mean, or control mean. The arbitrary units of size (e.g. area) may be trivially converted to physical measurements by the addition of a physical reference to the imaging process. For instance, a physical reference of known area can be included in both top and side imaging processes. Based on the area of these physical references a conversion factor can be determined to allow conversion from pixels to a unit of area such as square centimeters (cm²). The physical reference may or may not be an independent sample. For instance, the pot, with a known diameter and height, could serve as an adequate physical reference.

Color Classification

The imaging technology may also be used to determine plant color and to assign plant colors to various color classes. The assignment of image colors to color classes is an inherent feature of the LemnaTec software. With other image analysis software systems color classification may be determined by a variety of computational approaches.

For the determination of plant size and growth parameters, a useful classification scheme is to define a simple color scheme including two or three shades of green and, in addition, a color class for chlorosis, necrosis and bleaching, should these conditions occur. A background color class which includes non plant colors in the image (for example pot and soil colors) is also used and these pixels are specifically excluded from the determination of size. The plants are analyzed under controlled constant illumination so that any change within one plant over time, or between plants or different batches of plants (e.g. seasonal differences) can be quantified.

In addition to its usefulness in determining plant size growth, color classification can be used to assess other yield component traits. For these other yield component traits additional color classification schemes may be used. For instance, the trait known as "staygreen", which has been associated with improvements in yield, may be assessed by a color classification that separates shades of green from shades of yellow and brown (which are indicative of senescing

tissues). By applying this color classification to images taken toward the end of the T0 or T1 plants' life cycle, plants that have increased amounts of green colors relative to yellow and brown colors (expressed, for instance, as Green/Yellow Ratio) may be identified. Plants with a significant difference in this Green/Yellow ratio can be identified as carrying transgenes which impact this important agronomic trait.

The skilled plant biologist will recognize that other plant colors arise which can indicate plant health or stress response (for instance anthocyanins), and that other color classification schemes can provide further measures of gene action in traits related to these responses.

Plant Architecture Analysis

Transgenes which modify plant architecture parameters may also be identified using the present invention, including such parameters as maximum height and width, internodal distances, angle between leaves and stem, number of leaves starting at nodes and leaf length. The LemnaTec system software may be used to determine plant architecture as follows. The plant is reduced to its main geometric architecture in a first imaging step and then, based on this image, parameterized identification of the different architecture parameters can be performed. Transgenes that modify any of these architecture parameters either singly or in combination can be identified by applying the statistical approaches previously described.

Pollen Shed Date

Pollen shed date is an important parameter to be analyzed in a transformed plant, and may be determined by the first appearance on the plant of an active male flower. To find the male flower object, the upper end of the stem is classified by color to detect yellow or violet anthers. This color classification analysis is then used to define an active flower, which in turn can be used to calculate pollen shed date.

Alternatively, pollen shed date and other easily visually detected plant attributes (e.g. pollination date, first silk date) can be recorded by the personnel responsible for performing plant care. To maximize data integrity and process efficiency this data is tracked by utilizing the same barcodes utilized by the LemnaTec light spectrum digital analyzing device. A computer with a barcode reader, a palm device, or a notebook PC may be used for ease of data capture recording time of observation, plant identifier, and the operator who captured the data.

Orientation of the Plants

Mature maize plants grown at densities approximating commercial planting often have a planar architecture. That is, the plant has a clearly discernable broad side, and a narrow side. The image of the plant from the broadside is determined.

- 5 To each plant a well defined basic orientation is assigned to obtain the maximum difference between the broadside and edgewise images. The top image is used to determine the main axis of the plant, and an additional rotating device is used to turn the plant to the appropriate orientation prior to starting the main image acquisition.

EXAMPLE 19

10 Screening of Gaspe Bay Flint Derived Maize Lines

Under Nitrogen Limiting Conditions

- Transgenic plants will contain two or three doses of Gaspe Flint-3 with one dose of GS3 (GS3/(Gaspe-3)2X or GS3/(Gaspe-3)3X) and will segregate 1:1 for a dominant transgene. Plants will be planted in Turface, a commercial potting
15 medium, and watered four times each day with 1 mM KNO₃ growth medium and with 2 mM KNO₃, or higher, growth medium (see Fig.16). Control plants grown in 1 mM KNO₃ medium will be less green, produce less biomass and have a smaller ear at anthesis (see Fig.17 for an illustration of sample data).

- Statistics are used to decide if differences seen between treatments are really
20 different. Fig.17 illustrates one method which places letters after the values. Those values in the same column that have the same letter (not group of letters) following them are not significantly different. Using this method, if there are no letters following the values in a column, then there are no significant differences between any of the values in that column or, in other words, all the values in that column are
25 equal.

- Expression of a transgene will result in plants with improved plant growth in 1 mM KNO₃ when compared to a transgenic null. Thus biomass and greenness will be monitored during growth and compared to a transgenic null. Improvements in growth, greenness and ear size at anthesis will be indications of increased nitrogen
30 tolerance.

EXAMPLE 20

Yield Analysis of Maize Lines with *RUM1* or *RUM1*-like Genes

A recombinant DNA construct containing a *RUM1* or *RUM1*-like Gene can be introduced into a maize line either by direct transformation or introgression from a separately transformed line.

Transgenic plants, either inbred or hybrid, can undergo more vigorous field-based experiments to study yield enhancement and/or stability under various environmental conditions, such as variations in water and nutrient availability.

Subsequent yield analysis can be done to determine whether plants that contain the *RUM1* or *RUM1*-like gene have an improvement in yield performance under various environmental conditions, when compared to the control plants that do not contain the *RUM1* or *RUM1*-like gene. Reduction in yield can be measured for both. Plants containing the *RUM1* or *RUM1*-like gene have less yield loss relative to the control plants, preferably 50% less yield loss.

EXAMPLE 21

Assays to Determine Alterations of Root Architecture in Maize

Transgenic maize plants are assayed for changes in root architecture at seedling stage, flowering time or maturity. Assays to measure alterations of root architecture of maize plants include, but are not limited to the methods outlined below. To facilitate manual or automated assays of root architecture alterations, corn plants can be grown in clear pots.

- 1) Root mass (dry weights). Plants are grown in Turface, a growth media that allows easy separation of roots. Oven-dried shoot and root tissues are weighed and a root/shoot ratio calculated.
- 2) Levels of lateral root branching. The extent of lateral root branching (e.g. lateral root number, lateral root length) is determined by sub-sampling a complete root system, imaging with a flat-bed scanner or a digital camera and analyzing with WinRHIZOTM software (Regent Instruments Inc.).
- 3) Root band width measurements. The root band is the band or mass of roots that forms at the bottom of greenhouse pots as the plants mature. The thickness of the root band is measured in mm at maturity as a rough estimate of root mass.

4) Nodal root count. The number of crown roots coming off the upper nodes can be determined after separating the root from the support medium (e.g. potting mix). In addition the angle of crown roots and/or brace roots can be measured. Digital analysis of the nodal roots and amount of branching of nodal roots form another extension to the aforementioned manual method. All data taken on root phenotype are subjected to statistical analysis, normally a t-test to compare the transgenic roots with that of non-transgenic sibling plants. One-way ANOVA may also be used in cases where multiple events and/or constructs are involved in the analysis.

EXAMPLE 22

Subcellular localization of *RUM1* and *RUL*

The Aux/IAA proteins of *Arabidopsis* and rice have been shown to be localized to the nucleus [Abel et al. (1994) *Proc Natl Acad Sci USA* 91:326-330; Thakur et al. (2005) *Biochim Biophys Acta* 1730:196-205]. Two types of putative nuclear localization signals (NLS) that are conserved in most of the rice Aux/IAA proteins [Jain et al. (2006) *Funct Integr Genomics* 6:47-59] are also present in the maize *RUM1* and *RUL* proteins. A bipartite NLS comprises residues KR, at amino acid residues 80 and 84 in *RUM1* and *RUL*, respectively and residues NYRKN, at amino acid residues 122 and 125 in *RUM1* and *RUL*, respectively. A SV40-type NLS comprises residues RKLKIMR at amino acid residues 244 and 247 in *Rum1* and *RUL*, respectively.

In order to confirm that the *RUM1* and the *RUL* proteins localize to the nucleus, one can analyze the transient expression of the respective proteins in onion epidermal cells. First, vectors carrying full length cDNAs driven by the CaMV 35S promoter and fused translationally to the YFP reporter gene (Clontech) are constructed, and then introduced into onion epidermal cells by particle bombardment (Scott A. et al. (1999) *Biotechniques* 26(6):1125, 1128-32).

EXAMPLE 23

Analysis of the transcriptional repressor activity of *RUM1* and *RUL* proteins

The Aux/IAA proteins show a conserved LxLxL motif which has been shown to act as a transcriptional repressor domain [Tiwari et al (2004) *Plant Cell* 16:533-543]. The LxLxL motif is also present in the *RUM1* and *RUL* proteins at residue 42 in *RUM1* and 40 in *RUL* (Fig.18).

In order to determine if RUM1 and RUL are transcriptional repressors, one can analyze their repressor activity by protoplast transfection assay. In this method, an *Arabidopsis* leaf mesophyll protoplast transfection assay system and a reporter construct containing the firefly luciferase reporter gene (pGL3, Promega, Madison WI, 53711) driven by the CaMV 35S minimal promoter (nucleotides -46 to -1) with four GAL4 DNA binding sequences (SEQ ID NO:64) are used. The luciferase reporter is co-transfected with one effector gene encoding a chimeric protein consisting of the yeast GAL4 DNA binding domain (amino acids 1 to 147 from pGBKT7, Clontech) fused in frame to either the *RUM1*, or the *RUL* cDNAs. Effector genes are driven by a duplicated CaMV 35S enhancer sequence (nucleotides -206 to 46) followed by the CaMV 35S minimal promoter. A construct containing only the 35S promoter and the GAL4 DBD is used as an effector control. Effector plasmids (5 µg) are cotransfected with reporter plasmids (10 µg) at a ratio of 1:2. The efficiency of transfection is normalized by adding 100 ng of a *pUbiquitin:Renilla LUC* reporter gene (phRL-TK, Promega, Madison WI, 53711), (Tiwari et al. (2005) *Methods in Mol Biol* 323: 237-244). If RUM1 and RUL function as transcriptional repressors, it is expected that the RUM1 and RUL effectors will reduce the relative luciferase activity of the reporter in comparison to the effector control.

EXAMPLE 24

Composition of cDNA Libraries:

Isolation and Sequencing of cDNA Clones

cDNA libraries representing mRNAs from various tissues of *Brassica napus* (canola), *Glycine max* (soybean), and *Triticum aestivum* (wheat) were prepared. The characteristics of the libraries are described below.

TABLE 2

cDNA Libraries from Canola, Soybean and Wheat.

Library	Tissue	Clone
ebb1c	Immature buds of Canola Rf gene knock out mutant line, 02SM2. Isolation of genes involved in CMS restoration.	ebb1c.pk008.p9: fis
smj1c	Characterization of IPT transcripts from transgenic soybean. The lead Yield	smj1c.pk013.h7.f: fis smj1c.pk007.k12.f: fis

	Enhancement (Soy YE2.1) construct is expressing Agrobacterium isopentenyl transferase gene, and we need to characterize all transcripts from the transgene.	
wdk1c	Wheat (Triticum aestivum L.) developing kernel, 3 days after anthesis.	wdk1c.pk023.b8: fis

cDNA libraries may be prepared by any one of many methods available. For example, the cDNAs may be introduced into plasmid vectors by first preparing the cDNA libraries in Uni-ZAP™ XR vectors according to the manufacturer's protocol (Stratagene Cloning Systems, La Jolla, CA). The Uni-ZAP™ XR libraries are converted into plasmid libraries according to the protocol provided by Stratagene. Upon conversion, cDNA inserts will be contained in the plasmid vector pBluescript. In addition, the cDNAs may be introduced directly into precut Bluescript II SK(+) vectors (Stratagene) using T4 DNA ligase (New England Biolabs), followed by transfection into DH10B cells according to the manufacturer's protocol (GIBCO BRL Products). Once the cDNA inserts are in plasmid vectors, plasmid DNAs are prepared from randomly picked bacterial colonies containing recombinant pBluescript plasmids, or the insert cDNA sequences are amplified via polymerase chain reaction using primers specific for vector sequences flanking the inserted cDNA sequences. Amplified insert DNAs or plasmid DNAs are sequenced in dye-primer sequencing reactions to generate partial cDNA sequences (expressed sequence tags or "ESTs"; see Adams et al., (1991) *Science* 252:1651-1656). The resulting ESTs are analyzed using a Perkin Elmer Model 377 fluorescent sequencer.

Full-insert sequence (FIS) data is generated utilizing a modified transposition protocol. Clones identified for FIS are recovered from archived glycerol stocks as single colonies, and plasmid DNAs are isolated via alkaline lysis. Isolated DNA templates are reacted with vector primed M13 forward and reverse oligonucleotides in a PCR-based sequencing reaction and loaded onto automated sequencers. Confirmation of clone identification is performed by sequence alignment to the original EST sequence from which the FIS request is made.

Confirmed templates are transposed via the Primer Island transposition kit (PE Applied Biosystems, Foster City, CA) which is based upon the *Saccharomyces cerevisiae* Ty1 transposable element (Devine and Boeke (1994) *Nucleic Acids Res.* 22:3765-3772). The *in vitro* transposition system places unique binding sites randomly throughout a population of large DNA molecules. The transposed DNA is then used to transform DH10B electro-competent cells (Gibco BRL/Life Technologies, Rockville, MD) via electroporation. The transposable element contains an additional selectable marker (named DHFR; Fling and Richards (1983) *Nucleic Acids Res.* 11:5147-5158), allowing for dual selection on agar plates of only those subclones containing the integrated transposon. Multiple subclones are randomly selected from each transposition reaction, plasmid DNAs are prepared via alkaline lysis, and templates are sequenced (ABI Prism dye-terminator ReadyReaction mix) outward from the transposition event site, utilizing unique primers specific to the binding sites within the transposon.

Sequence data is collected (ABI Prism Collections) and assembled using Phred and Phrap (Ewing et al. (1998) *Genome Res.* 8:175-185; Ewing and Green (1998) *Genome Res.* 8:186-194). Phred is a public domain software program which re-reads the ABI sequence data, re-calls the bases, assigns quality values, and writes the base calls and quality values into editable output files. The Phrap sequence assembly program uses these quality values to increase the accuracy of the assembled sequence contigs. Assemblies are viewed by the Consed sequence editor (Gordon et al. (1998) *Genome Res.* 8:195-202).

In some of the clones the cDNA fragment corresponds to a portion of the 3'-terminus of the gene and does not cover the entire open reading frame. In order to obtain the upstream information one of two different protocols are used. The first of these methods results in the production of a fragment of DNA containing a portion of the desired gene sequence while the second method results in the production of a fragment containing the entire open reading frame. Both of these methods use two rounds of PCR amplification to obtain fragments from one or more libraries. The libraries some times are chosen based on previous knowledge that the specific gene should be found in a certain tissue and some times are randomly-chosen. Reactions to obtain the same gene may be performed on several libraries in parallel or on a pool of libraries. Library pools are normally prepared using from 3 to 5

different libraries and normalized to a uniform dilution. In the first round of amplification both methods use a vector-specific (forward) primer corresponding to a portion of the vector located at the 5'-terminus of the clone coupled with a gene-specific (reverse) primer. The first method uses a sequence that is complementary to a portion of the already known gene sequence while the second method uses a gene-specific primer complementary to a portion of the 3'-untranslated region (also referred to as UTR). In the second round of amplification a nested set of primers is used for both methods. The resulting DNA fragment is ligated into a pBluescript vector using a commercial kit and following the manufacturer's protocol. This kit is selected from many available from several vendors including Invitrogen™ (Carlsbad, CA), Promega Biotech (Madison, WI), and Gibco-BRL (Gaithersburg, MD). The plasmid DNA is isolated by alkaline lysis method and submitted for sequencing and assembly using Phred/Phrap, as above.

EXAMPLE 25

Identification of cDNA Clones

cDNA clones encoding RUM1-like polypeptides were identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol.* 215:403-410; see also the explanation of the BLAST algorithm on the world wide web site for the National Center for Biotechnology Information at the National Library of Medicine of the National Institutes of Health) searches for similarity to sequences contained in the BLAST "nr" database (comprising all non-redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the last major release of the SWISS-PROT protein sequence database, EMBL, and DDBJ databases). The cDNA sequences obtained as described in Example 24 were analyzed for similarity to all publicly available DNA sequences contained in the "nr" database using the BLASTN algorithm provided by the National Center for Biotechnology Information (NCBI). The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the "nr" database using the BLASTX algorithm (Gish and States (1993) *Nat. Genet.* 3:266-272) provided by the NCBI. For convenience, the P-value (probability) of observing a match of a cDNA sequence to a sequence contained in the searched databases merely by chance as calculated by BLAST are reported herein as "pLog" values, which represent the

negative of the logarithm of the reported P-value. Accordingly, the greater the pLog value, the greater the likelihood that the cDNA sequence and the BLAST "hit" represent homologous proteins.

ESTs submitted for analysis are compared to the Genbank database as described above. ESTs that contain sequences more 5- or 3-prime can be found by using the BLASTn algorithm (Altschul et al (1997) *Nucleic Acids Res.* 25:3389-3402.) against the Du Pont proprietary database comparing nucleotide sequences that share common or overlapping regions of sequence homology. Where common or overlapping sequences exist between two or more nucleic acid fragments, the sequences can be assembled into a single contiguous nucleotide sequence, thus extending the original fragment in either the 5 or 3 prime direction. Once the most 5-prime EST is identified, its complete sequence can be determined by Full Insert Sequencing as described in Example 24. Homologous genes belonging to different species can be found by comparing the amino acid sequence of a known gene (from either a proprietary source or a public database) against an EST database using the tBLASTn algorithm. The tBLASTn algorithm searches an amino acid query against a nucleotide database that is translated in all 6 reading frames. This search allows for differences in nucleotide codon usage between different species, and for codon degeneracy.

EXAMPLE 26

Characterization of cDNA Clones Encoding RUM1 Polypeptides, RUL polypeptides and homologs thereof

The BLASTX search using the EST sequences from clones listed in Table 3 revealed similarity of the polypeptides encoded by the ORF to proteins from rice, Arabidopsis and soybean identified as belonging to the AUX-IAA family (NCBI General Identifier No's. 34911088, 125553286, 15229343, and 2388689, corresponding to SEQ ID NO's:65, 76, 74, and 75, respectively).

Shown in Table 3 and 4 are the literature and patent BLAST results, respectively, for individual ESTs ("EST"), the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), the sequences of contigs assembled from two or more ESTs ("Contig"), sequences of contigs assembled from an FIS and one or more ESTs ("Contig*"), or sequences encoding an entire protein derived from an FIS, a contig, or an FIS and PCR ("CGS"):

TABLE 3

BLAST Results (Literature) and Percent Identity for Sequences Encoding RUM1 and RUL polypeptides and homologs thereof.

5

Sequence	Status	BLAST pLOG Score to	% identity
B73-Mu-wt RUM1 (SEQ ID NO:24)	cgs	77 (NCBI GI No: 34911088 ,SEQ ID NO:65)	67.3 NCBI GI No: 34911088 (SEQ ID NO:65)
B73 RUM1 (SEQ ID NO:29)	cgs	78 (NCBI GI No: 34911088 ,SEQ ID NO:65)	67.3 NCBI GI No: 34911088 (SEQ ID NO:65)
B73 RUL (SEQ ID NO:39)	cgs	77 (NCBI GI No: 34911088 ,SEQ ID NO:65)	68.6 NCBI GI No: 34911088 (SEQ ID NO:65)
ebb1c.pk008.p9: fis (SEQ ID NO:67)	cgs	100 (NCBI GI No: 15229343, SEQ ID NO:74)	90.3(NCBI GI No: 15229343, SEQ ID NO:74)
smj1c.pk013.h7.f: fis (SEQ ID NO:69)	cgs	>180 (NCBI GI No: 2388689, SEQ ID NO:75)	95.6 (NCBI GI No: 2388689, SEQ ID NO:75)
smj1c.pk007.k12.f: fi s (SEQ ID NO:71)	cgs	>180 (NCBI GI No: 2388689, SEQ ID NO:75)	100 (NCBI GI No: 2388689, SEQ ID NO:75)
wdk1c.pk023.b8: fis (SEQ ID NO:73)	cgs	79 (NCBI GI No: 125553286 SEQ ID NO:76)	64.4 (NCBI GI No: 125553286 SEQ ID NO:76)

The BLASTX search using the sequences from clones listed in Table 1 below revealed similarity of the polypeptides encoded by the Table 3 shows the BLAST results for individual ESTs ("EST"), the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), the sequences of contigs assembled from two or more ESTs ("Contig"), sequences of contigs assembled from an FIS and

10

one or more ESTs ("Contig*"), or sequences encoding an entire protein derived from an FIS, a contig, or an FIS and PCR ("CGS").

TABLE 4

5 BLAST Results (patent) for Sequences Encoding Polypeptides Homologous to RUM1 and RUL Polypeptides and homologs thereof.

Sequence	Status	Reference	Blast pLog Score	% identity
B73-Mu-wt RUM1 (SEQ ID NO:24)	CGS	SEQ ID NO: 349502 in US2004214272	106	98.5
B73 RUM1 (SEQ ID NO:29)	CGS	SEQ ID NO: 349502 in US2004214272	106	99.3
B73 RUL (SEQ ID NO:39)	CGS	SEQ ID NO: 6770 in US2004034888-A1	106	100
ebb1c.pk008.p9:fis (SEQ ID NO:67)	CGS	G456 in US2007022495	101	90.3
smj1c.pk013.h7.f:fis (SEQ ID NO:69)	CGS	SEQ ID NO:23940 in US2006107345	>180	100
smj1c.pk007.k12.f:fis (SEQ ID NO:71)	CGS	SEQ ID NO:23940 in US2006107345	>180	100
wdk1c.pk023.b8:fis (SEQ ID NO:73)	CGS	SEQ ID NO:33260 in US2006107345	83	66.4

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed
 10 using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS*. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5.

WHAT IS CLAIMED IS:

1. A plant comprising in its genome a recombinant DNA construct comprising a polynucleotide operably linked to at least one regulatory element,
5 wherein said polynucleotide encodes a polypeptide having an amino acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73 and wherein said plant exhibits altered root architecture when compared to a control plant not comprising said recombinant DNA construct.
- 10 2. The plant of claim 1, wherein the plant is a maize plant or a soybean plant.
3. A plant comprising in its genome:
a recombinant DNA construct comprising:
 - 15 (a) a polynucleotide operably linked to at least one regulatory element, wherein said polynucleotide encodes a polypeptide having an amino acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73, or
 - (b) a suppression DNA construct comprising at least one regulatory element operably linked to:
 - 20 (i) all or part of: (A) a nucleic acid sequence encoding a polypeptide having an amino acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73, or (B) a full complement of the nucleic acid sequence of (b)(i)(A); or
 - 25 (ii) a region derived from all or part of a sense strand or antisense strand of a target gene of interest, said region having a nucleic acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to said all or part of a sense strand or antisense strand from which said region is derived, and wherein said target gene of interest encodes
 - 30 a RUM1 or RUM1-like polypeptide, and wherein said plant exhibits an alteration of at least one agronomic characteristic when compared to a control plant not comprising said recombinant DNA construct.

4. The plant of claim 3, wherein the plant is a maize plant or a soybean plant.

5. The plant of claim 3, wherein said plant exhibits said alteration of said at least one agronomic characteristic when compared, under varying environmental conditions, to said control plant not comprising said recombinant DNA construct.

6. The plant of claim 5, wherein said varying environmental condition is at least one selected from drought, nitrogen, insect or disease.

7. The plant of claim 5, wherein the plant is a maize plant or a soybean plant.

8. The plant of claim 6, wherein the plant is a maize plant or a soybean plant.

9. The plant of claim 7, wherein the plant is a maize plant or a soybean plant.

10. The plant of claim 3, wherein said at least one agronomic characteristic is selected from the group consisting of greenness, yield, growth rate, biomass, fresh weight at maturation, dry weight at maturation, fruit yield, seed yield, total plant nitrogen content, fruit nitrogen content, seed nitrogen content, nitrogen content in a vegetative tissue, total plant free amino acid content, fruit free amino acid content, seed free amino acid content, free amino acid content in a vegetative tissue, total plant protein content, fruit protein content, seed protein content, protein content in a vegetative tissue, drought tolerance, nitrogen uptake, root lodging, stalk lodging, plant height, ear length, and harvest index.

11. The plant of claim 10, wherein the plant is a maize plant or a soybean plant.

12. The plant of claim 3, wherein said plant exhibits an increase of said at least one agronomic characteristic when compared to said control plant.

13. The plant of claim 12, wherein the plant is a maize plant or a soybean plant.

14. A method of altering root architecture in a plant, comprising:
(a) introducing into a regenerable plant cell a recombinant DNA construct comprising a polynucleotide operably linked to at least one regulatory sequence, wherein the polynucleotide encodes a polypeptide having an amino acid

sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73; and

(b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the recombinant DNA construct and exhibits altered root architecture when compared to a control plant not comprising the recombinant DNA construct.

15. The method of claim 14, further comprising:

(c) obtaining a progeny plant derived from the transgenic plant, wherein said progeny plant comprises in its genome the recombinant DNA construct and exhibits altered root architecture when compared to a control plant not comprising the recombinant DNA construct.

16. A method of evaluating root architecture in a plant, comprising:

(a) introducing into a regenerable plant cell a recombinant DNA construct comprising a polynucleotide operably linked to at least one regulatory sequence, wherein the polynucleotide encodes a polypeptide having an amino acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73;

(b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the recombinant DNA construct; and

(c) evaluating root architecture of the transgenic plant compared to a control plant not comprising the recombinant DNA construct.

17. The method of claim 16, further comprising:

(d) obtaining a progeny plant derived from the transgenic plant, wherein the progeny plant comprises in its genome the recombinant DNA construct; and

(e) evaluating root architecture of the progeny plant compared to a control plant not comprising the recombinant DNA construct.

18. A method of evaluating root architecture in a plant, comprising:

(a) introducing into a regenerable plant cell a recombinant DNA construct comprising a polynucleotide operably linked to at least one regulatory sequence, wherein the polynucleotide encodes a polypeptide having an amino acid

sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73;

(b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the recombinant

5 DNA construct;

(c) obtaining a progeny plant derived from the transgenic plant, wherein the progeny plant comprises in its genome the recombinant DNA construct; and

(d) evaluating root architecture of the progeny plant compared to a
10 control plant not comprising the recombinant DNA construct.

19. A method of determining an alteration of an agronomic characteristic in a plant, comprising:

(a) introducing into a regenerable plant cell a recombinant DNA construct comprising a polynucleotide operably linked to at least one regulatory
15 sequence, wherein the polynucleotide encodes a polypeptide having an amino acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73;

(b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the recombinant
20 DNA construct; and

(c) determining whether the transgenic plant exhibits an alteration of at least one agronomic characteristic when compared to a control plant not comprising the recombinant DNA construct.

20. The method of claim 19, further comprising:

(d) obtaining a progeny plant derived from the transgenic plant, wherein the progeny plant comprises in its genome the recombinant DNA construct; and

(e) determining whether the progeny plant exhibits an alteration of at least one agronomic characteristic when compared to a control plant not
30 comprising the recombinant DNA construct.

21. The method of claim 19, wherein said determining step comprises determining whether the transgenic plant exhibits an alteration of at least one

agronomic characteristic when compared, under varying environmental conditions, to a control plant not comprising the recombinant DNA construct.

22. The method of claim 20, wherein said varying environmental condition is at least one selected from drought, nitrogen, insect or disease.

5 23. The method of claim 20, wherein said determining step (e) comprises determining whether the progeny plant exhibits an alteration of at least one agronomic characteristic when compared, under varying environmental conditions, to a control plant not comprising the recombinant DNA construct.

10 24. The method of claim 23, wherein said varying environmental condition is at least one selected from drought, nitrogen, insect or disease.

25. A method of determining an alteration of an agronomic characteristic in a plant, comprising:

(a) introducing into a regenerable plant cell a recombinant DNA construct comprising a polynucleotide operably linked to at least one regulatory
15 sequence, wherein the polynucleotide encodes a polypeptide having an amino acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73;

(b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the recombinant
20 DNA construct;

(c) obtaining a progeny plant derived from the transgenic plant, wherein the progeny plant comprises in its genome the recombinant DNA construct; and

(d) determining whether the progeny plant exhibits an alteration of
25 at least one agronomic characteristic when compared to a control plant not comprising the recombinant DNA construct.

26. The method of claim 25, wherein said determining step comprises determining whether the transgenic plant exhibits an alteration of at least one agronomic characteristic when compared, under varying environmental conditions,
30 to a control plant not comprising the recombinant DNA construct.

27. A method of determining an alteration of an agronomic characteristic in a plant, comprising:

(a) introducing into a regenerable plant cell a suppression DNA construct comprising at least one regulatory element operably linked to:

(i) all or part of: (A) a nucleic acid sequence encoding a polypeptide having an amino acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73, or (B) a full complement of the nucleic acid sequence of (a)(i)(A); or

(ii) a region derived from all or part of a sense strand or antisense strand of a target gene of interest, said region having a nucleic acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to said all or part of a sense strand or antisense strand from which said region is derived, and wherein said target gene of interest encodes a RUM1 or RUM1-like polypeptide;

(b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the suppression DNA construct; and

(c) determining whether the transgenic plant exhibits an alteration of at least one agronomic characteristic when compared to a control plant not comprising the suppression DNA construct.

28. The method of claim 27, wherein said determining step comprises determining whether the transgenic plant exhibits an alteration of at least one agronomic characteristic when compared, under varying environmental conditions, to a control plant not comprising the suppression DNA construct.

29. The method of claim 28, wherein said varying environmental condition is at least one selected from drought, nitrogen, insect or disease.

30. The method of claim 27, further comprising:

(d) obtaining a progeny plant derived from the transgenic plant, wherein the progeny plant comprises in its genome the suppression DNA construct; and

(e) determining whether the progeny plant exhibits an alteration of at least one agronomic characteristic when compared to a control plant not comprising the suppression DNA construct.

31. The method of claim 30, wherein said determining step (e) comprises determining whether the progeny plant exhibits an alteration of at least one agronomic characteristic when compared, under varying environmental conditions, to a control plant not comprising the suppression DNA construct.

5 32. The method of claim 31, wherein said varying environmental condition is at least one selected from drought, nitrogen, insect or disease.

33. A method of determining an alteration of an agronomic characteristic in a plant, comprising:

(a) introducing into a regenerable plant cell a suppression DNA
10 construct comprising at least one regulatory element operably linked to:

(i) all or part of: (A) a nucleic acid sequence encoding a polypeptide having an amino acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73, or (B) a full complement of the nucleic acid sequence of

15 (a)(i)(A); or

(ii) a region derived from all or part of a sense strand or antisense strand of a target gene of interest, said region having a nucleic acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to said all or part of a sense strand or antisense strand
20 from which said region is derived, and wherein said target gene of interest encodes a RUM1 or RUM1-like polypeptide;

(b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the suppression DNA construct and exhibits altered root architecture when compared to a control
25 plant not comprising the suppression DNA construct;

(c) obtaining a progeny plant derived from the transgenic plant, wherein the progeny plant comprises in its genome the suppression DNA construct; and

(d) determining whether the progeny plant exhibits an alteration of
30 at least one agronomic characteristic when compared to a control plant not comprising the suppression DNA construct.

34. The method of claim 33, wherein said determining step comprises determining whether the transgenic plant exhibits an alteration of at least one

agronomic characteristic when compared, under varying environmental conditions, to a control plant not comprising the recombinant DNA construct.

35. The method of claim 34, wherein said varying environmental condition is at least one selected from drought, nitrogen, insect or disease.

5 36. A method of altering root architecture in a plant, comprising:

(a) introducing into a regenerable plant cell a suppression DNA construct comprising at least one regulatory element operably linked to:

10 (i) all or part of: (A) a nucleic acid sequence encoding a polypeptide having an amino acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73, or (B) a full complement of the nucleic acid sequence of (a)(i)(A); or

15 (ii) a region derived from all or part of a sense strand or antisense strand of a target gene of interest, said region having a nucleic acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to said all or part of a sense strand or antisense strand from which said region is derived, and wherein said target gene of interest encodes a RUM1 or RUM1-like polypeptide; and

20 (b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the suppression DNA construct and wherein the transgenic plant exhibits altered root architecture when compared to a control plant not comprising the suppression DNA construct.

37. The method of claim 36, further comprising:

25 (c) obtaining a progeny plant derived from the transgenic plant, wherein said progeny plant comprises in its genome the recombinant DNA construct and wherein the progeny plant exhibits altered root architecture when compared to a control plant not comprising the suppression DNA construct.

38. A method of evaluating root architecture in a plant, comprising:

30 (a) introducing into a regenerable plant cell a suppression DNA construct comprising at least one regulatory element operably linked to:

(i) all or part of: (A) a nucleic acid sequence encoding a polypeptide having an amino acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29,

39, 67, 69, 71 or 73, or (B) a full complement of the nucleic acid sequence of (a)(i)(A); or

(ii) a region derived from all or part of a sense strand or antisense strand of a target gene of interest, said region having a nucleic acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to said all or part of a sense strand or antisense strand from which said region is derived, and wherein said target gene of interest encodes a RUM1 or RUM1-like polypeptide;

(b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the suppression DNA construct; and

(c) evaluating root architecture of the transgenic plant compared to a control plant not comprising the suppression DNA construct.

39. The method of claim 38, further comprising:

(d) obtaining a progeny plant derived from the transgenic plant, wherein the progeny plant comprises in its genome the suppression DNA construct; and

(e) evaluating root architecture of the progeny plant compared to a control plant not comprising the suppression DNA construct.

40. A method of evaluating root architecture in a plant, comprising:

(a) introducing into a regenerable plant cell a suppression DNA construct comprising at least one regulatory element operably linked to:

(i) all or part of: (A) a nucleic acid sequence encoding a polypeptide having an amino acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 24, 29, 39, 67, 69, 71 or 73, or (B) a full complement of the nucleic acid sequence of (a)(i)(A); or

(ii) a region derived from all or part of a sense strand or antisense strand of a target gene of interest, said region having a nucleic acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to said all or part of a sense strand or antisense strand from which said region is derived, and wherein said target gene of interest encodes a RUM1 or RUM1-like polypeptide;

(b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant comprises in its genome the suppression DNA construct;

(c) obtaining a progeny plant derived from the transgenic plant, wherein the progeny plant comprises in its genome the suppression DNA construct; and

(d) evaluating root architecture of the progeny plant compared to a control plant not comprising the suppression DNA construct.

41. An isolated polynucleotide comprising:

(i) a nucleic acid sequence encoding a polypeptide having an amino acid sequence of at least 85%, sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO:73; or

(ii) a full complement of the nucleic acid sequence of (i).

42. An isolated polynucleotide comprising:

(i) a nucleic acid sequence encoding a polypeptide having an amino acid sequence of at least 90%, sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO:73; or

(ii) a full complement of the nucleic acid sequence of (i).

43. An isolated polynucleotide comprising:

(i) a nucleic acid sequence encoding a polypeptide having an amino acid sequence of at least 95%, sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO:73; or

(ii) a full complement of the nucleic acid sequence of (i).

44. The polynucleotide of claim 1, wherein the polypeptide sequence comprises SEQ ID NO:73.

45. The polynucleotide of claim 1, wherein the nucleic acid sequence comprises SEQ ID NO:72.

46. An isolated nucleic acid fragment comprising a root-preferred maize NAS2 promoter.

47. An isolated nucleic acid fragment comprising a root-preferred maize promoter wherein said promoter consists essentially of the nucleotide sequence set forth in SEQ ID NO:51.

Figure 1

RUM1 Genetic Mapping with SSRs

Total SSRs analyzed: 258 F7/*rum1* polymorphic SSRs: 95

Marker	Bin	IBM2	Results
umc1071	1.01S	85.2	No linkage
umc1403	1.03S	210.6	No linkage
bnlg1203	1.03S	259.3	No linkage
umc1590	1.06C	517.0	No linkage
umc1278	1.07L	631.9	No linkage
umc1833	1.07L	647.8	No linkage
umc1446	1.08L	781.6	No linkage
umc1512	1.09L	812.3	No linkage
umc1306	1.09L	881.2	No linkage
bnlg1671	1.10L	913.4	No linkage
phi064	1.11L	1103.0	No linkage
umc1165	2.01S	47.4	No linkage
umc1823	2.02S	92.8	No linkage
umc1422	2.02S	156.6	No linkage
umc1448	2.04S	269.8	No linkage
umc1079	2.06L	369.3	No linkage
bnlg2077	2.07L	474.8	No linkage
umc2085	2.08L	544.4	No linkage
mnc0381	2.08L	572.4	No linkage
bnlg1647	3.02S	103.3	No linkage
umc1644	3.06L	473.1	Linkage
umc1690	3.07L	544.6	Linkage
umc1659	3.07L	544.6	Linkage
umc1489	3.07L	567.6	Linkage
umc1404	3.07L	568.3	Linkage
umc1844	3.08L	596.9	Linkage
umc2274	3.08L	608.6	Linkage
phi046	3.08L	612.0	Linkage
umc1915	3.08L	617.5	Linkage
umc2152	3.09L	738.7	Linkage
mnc0001	3.09L	759.9	Linkage
umc2048	3.10L	824.7	Linkage
umc1639	3.09L	824.7	No linkage
umc1594	3.09L	828.9	No linkage

Marker	Bin	IBM2	Results
umc1759	4.01S	81	No linkage
umc2176	4.03S	174.6	No linkage
bnlg1265	4.05C	268.4	No linkage
umc2027	4.06L	349.8	No linkage
bnlg2291	4.06L	392.2	No linkage
umc1775	4.08L	443.2	No linkage
umc2188	4.08L	554.1	No linkage
umc1573	4.09L	644.3	No linkage
umc1260	5.00S	68.1	No linkage
bnlg566	5.02S	150.9	No linkage
umc1892	5.03S	271.5	No linkage
umc1349	5.04L	346.5	No linkage
umc1171	5.04L	376.4	No linkage
umc1155	5.05L	410.8	No linkage
umc1524	5.06L	493.5	No linkage
umc108	5.07L	536.6	No linkage
umc1225	5.08L	641.4	No linkage
umc1143	6.00S	17.3	No linkage
umc2068	6.00S	46.1	No linkage
umc1133	6.01S	98.6	No linkage
umc1887	6.03L	166.8	No linkage
bnlg1154	6.05L	253	No linkage
bnlg1702	6.05L	320.7	No linkage
phi29852	6.07L	450.7	No linkage
bnlg1136	6.07L	531.8	No linkage
umc1653	6.07L	534.6	No linkage
mnc0171	7.00S	64.3	No linkage
umc2160	7.01S	118.5	No linkage
bnlg2203	7.01S	190.6	No linkage
umc2142	7.02L	246.3	No linkage
umc1408	7.03L	365.4	No linkage
umc1029	7.04L	444.7	No linkage
umc1407	7.05L	600.2	No linkage

Marker	Bin	IBM2	Results
umc1327	8.01S	55.0	No linkage
umc2004	8.02L	120.2	No linkage
umc1868	8.02L	200.3	No linkage
umc1735	8.03L	279.3	No linkage
umc1846	8.03L	357.9	No linkage
bnlg1031	8.06L	455.1	No linkage
umc1724	8.06L	457.3	No linkage
umc1056	8.08L	550.4	No linkage
umc1867	9.01S	24.3	No linkage
umc1258	9.02S	195.7	No linkage
umc1191	9.03C	232.8	No linkage
bnlg1714	9.04L	266.4	No linkage
umc1657	9.05L	344.8	No linkage
umc1804	9.07L	538.5	No linkage
umc1137	9.07L	603.5	No linkage
umc1380	10.00S	16.6	No linkage
umc1291	10.01S	58.8	No linkage
bnlg1451	10.02S	74.4	No linkage
umc1319	10.02S	82.2	No linkage
umc1432	10.02S	120.09	No linkage
umc1179	10.03C	208.5	No linkage
bnlg640	10.03C	225.7	No linkage
umc1077	10.04L	253.5	No linkage
umc1115	10.04L	295.9	No linkage
umc1272	10.04L	299.4	No linkage
umc1477	10.05L	366.3	No linkage
umc2122	10.06L	392.5	No linkage
bnlg1450	10.07L	483.7	No linkage

Figure 2

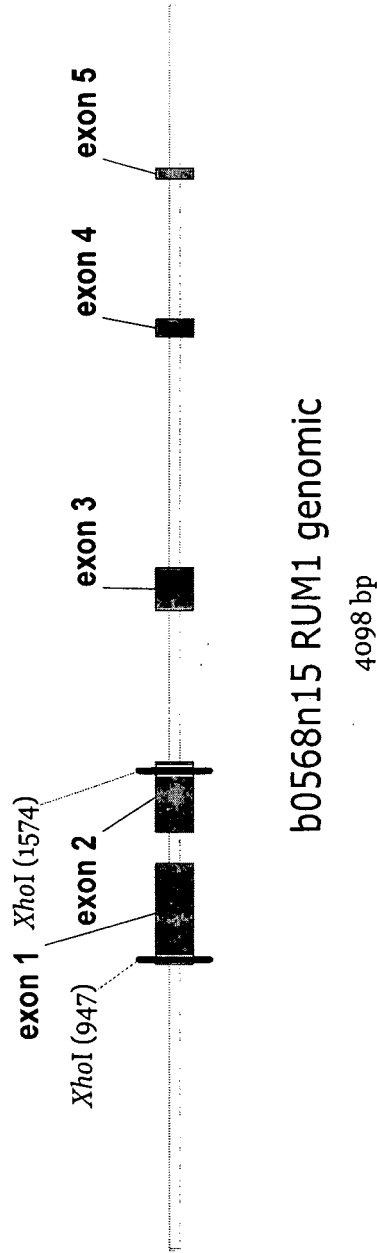
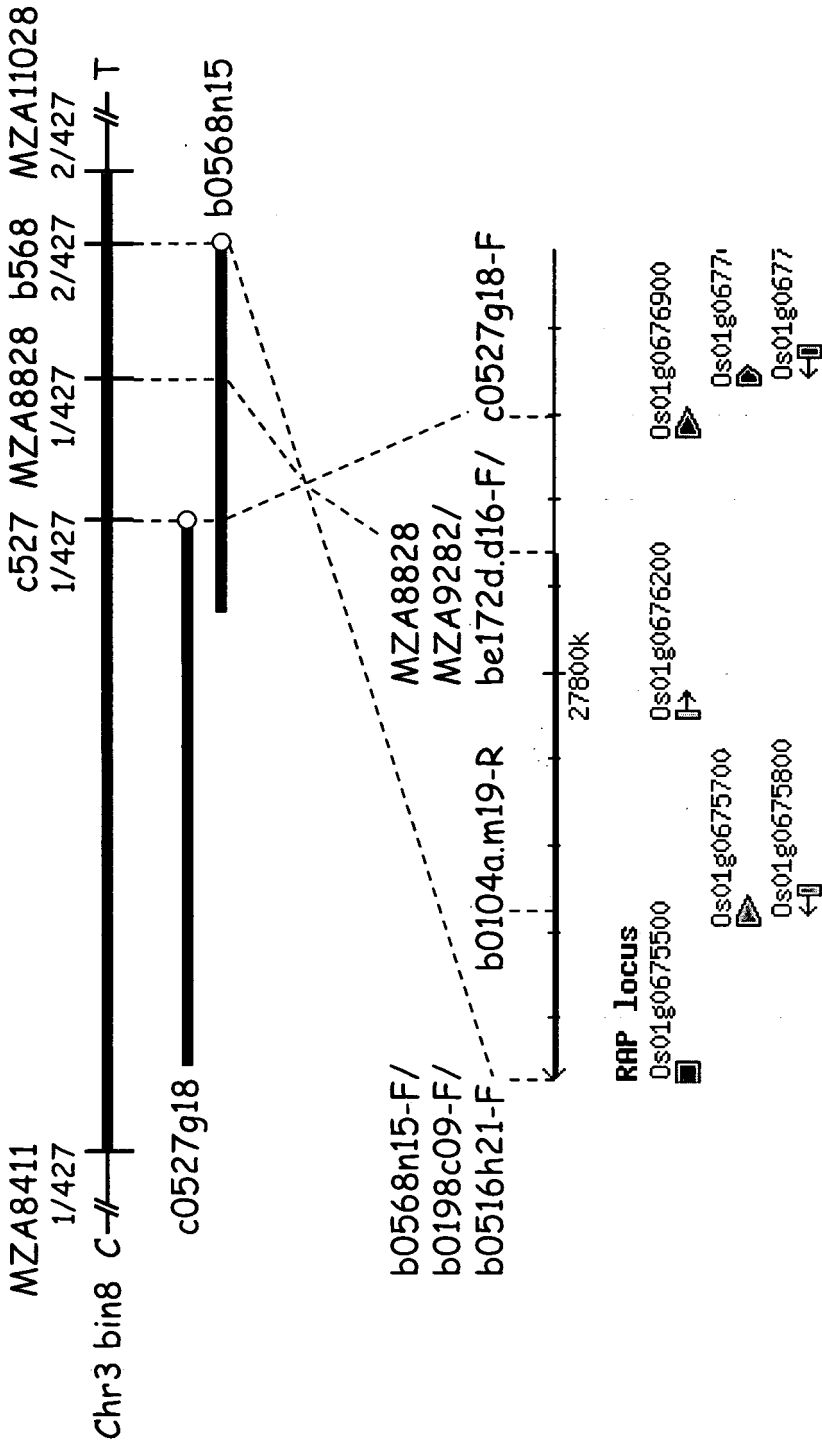


Figure 3
RUM1 Physical Map and Synteny with Rice



Rice Ch1: 27752889 ~ 27830748 bp

Figure 4

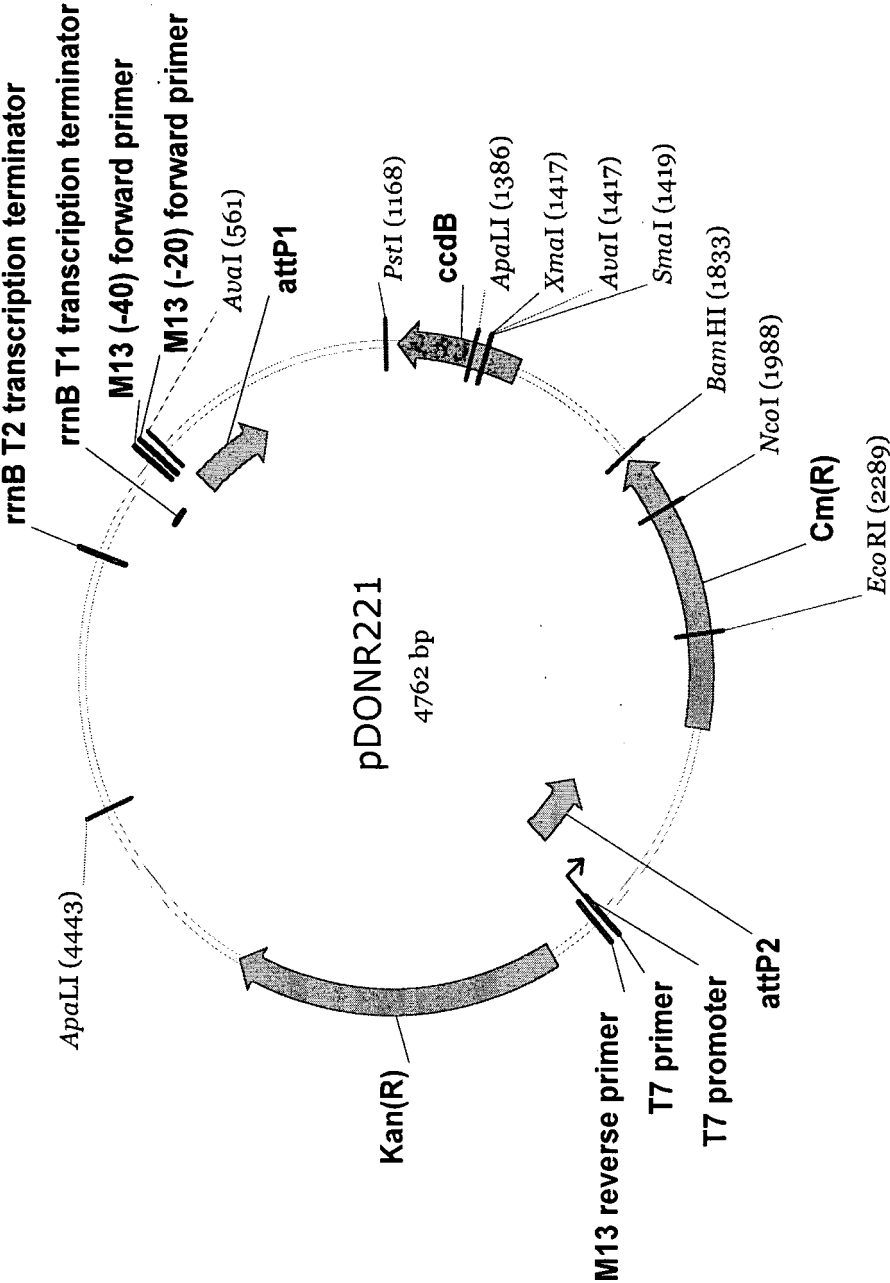


Figure 5

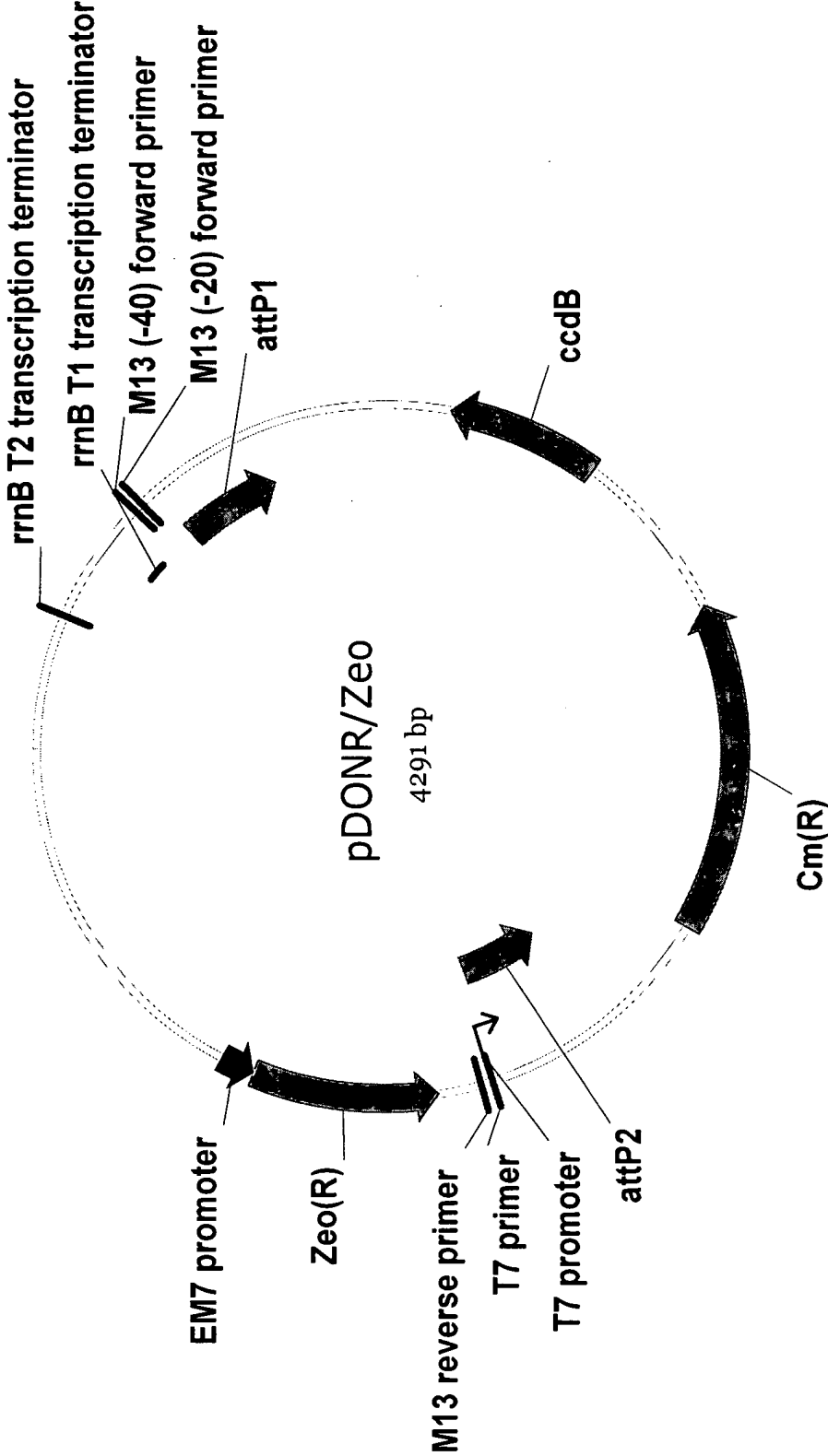


Figure 6

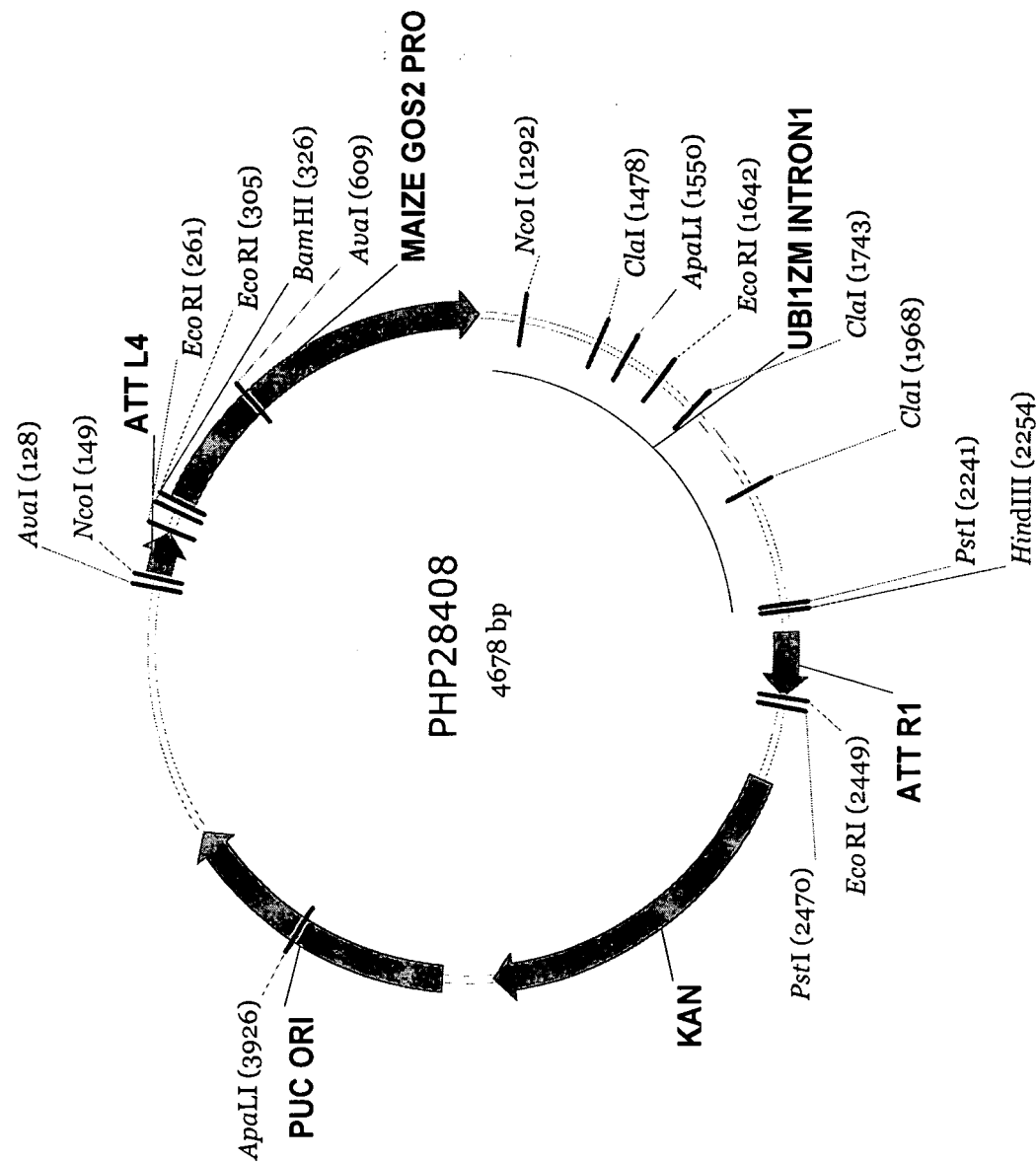


Figure 7

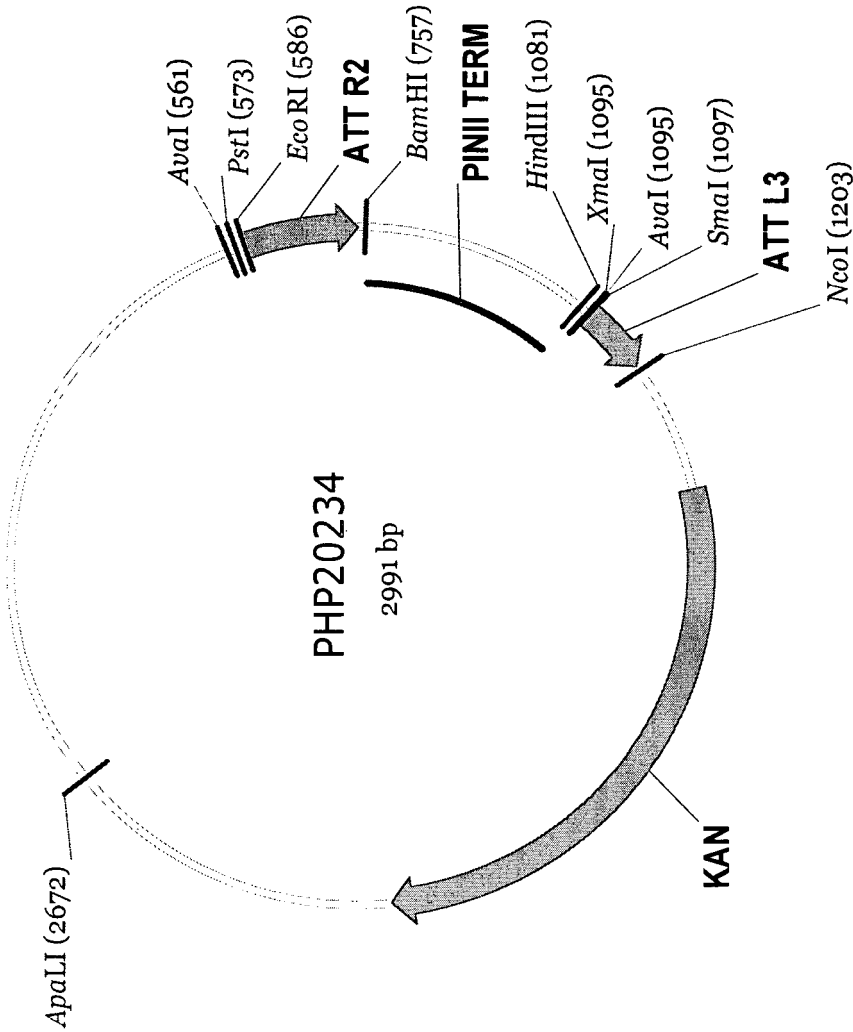
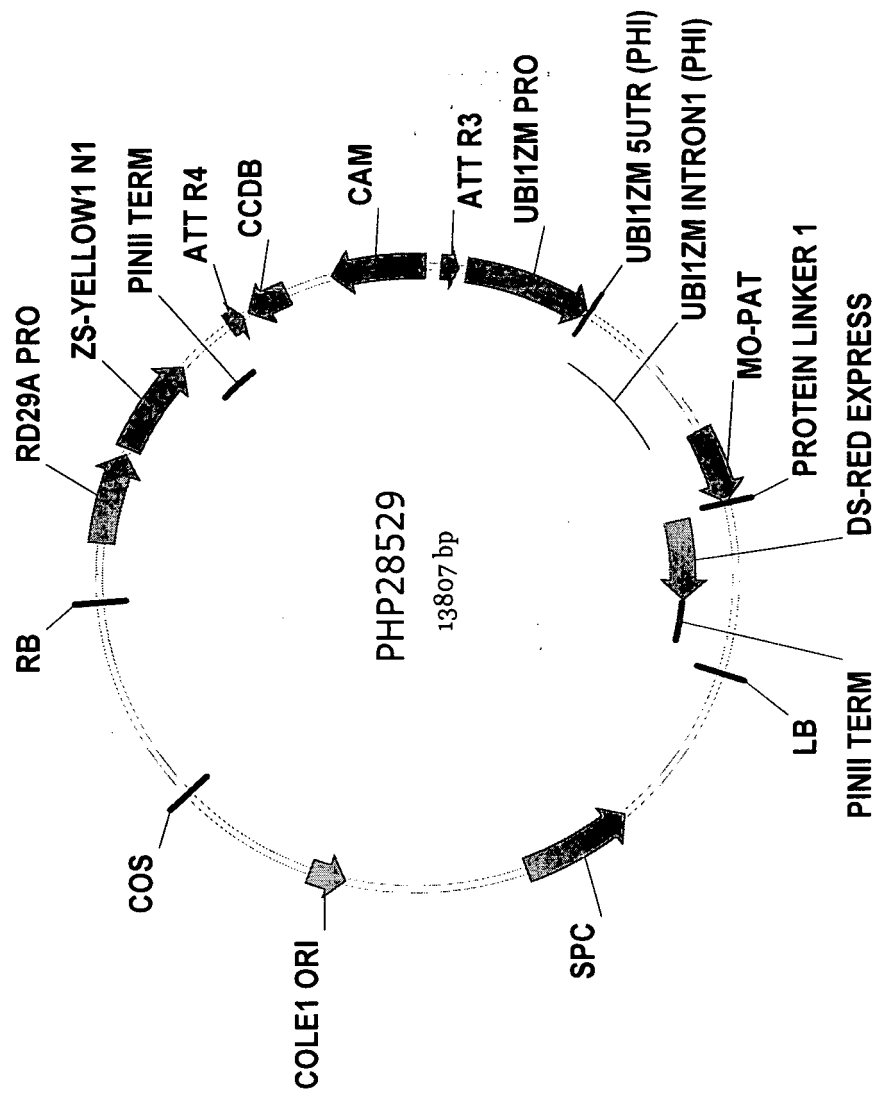


Figure 8



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Figure 9

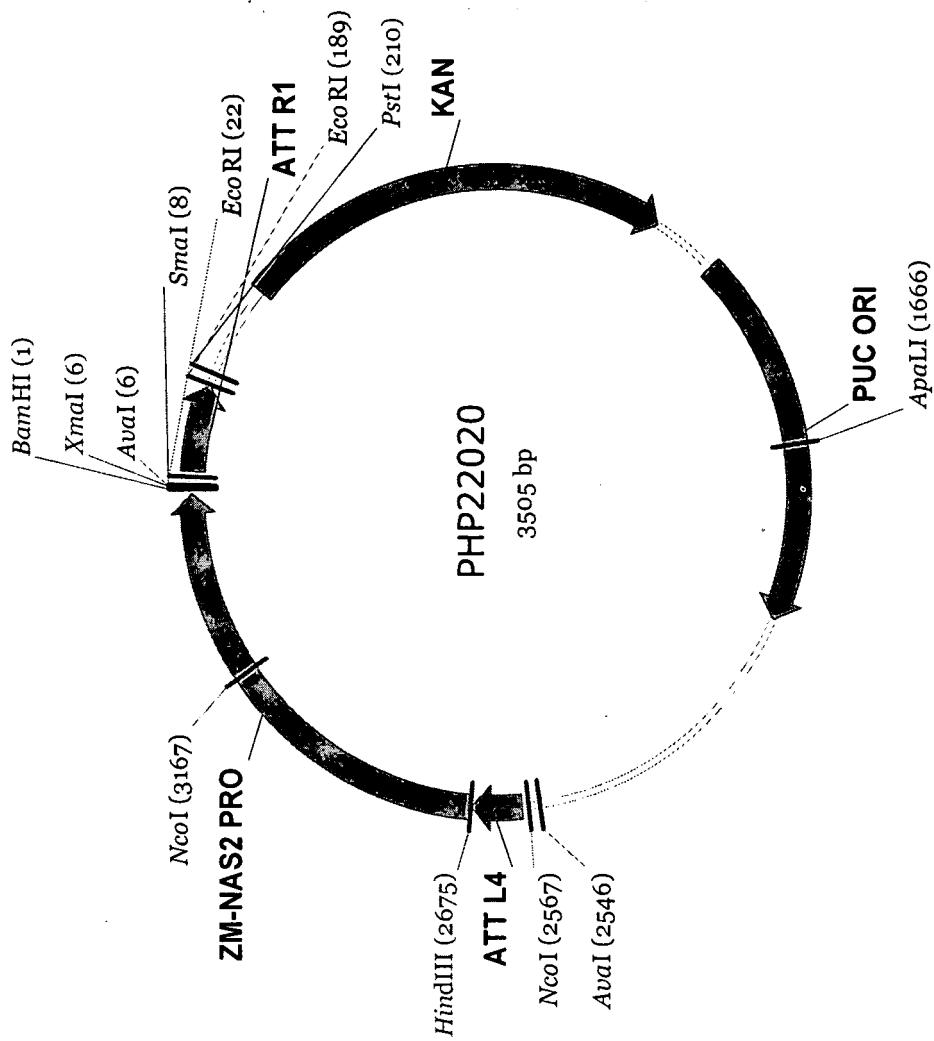


Figure 10

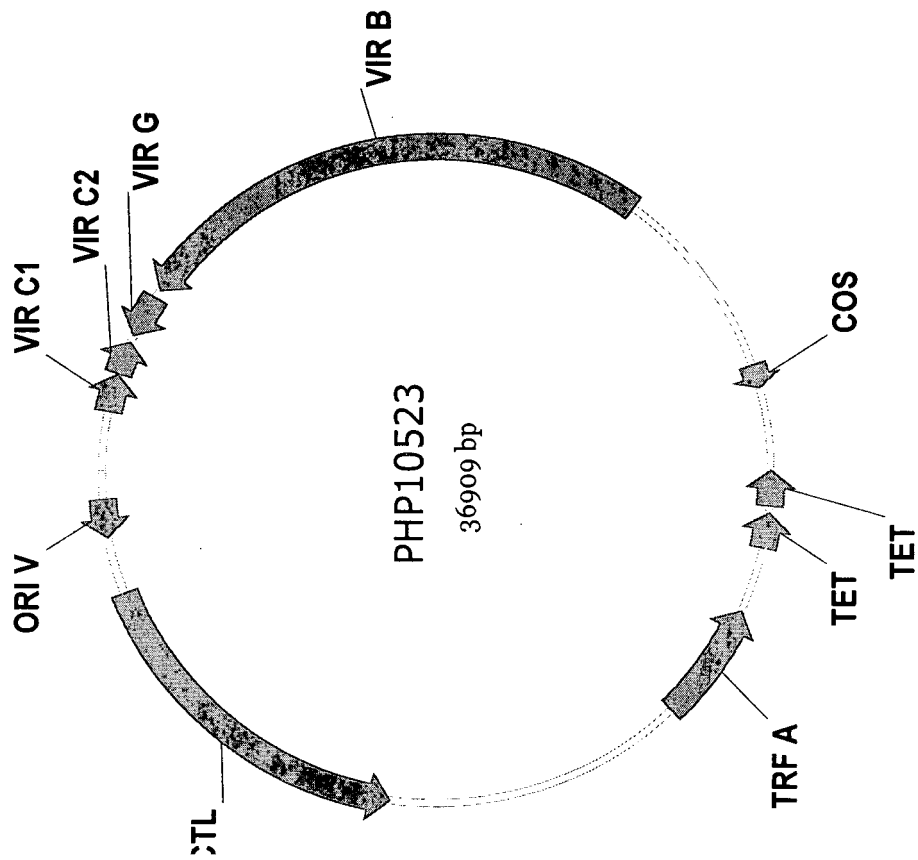


Figure 11

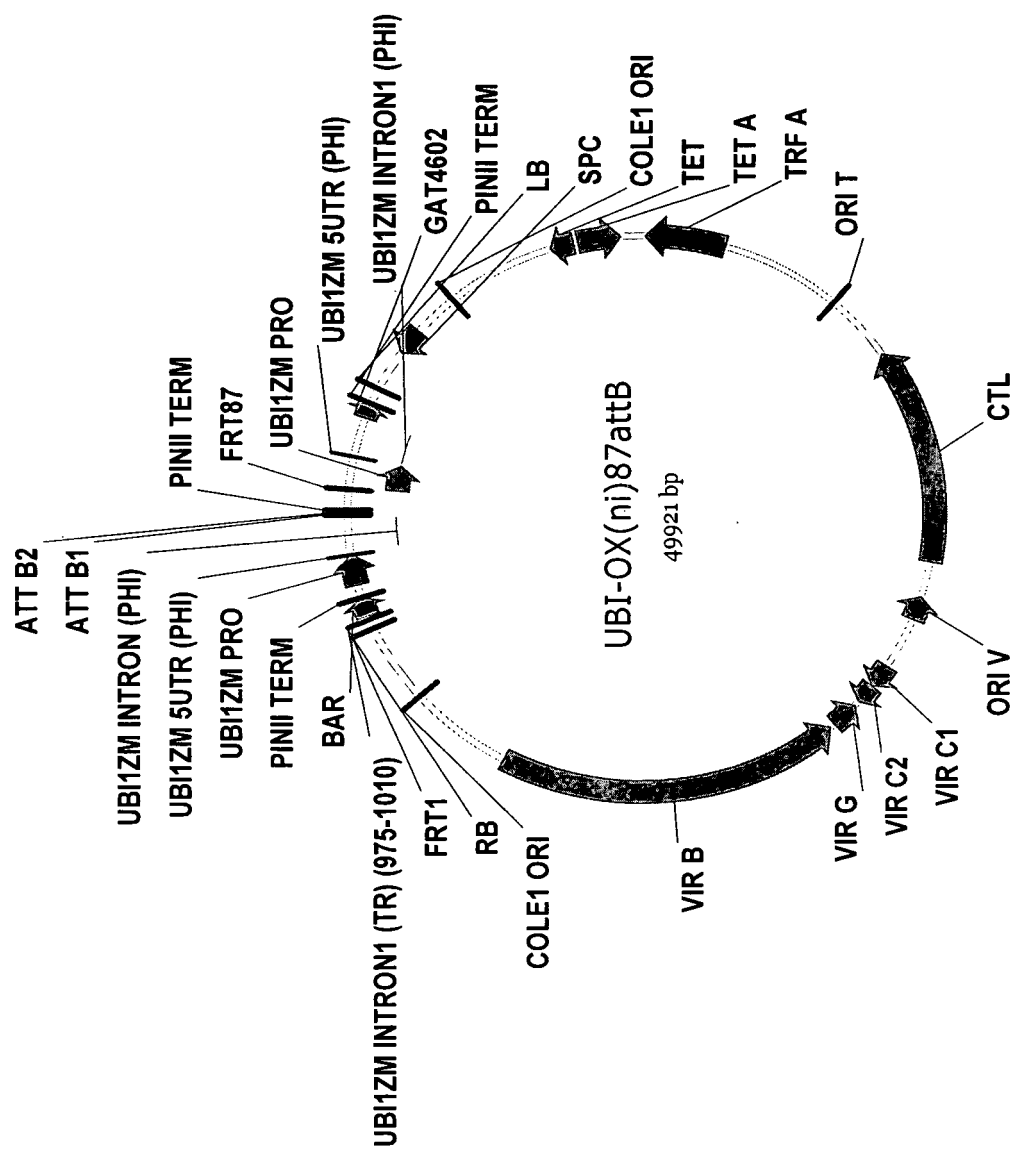


Figure 12

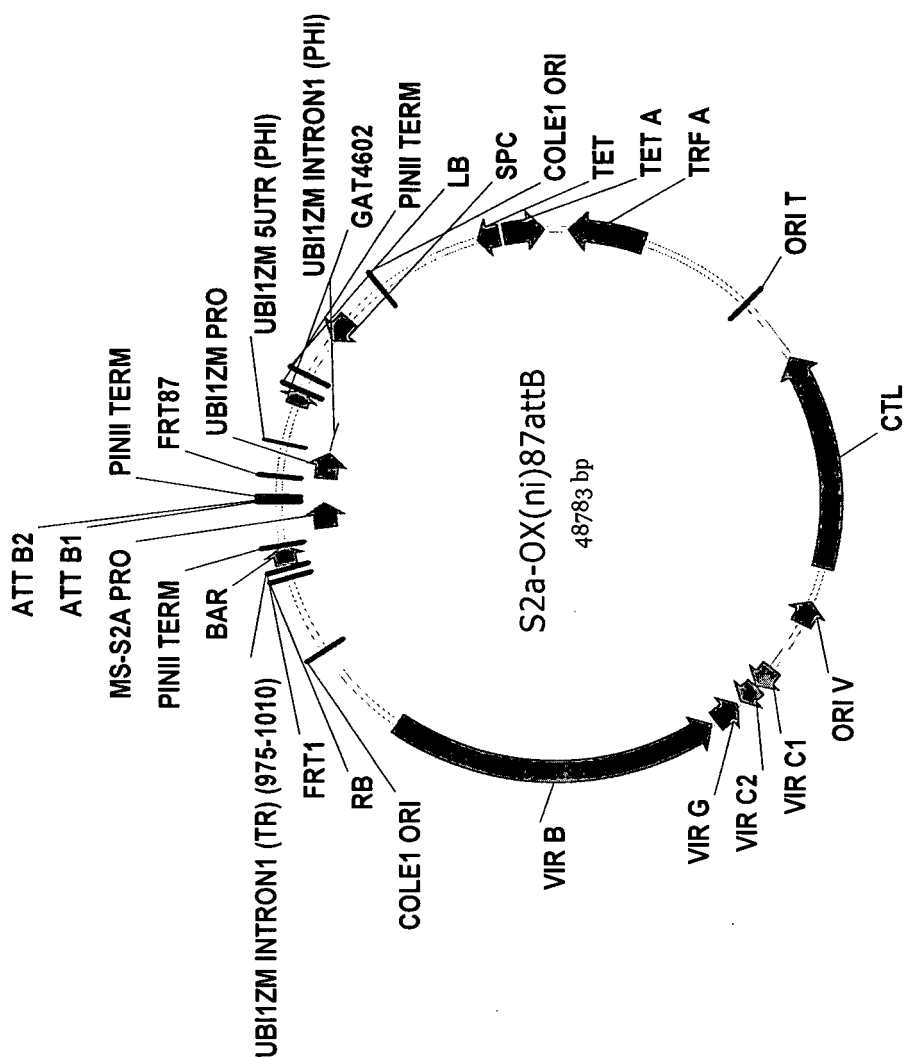
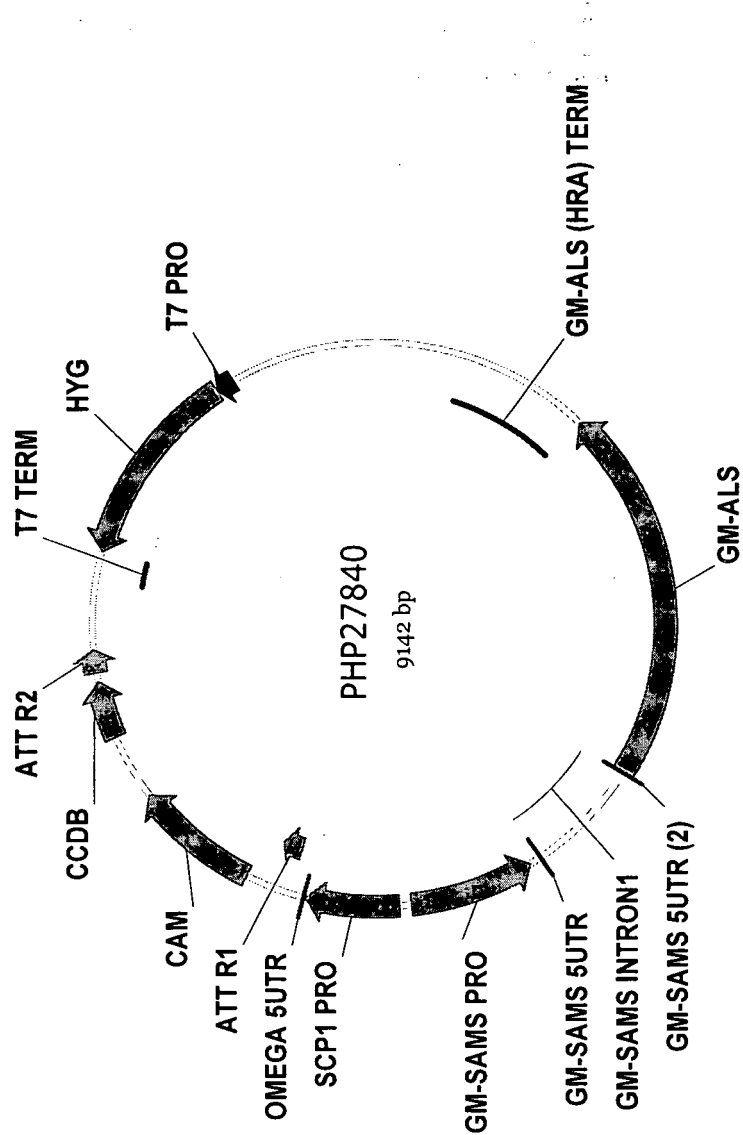


Figure 13



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Figure 14

```

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SEQ ID NO: 25
MSPPLEPHDYIGLSAAAAAAPTPTPTSSSSSSSPA--PRLTLRLGLPGSESPDRDRDC
SEQ ID NO: 34
MSPPLEPHDYIGLSAAAAAAPTPTPTSSSSSSSPA--PRLTLRLGLPGSESPDRDRDC
SEQ ID NO: 53
MSPPLEPHDYIGLSAAAAAAPTPTPTSSSSSSSPA--PRLTLRLGLPGSESPDRDRDC
SEQ ID NO: 51

* * * * *
--CEDVAATLSLGPLPAA---AAVSAKRAFPDPAQRFGASKASDA-----KQQASPA
SEQ ID NO: 52
--CEDVAATLSLGPLPAA---AAVSAKRAFPDPAQRFGASKASDA-----KQQASPA
SEQ ID NO: 25
DRCEDVAAALSLGPLPATPKAPAAVSAKRAFPDPAQRFGAAKASDD-----KQ-ASPA
SEQ ID NO: 34
--CEDVAATLSLGPLPAA---AAVSAKRAFPDPAQRFGASKASDA-----KQQASPA
SEQ ID NO: 53
---EVVAAGLTGLPLPTTTTAAAS---KRAFPDSSPRHGASSGSVAAAAACQDKAAPAAA
SEQ ID NO: 51

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PAAK-----SKAPAEAAASGAGPMYVKVSMDGAPYLRKVD
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SEQ ID NO: 51

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SEQ ID NO: 34
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SEQ ID NO: 53
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SEQ ID NO: 51

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Figure 15

% Identity

	SEQ ID NO: Rum1 original	SEQ ID NO: RUM1 B73	SEQ ID NO: RML	SEQ ID NO: Rum1-R	SEQ ID NO: Rice seq
SEQ ID NO: Rum1 original		99.3	87.0	97.1	67.3
SEQ ID NO: RUM1 B73			87.0	97.1	67.3
SEQ ID NO: RML				88.9	68.6
SEQ ID NO: Rum1-R					66.3
SEQ ID NO: Rice seq					

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Figure 16

**Modified Hoagland's solutions -
16X concentrations for semi-hydroponics maize growth.**

Nutrient	1 mM KNO ₃	2 mM KNO ₃	3 mM KNO ₃	4 mM KNO ₃
KNO ₃	16 mM	32 mM	48 mM	64 mM
KCl	48 mM	32 mM	16 mM	-----
KH ₂ PO ₄	11 mM	11 mM	11 mM	11 mM
MgSO ₄	16 mM	16 mM	16 mM	16 mM
CaCl ₂ ·2H ₂ O	16 mM	16 mM	16 mM	16 mM
Sprint 330	1.6 g/L	1.6 g/L	1.6 g/L	1.6 g/L
H ₃ BO ₃	24 μM	24 μM	24 μM	24 μM
5 mM MnCl ₂ ·4H ₂ O	8 μM	8 μM	8 μM	8 μM
5 mM ZnSO ₄ ·7 H ₂ O	8 M	8 μM	8 μM	8 μM
0.5 mM CuSO ₄ ·5 H ₂ O	800 nM	800 nM	800 nM	800 nM
0.5 mM H ₂ MoO ₄ ·H ₂ O	800 nM	800 nM	800 nM	800 nM

Dilute 16X with tap water and determine the pH of the final mixture.

Add 3-12 mL H₂SO₄ if the pH is above 6.5.

Optimum pH is 5.0 - 5.5

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Figure 17

The effect of different nitrate concentrations on the growth and development of Gaspe Bay Flint derived maize lines (see Example 10C).

[nitrate]	root (g dwt)	shoot (g dwt)	total vegetative (g dwt)	ear & husk (g dwt)	tassel (g dwt)	tiller #	tiller (g dwt)
1 week after emergence							
1 mM	0.070a	0.105b	0.175b				
2 mM	0.073a	0.137ab	0.209ab				
3 mM	0.056a	0.120ab	0.176ab				
4 mM	0.074a	0.157a	0.231a				
2 weeks after emergence							
1 mM	0.331ab	0.544c	0.875c				
2 mM	0.266b	0.951b	1.217b				
3 mM	0.352a	1.171a	1.523a				
4 mM	0.303ab	1.209a	1.512a				
3 weeks after emergence							
1 mM	0.757a	1.283b	2.040b	0.379c	0.239c	0.8c	0.080b
2 mM	0.785a	2.033a	2.819a	0.718a	0.363bc	2.3	0.506a
3 mM	0.664a	1.911a	2.574a	0.451bc	0.403ab	2.8ab	0.441a
4 mM	0.845a	2.129a	2.974a	0.650ab	0.506a	3.3a	0.688a
4 weeks after emergence							
1 mM	0.842b	2.010b	2.852b	1.318b	0.677b	*	*
2 mM	1.493a	3.772a	5.265a	3.130a	1.018a	*	*
3 mM	1.232ab	3.563a	4.795a	3.060a	0.875ab	*	*
4 mM	1.010b	2.943a	3.952a	2.787a	0.891ab	*	*

* Tillers removed 3 weeks after emergence

Means with similar letters are not different by protected Least Significant Difference (LSD) (0.05)