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An agency of Industry Canada

CA 2687442 A1 2009/01/08

(21) 2 687 442

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2008/06/27
(87) Date publication PCT/PCT Publication Date: 2009/01/08
(85) Entrée phase nationale/National Entry: 2009/11/17
(86) N° demande PCT/PCT Application No.: US 2008/068526
(87) N° publication PCT/PCT Publication No.: 2009/006276
(30) Priorité/Priority: 2007/06/29 (US60/947,117)

(51) Cl.Int./Int.Cl. *C12N 15/82* (2006.01),
A01H 5/00 (2006.01)

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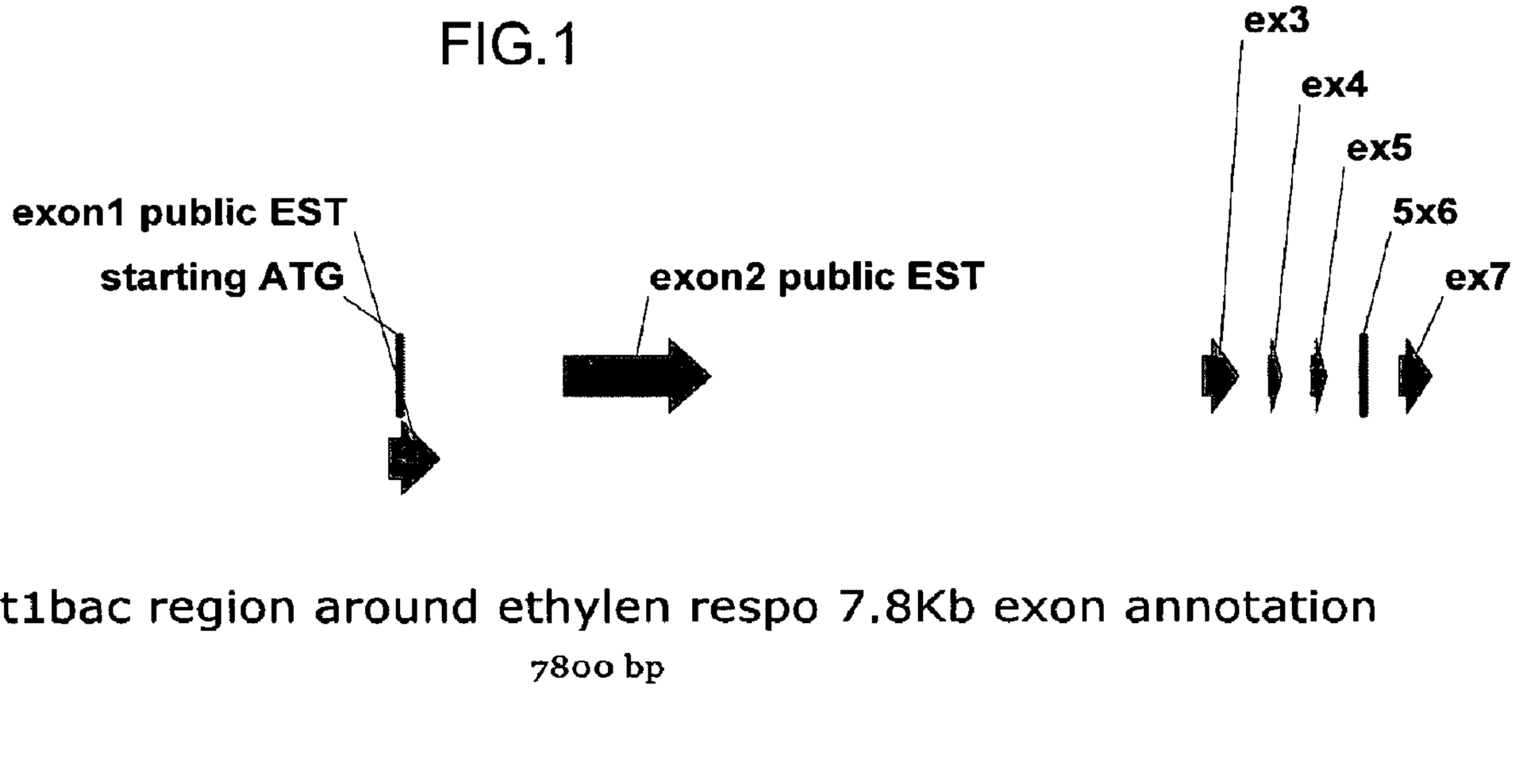
(54) Titre : PLANTES AVEC ARCHITECTURE DE RACINE MODIFIEE, COMPRENANT LE GENE RT1, CONSTRUCTIONS ASSOCIEES ET PROCEDES

(54) Title: PLANTS WITH ALTERED ROOT ARCHITECTURE, INVOLVING THE RT1 GENE, RELATED CONSTRUCTS AND METHODS

FIG.1



(57) Abrégé/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.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 January 2009 (08.01.2009)

PCT

(10) International Publication Number
WO 2009/006276 A1

(51) International Patent Classification:
C12N 15/82 (2006.01) *A01H 5/00* (2006.01)

(21) International Application Number:
PCT/US2008/068526

(22) International Filing Date: 27 June 2008 (27.06.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/947,117 29 June 2007 (29.06.2007) US

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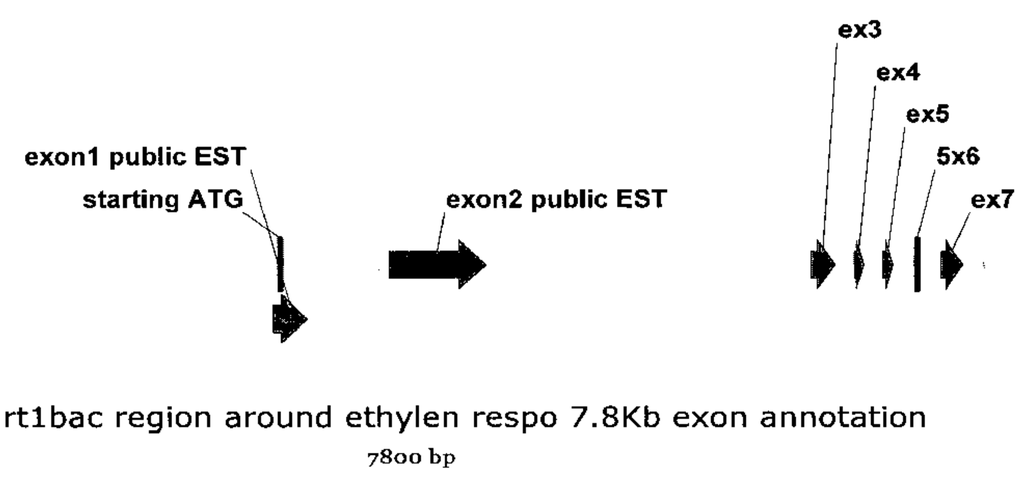
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*
— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

(54) Title: PLANTS WITH ALTERED ROOT ARCHITECTURE, INVOLVING THE *RT1* GENE, RELATED CONSTRUCTS AND METHODS

FIG.1



(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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TITLE

PLANTS WITH ALTERED ROOT ARCHITECTURE, INVOLVING THE *RT1* GENE, RELATED CONSTRUCTS AND METHODS

FIELD OF THE INVENTION

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

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 during different developmental stages have been described in maize (e.g. *rtcs* (rootless concerning crown and seminal roots), *lrt1* (lateral rootless1)), *rt1* ((rootless 1) (Hochholdinger et al.(2004) Annals of Botany 93: 359-368). The mutant *rt1* was the first mutant of root formation that was isolated and shows a reduced number of shoot-borne roots. The *rt1* mutant is missing all shoot-borne roots at the higher nodes while there is only a slight difference in the number of crown roots at the first two nodes. The mutation *rt1* is inherited as a monogenic recessive trait and maps on chromosome 3 (Maize GDB on the World Wide Web at maizegdb.org)

The *rt1* mutant was first described by Jenkins (Jenkins M T (1930) J Hered 21:79-80), but there,has been no molecular analysis of the nucleic acid encoding the protein associated with the *rt1* phenotype. Indeed, the identity of the protein encoded by *rt1* has not been reported, so far.

SUMMARY OF THE INVENTION

The present invention includes:

In one embodiment an isolated polynucleotide comprising: (i) 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: 13 or 21 and wherein 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.

In another embodiment an isolated 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: 13 or 21 and wherein expression of said polypeptide in a plant results in an altered plant root architecture when compared to a control plant not comprising said recombinant DNA construct.

In yet another embodiment an isolated polynucleotide comprising (i) a nucleic acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 12 or 20 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).

In yet another embodiment an isolated polynucleotide comprising (i) a nucleic acid sequence of at least 50% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 12 or 20 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 and wherein the polypeptide sequence comprises at least one motif selected from the group consisting of SEQ ID NOs:22 and 23, wherein said motif is a substantially conserved subsequence.

In another embodiment an isolated polynucleotide encoding a polypeptide, wherein expression of said polypeptide results in an altered root architecture and wherein the polypeptide sequence comprises at least one motif selected from the group consisting of SEQ ID NOs:22 and 23, wherein said motif is a substantially conserved subsequence.

In still another 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: 13, 17, 19, or 21, 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: 13, 17, 19, or 21, or

(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: 13, 17, 19, or 21, 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 RT1 or RT1-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 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: 13, 17, 19, or 21; 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; 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 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: 13, 17, 19, or 21; (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: 13, 17, 19,or 21; (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: 13, 17, 19, or 21; (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: 13, 17, 19, or 21; (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: 13, 17, 19, or 21, 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 RT1 or RT1-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: 13, 17, 19, or 21, 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 RT1 or RT1-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: 13, 17, 19, or 21; 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 RT1 or RT1-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: 13, 17, 19, or 21, 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 RT1 or RT1-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;

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.

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: 13, 17, 19, or 21, 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 RT1 or RT1-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 depicts the *RT1* gene on bac clone b0541.c13 (SEQ ID NO:9)

Fig.2 demonstrates induction of *RT1* transcripts after addition of Ethephon.

Figs.3A-3B show the multiple alignment of the full length amino acid sequences of RT1 protein from B73 (SEQ ID NO:13), the rice RT1 homolog (NCBI General identifier No. SEQ ID NO:17), the Arabidopsis RT1 homolog (NCBI General identifier No. SEQ ID NO:19) and the maize RT1 homolog from clone cfp7n.pk6.i3 (SEQ ID NO:21). 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. Two highly conserved motifs among all four sequences are underlined in the alignment. 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.4 shows a chart of the percent sequence identity for each pair of amino acid sequences displayed in Figs.3A-3B.

Fig.5 depicts the vector pDONOR^{TM}/Zeo.

Fig.6 depicts the vector pDONOR™221.

Fig.7depicts the vector PHP27840.

Fig.8 depicts the vector PHP23236.

Fig.9depicts the vector PHP10523.

Fig.10 depicts the vector PHP28408.

Fig.11 depicts the vector PHP20234.

Fig.12 depicts the vector PHP28529.

Fig.13 depicts the vector PHP22020.

Fig.14 depicts the vector PHP23112.

Fig.15 depicts the vector PHP23235.

Fig.16 depicts the vector PHP29635.

Fig.17 depicts the vector pIIOXS2a-FRT87(ni)m.

Fig.18 is the growth medium used for semi-hydroponics maize growth in Example 19.

Fig.19 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.20 a-c show a comparison of *rt1* and wild type plants grown in the field, greenhouse or hydroponic conditions.

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 marker MZA8757-F81 used in Example 2.

SEQ ID NO:2 is the reverse primer for marker MZA8757-R593 used in Example 2.

SEQ ID NO:3 is the forward primer for marker MZA15417-F132 used in Example 2.

SEQ ID NO:4 is the reverse primer for marker MZA15417-R607 used in Example 1.

SEQ ID NO:5 is the forward primer for CAP marker b0541 used in Example 2.

SEQ ID NO:6 is the reverse primer for CAP marker b0541used in Example 2.

SEQ ID NO:7 is the forward primer for CAP marker b0461 used in Example 2

SEQ ID NO:8 is the reverse primer for CAP marker b0461 used in Example 2.

SEQ ID NO:9 is the sequence of the candidate gene derived from BAC b0541.c13 described in Example 3.

SEQ ID NO:10 is the forward primer RT1 3006F used in Example 3.

SEQ ID NO:11 is the reverse primer RT1 17631R used in Example 3.

SEQ ID NO:12 is the B73 cDNA of RT1 described in Example 3.

SEQ ID NO:13 is the RT1 amino acid sequence encoded by nucleotides 50 through 1382 (Stop) of SEQ ID NO:12.

SEQ ID NO:14 is the forward primer 4405F used in Example 5.

SEQ ID NO:15 is the reverse primer etr4Rnew used in Example 5.

SEQ ID NO:16 is the nucleotide sequence encoding the closest polypeptide RT1 homolog from rice.

SEQ ID NO:17 corresponds to the RT1 amino acid sequence homolog encoded by nucleotides 91 through 1263 (Stop) of SEQ ID NO:16 and is set forth in NCBI General identifier No.115434026.

SEQ ID NO:18 is the nucleotide sequences encoding the closest polypeptide RT1 homolog from Arabidopsis.

SEQ ID NO:19 corresponds to the RT1 amino acid sequence homolog encoded by nucleotides 132 through 1493 (Stop) of SEQ ID NO:18 and is set forth in NCBI General identifier No.15217667.

SEQ ID NO:20 is an EST corresponding to a maize homolog of the maize RT1 sequence.

SEQ ID NO:21 is the amino acid sequence encoded by SEQ ID NO:20.

SEQ ID NO:22 corresponds to Motif I in the alignment shown in Figs.3A-3B.

SEQ ID NO:23 correpsonds to Motif II in the alignment shown in Figs.3A-3B.

SEQ ID NO:24 is the attB1 sequence described in Example 9.

SEQ ID NO:25 is the attB2 sequence described in Example 9.

SEQ ID NO:26 is the sequence of the forward primer VC062 described in Example 9.

SEQ ID NO:27 is the sequence of the reverse primer VC063 described in Example 9.

SEQ ID NO:28 is the sequence of vector pDONOR™/Zeo described in Example 9.

SEQ ID NO:29 is the sequence of vector pDONOR™/221 described in Example 9.

SEQ ID NO:30 is the sequence of PHP27840 described in Example 9.

SEQ ID NO:31 is the sequence of PHP23236 described in Example 9.

SEQ ID NO:32 is the sequence of PHP10523.

SEQ ID NO:33 is the sequence of the NAS2 promoter.

SEQ ID NO:34 is the sequence of the GOS2 promoter.

SEQ ID NO:35 is the sequence of the ubiquitin promoter.

SEQ ID NO:36 is the sequence of the PINII terminator.

SEQ ID NO:37 is the sequence of PHP28408.

SEQ ID NO:38 is the sequence of PHP20234.

SEQ ID NO:39 is the sequence of PHP28529.

SEQ ID NO:40 is the sequence of PHP22020.

SEQ ID NO:41 is the sequence of PHP23112.

SEQ ID NO:42 is the sequence of PHP23235.

SEQ ID NO:43 is the sequence of PHP29635.

SEQ ID NO:44 is the sequence of pIIOXS2a-FRT87(ni)m.

SEQ ID NO:45 is the sequence of the S2A promoter.

DETAILED DESCRIPTION

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.

The term “root architecture’’ refers to the arrangement of different plant parts that comprise the root. The terms “root architecture”, “root structure”, “root system” and “root system architecture” are used interchangeably herein.

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 taproot 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.

The term “altered root architecture” refers to changes in 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 changes in one or more measurable parameters, including and not limited to, the diameter, length, number, angle or surface of one or more of the root system parts, including and 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.

“Agronomic characteristics” is a measurable parameter including and 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, 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, root penetration, plant height, ear length, and harvest index.

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

“rt1” (rootless 1) *refers to the nucleotide sequence of the Zea Mays* mutant.

“rt1” refers to the polypeptide of the *Zea Mays* mutant.

"*RT1*" refers to the *Zea Mays RT1* wild type gene and cDNA and includes without limitation SEQ ID NO:9 and SEQ ID NO:12, respectively. "RT1 " refers to the *Zea Mays* RT1 wild type protein encoded by the exons of SEQ ID NO:9 and by the cDNA of SEQ ID NO:12.

"*RT1*-like" refers to the nucleotide homologs of the maize *RT1* sequence and corresponds to a rice, Arabidopsis, and additional maize sequence including without limitation the nucleotide sequences of SEQ ID NO:16, 18, and 20, respectively.

"RT1-like" refers to the polypeptide homologs of the maize RT1 protein and include without limitation the amino acid sequences of SEQ ID NO:17, 19 and 21, corresponding to an additional rice, Arabidopsis and additional maize homolog, respectively.

"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), the soil type, or the presence of insects or disease.

"Varying environmental conditions" refer to changes in the environmental conditions under which the plant is grown, including and not limited to water availability, nutrient availability (for example nitrogen or phosphate), soil type, or 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 (i.e. disposition of a hybrid to be affected by pests that result in root lodging), environmental stress (drought, flooding), insect and disease injury. Root lodging can be attributed to corn rootworm injury in some cases.

"Root penetration" refers to the rate and depth of penetration of the plant root into the soil.

"Soil type" refers in terms of soil texture to the different sizes of particles, including and not limited to mineral particles, in a particular sample. The term "soil type" also refers to the compactness of the soil under changing physical conditions including and not limited to water content and tilling. In general, soil is made up in part of finely ground rock particles, grouped according to size as sand, silt, and clay. Each size plays a significantly different role. For example, the largest particles, sand, determine aeration and drainage characteristics, while the tiniest, sub-

microscopic clay particles, are chemically active, binding with water and plant nutrients. The ratio of these sizes determines soil type: clay, loam, clay-loam, silt-loam, and so on.

"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". and "nucleic acid fragment" are used interchangeably to refer to 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 and 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.

“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 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 the introduction of a heterologous nucleic acid and a cell derived from a cell so modified. It 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.

"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 than that normally found in nature.

The terms "regulatory sequence(s)" and "regulatory element(s)" are used interchangeably herein.

"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 include and are not limited to, promoters, translation leader sequences, introns, polyadenylation recognition sequences and the like.

"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 and 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 and 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.

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%, 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: 13 or 21 and wherein 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.

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%, 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: 13 or 21 and wherein expression of said polypeptide in a plant results in an altered plant root 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%, 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: 13 or 21 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 RT1 or RT1-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%, 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: 13, 17, 19, or 21 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%, 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:12, 16, 18, or 20 or (ii) a full complement of the nucleic acid sequence of (i).

Figs.3A-3B shows the multiple alignment of the full length amino acid sequences of B73 RT1 (SEQ ID NO:13), rice RT1 homolog (SEQ ID NO:17), Arabiopsis RT1 homolog (SEQ ID NO:19), and the maize RT1 homolog from clone cfp7n.pk6.i3 (SEQ ID NO:21). 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. Two highly conserved sequence motifs are shown underlined in the alignment. 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.4 shows a chart of the percent sequence identity for each pair of amino acid sequences displayed in Figs.3A-3B.

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 RT1 or RT1-like protein. Preferably, the RT1 or RT1-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%, 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: 13 or 21 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%, 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 RT1 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%, 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:12 or 20 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%, 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% 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 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 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 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.

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 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 (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., 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 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 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) 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 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 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 trysin 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 Ciml which is specific to the nucleus of developing maize kernels. Ciml 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 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 and not limited to, translation leader sequences, introns, and polyadenylation recognition 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 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 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 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.

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)).

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, millet, 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). A 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 exihibit 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: 13, 17, 19, or 21, 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 RT1 or RT1-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 RT1 or RT1-like protein is from *Arabidopsis thaliana*, *Zea mays*, *Glycine max*, *Glycine tabacina*, *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%, 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 RT1 or RT1-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 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%, 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: 13, 17, 19, or 21, 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 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.

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.

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, 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, root penetration and harvest index.

Greenness, harvest index, yield, biomass, resistance to root lodging, improved root penetration are particularly preferred agronomic characteristic for alteration. Further, these agronomic characteristics preferably are increased relative to the control.

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 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 alterations in root architecture include but are not limited to alterations in vigor, growth, size, yield, biomass, improved root penetration 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 plant. Improved root penetration is an additional measure to determine alterations in root architecture.

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%, 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: 13, 17, 19, or 21,

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%, 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: 13, 17, 19, or 21 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 RT1 or RT1-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 on 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: 13, 17, 19, or 21 (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%, 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: 13, 17, 19, or 21, 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.

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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%, 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: 13, 17, 19, or 21 (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.

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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 RT1 or RT1-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 on 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: 13, 17, 19, or 21 (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; 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 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, 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: 13, 16, 17, or 19, 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; 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 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 RT1 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%, 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: 13, 16, 17, or 19 (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: 13, 17, 19, or 21 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 RT1 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 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 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 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) 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 or both.

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

Analysis of the *rt1* phenotype, root lodging and root penetration

The phenotype of *rt1* homozygote plants is variable and depending on the growing conditions. As shown in Fig 20 a-c, *rt1* plants grown in the field have a very strong depletion of the root system and show a remarkably root lodging phenotype, while the same plants grown in greenhouse or hydroponic conditions show only a moderate alteration in root architecture. When *rt1* plants are grown in the greenhouse using hard soil collected from the field, they still show a reduction in the root system (Fig 20c), indicating that the *rt1* locus is necessary for the penetration of the crown roots in the soil and the interaction of the roots with the soil. *rt1* mutants are unable to push through hard soils.

Resistance to root lodging can be measured following mechanical perturbances under a variety of field conditions, such as vertical root pulling resistance (Beck et al. (1987); Crop Sci. 27:356-358), mechanized push (Kato et al. (1999) Maydica 44, 167-174) or a portable electronic design described by Fouere (Fouere et al. (1995), Agronomy J. 87: 1010-1024.

The design consists of a portable electronic device that measures horizontal pushing resistance on individual plants. The apparatus simultaneously records the angle of inclination and the resistance torque of the plant during an artificial pushing test. The device consists of a support, a force sensor, an angle sensor, and a control head. Data logging is possible by using a microprocessor-based system. Data may be transferred to a computer using an RS232 serial transfer protocol. The time required for the test in field conditions is approximately 1 min per plant. Preliminary results obtained on 14 maize genotypes grown in three field environments showed that genotypes susceptible to root lodging were characterized by low average values of their maximum resistance torque.

Root penetration into the soil can be measured, for example, by comparing seedling emergence and/or plant growth of *RT1* transgenic and wild type plants on various soil types, e.g. tilled versus untilled soil. Untilled soils have higher resistance to root penetration and therefore reduce seedling emergence and subsequent plant growth. Soil types can be measured for example by using a cone penetrometer, measuring the tip resistance, sleeve friction and/or pore water pressure. The measurements can be made at different stages during plant growth

and the difference in the rate of root penetration into the soil between a *RT1* transgenic line and a nontransgenic (wild type) line can be recorded.

EXAMPLE 2

Map-based Cloning of *RT1*

The genetic data derived from the MaizeGDB (Maize Genetics and Genomics Database) showed that the *rt1* mutant maps on chromosome 3 bin 04 of corn. Based on this information we retrieved from the public database several SSR primers and used them to genotype 88 *rt1* plants derived from an F2 cross between the original *rt1* line (unknown background, Jenkins M T, 1930) and the inbred line B73. Homozygous *rt1/rt1* plants were scored as lodged plants when grown in the field for 30 days or more. DNA was extracted from those plants using standard molecular biology procedures. The public PCR-based DNA SSR marker UMC1908 (MaizeGDB) was found at 1.7cM from the *rt1* locus (3 recombinations on 88 individuals).

In order to fine map the *rt1* mutation, two mapping populations and their corresponding corn seeds, segregating for the *rt1* gene, were utilized. The first mapping populations consisted of 1500 BC2S1 plants derived by selfing a cross between a *rt1* plant, derived from the above mentioned F2 population segregating for the *rt1* locus, and the inbred line B73 (segregation ration 3:1). The second mapping populations consisted of 520 plants derived by backcrossing the above mentioned cross with the *rt1* plant, parent of the cross (segregation ration 1:1).

To obtain plants that carry recombination near the *rt1* locus, two sequence-based DNA markers, from the DuPont proprietary sequences of known map position were used.

Primers MZA8757-F81 (SEQ ID NO:1) and MZA8757-R593 (SEQ ID NO:2) were used to amplify and sequence the Mza8757 marker locus carrying a SNP A/C between the *rt1* parent and the B73 parent.

Primers MZA15417-F132 (SEQ ID NO:3) and MZA15417-R607 (SEQ ID NO:4) were used to amplify and sequence the Mza 5417 marker locus carrying a SNP A/G between the *rt1* parent and the B73 parent. Both markers reside on a physical BAC contig named 306 (Dupont Genomix database).

Only 62 plants showing a crossing over event between the two flanking markers (homozygotes at one marker and heterozygotes at the other marker) were

kept and selfed. The progeny of those plants was subsequently screened for the phenotype in order to confirm the position of the *rt1* gene relatively to the flanking markers.

New CAPS (Cleaved Amplified Polymorphic Site) markers were designed using available physically placed MZA sequences and BAC-end sequences of the BACs constituting the region of contig 306 surrounded by markers Mza 15417 (left side) and Mza 8757 (right side).

CAPS primers were used in a PCR reaction containing 25ng of DNA. CAPS marker amplifications were performed in a 25 ul PCR reaction using the Qiagen HotStart mix and 25 ng DNA. The thermal cycle conditions were: 95°C 15min (1 cycle), 94°C 45 sec, 56°C 45 sec, 72°C 45 sec, (35 cycles) 72°C 7 min.

3 ul of the amplification product were used for a restriction digest (total volume of 15 ul) with the appropriate restriction enzyme. Restriction reaction was carried out at the appropriate temperature for one hour. Restricted amplification products were examined on 3% agarose gels.

CAPS marker b0541 (b0541 forward primer, SEQ ID NO:5 and b0541 reverse primer, SEQ ID NO:6) was designed based on the BAC-end sequence of clone BAC b0541.c13. This primer set amplifies a region of 250 bp, showing polymorphism between B73 and *rt1* following restriction with the 6-cutter enzyme EcoRI.

CAPS marker b0461 (b0461 forward primer, SEQ ID NO:7 and b0461 reverse primer, SEQ ID NO:8): was designed based on the BAC-end sequence of clone BAC b0461.g10. This primer set amplifies a region of about 350 bp, showing polymorphism between B73 and *rt1* following restriction with the 6-cutter enzyme NcoI.

By screening the 62 previously obtained recombinants with CAPS b0541, only 3 recombination breakpoints were found, while 2 recombinants were found on the other side, using the CAPS b0461.

BAC b0541.c13 and BAC b0461.g10 and are public BAC clones for which the available fingerprinting data show overlap.

EXAMPLE 3

Identification of the *RT1* Gene

In order to identify the *RT1* gene that was mapped to the region comprising the two overlapping BAC clones, BAC b0541.c13 was sequenced. 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 estimated 165 Kb of sequence of BAC b0541.c13 was searched for the presence of open reading frames, and 4 regions, showing similarities to genes based on prediction models performed by FGENESH (Softberry, Inc. Mount Kisco, NY, USA) were identified.

In particular, one candidate gene showing homology to a "putative Ethylene Responsive Protein" was considered for further evaluation. The sequence of the gene derived from BAC b0541.c13 (B73 genotype) is shown SEQ ID NO:9 (*RT1* ethylene responsive gene 7800bp) and in Fig.1 Total RNA was extracted from developing maize roots from the B73 line and the *rt1* line using a TRIazol® Reagent obtained from Life Technologies Inc., Rockville, MD, 20849 (GIBCO-BRL) that contains phenol and guanidine thiocyanate. RT-PCR was performed with cDNA that was synthesized with Superscript III (Invitrogen, Carlsbad, CA) reverse transcriptase from 1 µg DNase treated total RNA. PCR was performed in a Perkin Elmer 9700 thermocycler using the GC-2 Advantage kit (BD Biosciences) and a PCR program of 94 °C for 3 min, followed by 27 cycles of 94°C for 30 sec, 58°C for 30 sec, 68°C for 1 min, and a final step of 68°C for 3 min. Primers designed based on the genomic sequence described in Fig.1, SEQ ID NO:9 and specific to the 5' and 3' end of *RT1* (RT1 3006F (SEQ ID NO:10) andRT17631R (SEQ ID NO:11), respectively, were used in the PCR reaction. Only the B73 wild type line generated a PCR product, indicating that the *rt1* line is missing the *RT1* mRNA, confirming that the lack of the *RT1* transcript is responsible for the *rt1* phenotype. The PCR product was cloned into the pPCR®II-Topo® nt vector (Invitrogen™) and sequenced to confirm identity. The B73 cDNA of *RT1* is shown in SEQ ID NO:12. The RT1 amino acid sequence encoded by nucleotides 50 through 1382 (Stop codon) of SEQ ID NO:12 is shown in SEQ ID NO:13.

EXAMPLE 4

Cloning the *RT1* cDNA

Total RNA can be extracted from developing maize using a TRIazol® Reagent obtained from Life Technologies Inc., Rockville, MD, 20849 (GIBCO-BRL) that contains phenol and guanidine thiocyanate. Poly A mRNA can be purified from total RNA with mRNA Purification kits obtained from Amersham Pharmacia Biotech

Inc., Piscataway, NJ, 08855, which consists of oligo (dT)-cellulose spin columns. To make the cDNA library, 5.5 ug of polyA RNA can be used for cDNA synthesis kits, which can be obtained from Stratagene, La Jolla, CA, 92037. Superscript® reverse transcriptase can be obtained from Life Technologies Inc., Rockville, MD, 20849 (GIBCO-BRL). BRL cDNA Size Fraction Columns (GIBCO-BRL) can be used to fractionate the cDNA by size, fractions can be precipitated, resuspended and ligated with 1 ug of the Uni-ZAP XR vector. After ligation it can be packaged in Gigapack III Gold® packaging extract obtained from Stratagene, La Jolla, CA, 92037. The unamplified library titer can be estimated. An appropriate amount can be used for amplification purposes to produce amplified cDNA.

Screening for the *RT1* cDNA follows standard protocols well known to those skilled in the art (Ausubel et al. 1993, "Current Protocols in Molecular Biology" John Wiley & Sons, USA, or Sambrook et al. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press). Briefly, 1.5 X 10^6 phage clones can be plated, then transferred to nylon membranes, which then will be subjected to hybridization with radioactively labeled *RT1* probe. Positives are isolated and examined for their identity as RT1 cDNAs through PCR with RT1-specific primers. The longest cDNA clones that give positive results from the PCR reaction are isolated and sequenced.

EXAMPLE 5

Ethylene induction of the *RT1* gene

Promotor analysis of the *RT1* gene via the PLACE database (Higo et al. (1999) *Nucleic Acids Res.* 27, 297-300) revealed two ERELEE4 ethylene response element (TTTGAATTT and TTTGAAAT) motives. In order to determine if the *RT1* gene is induced by Ethylene treatment, B73 seedlings were germinated in paper rolls for 11 d in a phytochamber at a 60% humidity, at 28°C, under a 16 h light, 8 h dark regime, then transferred to a $1.5x10^{-4}$ M Ethephon solution (Sigma Aldrich). Control plants were grown in distilled water. RNA was isolated from primary roots after 0, 1, 2.5 and 4 hours of Ethephon exposure and from control plants at the same time points. PCR reactions were performed with *RT1* specific oligonucleotide primers (4405F, SEQ ID NO:14 and etr4Rnew, SEQ ID NO:15). Histone 2A (Genebank AAB04687) was used as control. Fig.2 shows the induction of the *RT1* gene at 2.5 hrs of treatment.

EXAMPLE 6

Genetic Confirmation of the *RT1* gene

The genetic confirmation that the *RT1* isolated nucleic acid fragment encodes the polypeptide responsible for altering root structure can be accomplished by transforming *rt1* mutants with the isolated *RT1* cloned sequence.

RT1 homologs from other crop species can also be tested in this system by obtaining full-gene sequences, ligation to an appropriate promoter, such as the *RT1* promoter and complementing the maize *rt1* mutant.

In order to confirm possible tissue-specific expression of the *RT1* gene, the presence of the *RT1* transcript in various tissues can be analyzed by RNA blot analysis and in situ hybridization.

One method for transforming DNA into cells of higher plants that is available to those skilled in the art is high-velocity ballistic bombardment using metal particles coated with the nucleic acid constructs of interest (see Klein et al. Nature (1987) (London) 327:70-73, and see U.S. Patent No. 4,945,050). A Biolistic PDS-1000/He (BioRAD Laboratories, Hercules, CA) can be used for these complementation experiments. The particle bombardment technique can be used to transform the *RT1* mutant with the cloned *RT1* wild type sequence [nucleotides 50 through 1382 (Stop) of SEQ ID NO:12], encoding a functional RT1 protein.

The bacterial hygromycin B phosphotransferase (Hpt II) gene from *Streptomyces hygroscopicus* that confers resistance to the antibiotic hygromycin can be used as the selectable marker for the maize transformation. In the vector, pML18, the Hpt II gene can be engineered with the 35S promoter from Cauliflower Mosaic Virus and the termination and polyadenylation signals from the octopine synthase gene of *Agrobacterium tumefaciens*. pML18 was described in WO 97/47731, which was published on December 18, 1997, the disclosure of which is hereby incorporated by reference.

Embryogenic maize callus cultures derived serve as source material for transformation experiments. This material can be generated by germinating sterile maize seeds on a callus initiation media (MS salts, Nitsch and Nitsch vitamins, 1.0 mg/l 2,4-D and 10 μM $AgNO_3$) in the dark at 27-28°C. Embryogenic callus proliferating from the scutellum of the embryos is then transferred to CM media (N6 salts, Nitsch and Nitsch vitamins, 1 mg/l 2,4-D, Chu et al., 1985, *Sci. Sinica* 18:

659-668). Callus cultures are maintained on CM by routine sub-culture at two week intervals and used for transformation within 10 weeks of initiation.

Callus can be prepared for transformation by subculturing 0.5-1.0 mm pieces approximately 1 mm apart, arranged in a circular area of about 4 cm in diameter, in the center of a circle of Whatman #541 paper placed on CM media. The plates with callus are incubated in the dark at 27-28°C for 3-5 days. Prior to bombardment, the filters with callus are transferred to CM supplemented with 0.25 M mannitol and 0.25 M sorbitol for 3 hr in the dark. The petri dish lids are then left ajar for 20-45 minutes in a sterile hood to allow moisture on tissue to dissipate.

Each genomic DNA fragment is co-precipitated with pML18 containing the selectable marker for maize transformation onto the surface of gold particles. To accomplish this, a total of 10 μg of DNA at a 2:1 ratio of trait:selectable marker DNAs are added to 50 μl aliquot of gold particles that are resuspended at a concentration of 60 mg ml^{-1}. Calcium chloride (50 μl of a 2.5 M solution) and spermidine (20 μl of a 0.1 M solution) are then added to the gold-DNA suspension as the tube was vortexed for 3 min. The gold particles are centrifuged in a microfuge for 1 sec and the supernatant removed. The gold particles are then washed twice with 1 ml of absolute ethanol and then resuspended in 50 ml of absolute ethanol and sonicated (bath sonicator) for one second to disperse the gold particles. The gold suspension is incubated at -70°C for five minutes and sonicated (bath sonicator) if needed to disperse the particles. Six μl of the DNA-coated gold particles are then loaded onto mylar macrocarrier disks and the ethanol is allowed to evaporate.

At the end of the drying period, a petri dish containing the tissue is placed in the chamber of the PDS-1000/He. The air in the chamber is then evacuated to a vacuum of 28-29 inches Hg. The macrocarrier is accelerated with a helium shock wave using a rupture membrane that bursts when the He pressure in the shock tube reaches 1080-1100 psi. The tissue is placed approximately 8 cm from the stopping screen and the callus was bombarded two times. Two to four plates of tissue are bombarded in this way with the DNA-coated gold particles. Following bombardment, the callus tissue is transferred to CM media without supplemental sorbitol or mannitol.

Within 3-5 days after bombardment the callus tissue is transferred to SM media (CM medium containing 50 mg/l hygromycin). To accomplish this, callus tissue is transferred from plates to sterile 50 ml conical tubes and weighed. Molten top-agar at 40° C is added using 2.5 ml of top agar/100 mg of callus. Callus clumps are broken into fragments of less than 2 mm diameter by repeated dispensing through a 10 ml pipette. Three ml aliquots of the callus suspension are plated onto fresh SM media and the plates are incubated in the dark for 4 weeks at 27-28°C. After 4 weeks, transgenic callus events are identified, transferred to fresh SM plates and grown for an additional 2 weeks in the dark at 27-28°C.

Growing callus can then be transferred to RM1 media (MS salts, Nitsch and Nitsch vitamins, 2% sucrose, 3% sorbitol, 0.4% gelrite +50 ppm hyg B) for 2 weeks in the dark at 25°C. After 2 weeks the callus can be transferred to RM2 media (MS salts, Nitsch and Nitsch vitamins, 3% sucrose, 0.4% gelrite + 50 ppm hyg B) and placed under cool white light (~40 $\mu Em^{-2}s^{-1}$) with a 12 hr photoperiod at 25°C and 30-40% humidity. After 2-4 weeks in the light, callus can begin to organize, and form shoots. Shoots can be removed from surrounding callus/media and gently transferred to RM3 media (1/2 x MS salts, Nitsch and Nitsch vitamins, 1% sucrose + 50 ppm hygromycin B) in phytatrays (Sigma Chemical Co., St. Louis, MO) and incubation can be continued using the same conditions as described in the previous step.

Plants can then be transferred from RM3 to 4” pots containing Metro mix 350 after 2-3 weeks, when sufficient root and shoot growth has occurred. The seed obtained from the transgenic plants can be examined for genetic complementation of the *RT1* mutation with the wild-type genomic DNA containing the *RT1* gene.

EXAMPLE 7

Characterization of cDNA Clones Encoding RT1 homologs

The BLASTX search using the EST sequences from clones listed in Table 1 revealed similarity of the polypeptides encoded by the ORF to proteins from rice and Arabidopsis. The nucleotide sequence encoding the closest polypeptide RT1 homolog from rice is shown in SEQ ID NO:16 and the corresponding amino acid sequence is set forth in NCBI General Identifier No: 115434026, SEQ ID NO:17). The nucleotide sequence encoding the closest polypeptide RT1 homolog from Arabidopsis is shown in SEQ ID NO:18 and the corresponding amino acid sequence

is set forth in NCBI General Identifier No: 15217667, SEQ ID NO:19). The proteins from rice and Arabidopsis can be localized to Os01g01600 (TIGR) and At1g27660 (TAIR), respectively.

Shown in Table 1 and 2 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 P("CGS"). Also shown are the percent identities for sequences encoding RT1 and homologs thereof.

TABLE 1

BLAST Results (Literature) and Percent Identity for Sequences Encoding RT1 and homologs thereof.

Sequence	B73-*RT1* (SEQ ID NO:12)	B73-RT1 (SEQ ID NO:13)
Status	cgs	protein
BLAST pLOG Score to SEQ ID NO:16)	49	N/A[1]
BLAST pLOG Score to NCBI GI No:15217667 (SEQ ID NO:18)	37	N/A
% identity to NCBI GI No: 115434026 (SEQ ID NO:17)	N/A	39.7
% identity to NCBI GI No: 15217667 (SEQ ID NO:19)	N/A	26.0

[1]N/A = non – applicable.

The BLASTX search using the Maize *RT1* sequence (SEQ ID NO:12) revealed similarity to polypeptides homologous to RT1 from *Oryza sativa* (GI No. 115434026, SEQ ID NO:17) and to *Arabidopsis thaliana* (GI No. 15217667 SEQ ID NO:19) Shown in Table 1 and 2 are 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 EST, FIS or PCR sequences ("Contig"), or sequences encoding an entire or functional protein derived from an FIS or a contig ("CGS"):

TABLE 3

BLAST Results (patent) for Sequences Encoding Polypeptides Homologous to RT1.

Sequence	Status	Reference	Blast pLog Score	% identity
B73-*RT1* (SEQ ID NO: 13)	CGS	SEQ ID NO: 564 in WO2004031349-a2	38	29.3

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 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.

An additional maize RT1 homolog was found in the DuPont proprietary database. Maize clone cfp7n.pk6.i3 (SEQ ID NO:20 encodes an RT1-like protein (SEQ ID NO:21) that has 47.2% identity to the B73 RT1 protein (SEQ ID NO:13) based on the Clustal method of alignment. An alignment of the maize RT1 protein and the rice, Arabidospsis and the maize homolog from clone cfp7n.pk6.i amino acid sequences (SEQ ID NO: 13; 17, 19, and 21) is shown jn Figs. 3A-3B. Two sequence motifs (Motif I, SEQ ID NO:22 and Motif II, SEQ ID NO:23 in the alignment) are highly conserved in all four sequences and are shown underlined in the alignment.

EXAMPLE 8

Knockout analysis of the Arabidopsis *RT1*-like gene

In order to define the function of the *RT1* gene in Arabidopsis, several Knockout lines, containing a T-DNA insertion in the At1g27660 locus (see Example

7) can be retrieved from the Salk Institute Genome Analysis Laboratory (SIGnAL) database. In particular, seeds from the two lines Salk_102156 and Salk_001968, segregating for the presence of the T-DNA insertion within the second intron of the gene, can be retrieved and planted. Seedlings can be genotyped using primers flanking the T-DNA insertions following the instructions provided by the database and roots of plants containing the T-DNA insertion in a homozygote state can be phenotyped using the software WinRHIZO® (Regent Instruments Inc). WinRHIZO® is an image analysis system specifically designed for root measurement which uses the contrast in pixels to distinguish the light root from the darker background (see also Example 21).

EXAMPLE 9

Preparation of a Plant Expression Vector Containing the *RT1* gene or homologs thereof

Sequences homologous to the *RT*1 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 *RT1* gene (SEQ ID NO:12), or *RT1*-like genes, such as the one disclosed in SEQ ID NO:20, 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 *RT1* (extending from nts 50-1382 of SEQ ID NO:12) or a *RT1* homolog (for example the sequence extending from nts 83-1540 of SEQ ID NO:20), gene-specific primers can be designed. RT-PCR can be used with plant RNA to obtain a nucleic acid fragment containing the RT1 protein-coding region flanked by attB1 (SEQ ID NO:24) and attB2 (SEQ ID NO:25) 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 RT1 (SEQ ID NO:12 or a polypeptide homolog (such as the RT1 homolog encoded by SEQ ID NO:20), 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:26) and the reverse primer VC063 (SEQ ID NO:27) 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.5; SEQ ID NO:28) or pDONR™221 (Invitrogen™, Fig. 6; SEQ ID NO:29) 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 *RT1* or *RT1-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.7; SEQ ID NO:30) or PHP23236 (Fig. 8; SEQ ID NO:31), 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 *RT1* or homologs thereof

Soybean plants can be transformed to over-express the *RT1* and homologs thereof, such as for example the *RT1-like* gene shown in SEQ ID NO:20 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 (Fig. 7, SEQ ID NO:30) 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 ºC 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 $CaCl_2$ (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 *RT1* 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 *RT1* Gene and Homologs thereof Using Particle Bombardment

Maize plants can be transformed to overexpress *RT1* and *RT1-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 µL 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 µL of ethanol. An aliquot (5 µL) of the DNA-coated gold particles can be placed in the center of a Kapton™ 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 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 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 bialophos. After six weeks, areas 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

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 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 *RT1* gene functions in maize to alter root architecture.

Furthermore, a recombinant DNA construct containing the *RT1* 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, improved root penetration and/or resistance to root lodging under various environmental and soil conditions (e.g. variations in nutrient and water availability).

Subsequent yield analysis can also be done to determine whether plants that contain the *RT1* gene have an improvement in yield performance, when compared to the control (or reference) plants that do not contain the *RT1* gene. Plants containing the *RT1* 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.9, SEQ ID NO:32), are thawn 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 H_2O) is mixed with the thawn *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 SOCmedium) 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 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.

<u>Identification of transformants:</u>

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 #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 ddH_2O as per above.

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

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 SalI (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 SalI 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.

EXAMPLE 13

Agrobacterium mediated Transformation into Maize

Maize plants can be transformed to overexpress *RT1 and RT1-like genes* 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 innoculation, 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 20ºC, 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 ºC. 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

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 ºC, 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 ºC in the light (about 80 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

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.
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.
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).

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 *RT1* or the *RT1L* gene have an improvement of at least one agronomic characteristic, when compared to the control (or reference) plants that do not contain *RT1* or the *RT1L* gene. The alterations may also be studied under various environmental conditions.

EXAMPLE 14

Construction of Maize expression vectors with the *RT1* and *RT1-like* Genes using *Agrobacterium* mediated Transformation

Maize expression vectors can be prepared with the *RT1* (SEQ ID NO:12) and *RT1-like* genes *(*SEQ ID NO:20) under the control of the NAS2 (SEQ ID NO:33), GOS 2 (SEQ ID NO:34) or Ubiquitin (UBI1ZM; SEQ ID NO:35) promoter. PINII is the terminator (SEQ ID NO:36)

Using Invitrogen's ™ Gateway® technology the entry clone, created as described in Example 9, containing the maize *RT1* gene or maize *RT-ike* gene can be used in separate Gateway® LR reactions with:

1) the constitutive maize GOS2 promoter entry clone PHP28408 (Fig.10, SEQ ID NO:37) and the PinII Terminator entry clone PHP20234 (Fig.11, SEQ ID NO:38), into the destination vector PHP28529 (Fig.12 , SEQ ID NO:39).

2) the root maize NAS2 promoter entry clone PHP22020 (Fig.13,SEQ ID NO:40) and the PinII Terminator entry clone PHP20234 (Fig.11, SEQ ID NO:38) into the destination vector PHP28529 (Fig.12, SEQ ID NO:39).

3) the constitutive maize UBI1ZM promoter entry clone PHP23112 (Fig.14,SEQ ID NO:41) and the PinII Terminator entry clone PHP20234 (Fig.11, SEQ ID NO:38) into the destination vector PHP28529 (Fig.12, SEQ ID NO:39).

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

1) RD29A promoter::yellow fluorescent protein::PinII terminator cassette for Arabidospis 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 *RT1* and *RT1-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 *RT1* and *RT1-like* genes using *Agrobacterium* mediated Transformation

The final vectors (Example 14) can then electroporated separately into LBA4404 *Agrobacterium* containing PHP10523 (Fig. 9; SEQ ID NO:32, 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 18.

EXAMPLE 16

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

Destination vector PHP23236 (Fig.8, SEQ ID NO:31) was obtained by transformation of *Agrobacterium* strain LBA4404 containing plasmid PHP10523 (Fig.9, SEQ ID NO:32) with plasmid PHP23235 (Fig.15, SEQ ID NO:42) 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:35).

PHP29635 (Fig.16, SEQ ID NO:43) was obtained by transformation of *Agrobacterium* strain LBA4404 containing plasmid PHP10523 with plasmid PIIOXS2a-FRT87(ni)m (Fig.17, SEQ ID NO:44) 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 10 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:45).

EXAMPLE 17

Preparation of plasmids containing *RT1* or *RT1-like* genes for transformation of Gaspe Bay Flint Derived Maize Lines

Using Invitrogen's Gateway® Recombination technology, entry clones containing the *RT1* or *RT1*-like genes can be created, as described in Example 10 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 *RT1* and *RT1-like* Genes

Maize plants can be transformed to over-express the *RT1* and *RT1*-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 and not limited to inoculation type procedures using *Agrobacterium* based vectors. 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 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 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 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. 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 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 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.

<u>Imaging Instrumentation</u>

Any suitable imaging instrumentation may be used, including and 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 or 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 (cm^2). 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. 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

Screening of Gaspe Bay Flint Derived Maize Lines Under Nitrogen Limiting Conditions

Nitrogen utilization efficacy can be tested in the field by planting maize lines on nitrogen depleted soil or in the greenhouse using the experimental conditions as described herein. 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 medium, and watered four times each day with 1 mM KNO_3 growth medium

and with 2 mM KNO_3, or higher, growth medium (see Fig.18). Control plants grown in 1 mM KNO_3 medium will be less green, produce less biomass and have a smaller ear at anthesis (see Fig.19 for an illustration of sample data).

Statistics are used to decide if differences seen between treatments are really different. Fig.19 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 equal.

Expression of a transgene will result in plants with improved plant growth in 1 mM KNO_3 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 tolerance.

EXAMPLE 20

Yield Analysis of Maize Lines with *RT1* or *RT1*-like Genes

A recombinant DNA construct containing a *RT1* or *RT1*-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 *RT1* or *RT1*-like gene have an improvement in yield performance under various environmental conditions, when compared to the control plants that do not contain the *RT1* or *RT1*-like gene. Reduction in yield can be measured for both. Plants containing the *RT1* or *RT1*-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 WinRHIZO™ 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

Screening of Gaspe Bay Flint Derived Maize Lines for Drought Tolerance

Transgenic Gaspe Bay Flint derived maize lines containing the *RT1* or *RT1*-like gene can be screened for tolerance to drought stress in the following manner.

Transgenic maize plants are subjected to well-watered conditions (control) and to drought-stressed conditions. Transgenic maize plants are screened at the T1 stage or later.

Stress is imposed starting at 10 to 14 days after sowing (DAS) or 7 days after transplanting, and is continued through to silking. Pots are watered by an

automated system fitted to timers to provide watering at 25 or 50% of field capacity during the entire period of drought-stress treatment. The intensity and duration of this stress will allow identification of the impact on vegetative growth as well as on the anthesis-silking interval.

Potting mixture: A mixture of 1/3 turface (Profile Products LLC, IL, USA), 1/3 sand and 1/3 *SB300* (Sun Gro Horticulture, WA, USA) can be used. The *SB300* can be replaced with *Fafard Fine-Germ* (Conrad Fafard, Inc., MA, USA) and the proportion of sand in the mixture can be reduced. Thus, a final potting mixture can be 3/8 (37.5%) turface, 3/8 (37.5%) *Fafard* and ¼ (25%) sand.

Field Capacity Determination: The weight of the soil mixture (w1) to be used in one S200 pot (minus the pot weight) is measured. If all components of the soil mix are not dry, the soil is dried at 100°C to constant weight before determining w1. The soil in the pot is watered to full saturation and all the gravitational water is allowed to drain out. The weight of the soil (w2) after all gravitational water has seeped out (minus the pot weight) is determined. Field capacity is the weight of the water remaining in the soil obtained as w2-w1. It can be written as a percentage of the oven-dry soil weight.

Stress Treatment: During the early part of plant growth (10 DAS to 21 DAS), the well-watered control has a daily watering of 75% field capacity and the drought-stress treatment has a daily watering of 25% field capacity, both as a single daily dose at or around 10 AM. As the plants grow bigger, by 21 DAS, it will become necessary to increase the daily watering of the well-watered control to full field capacity and the drought stress treatment to 50% field capacity.

Nutrient Solution: A modified Hoagland's solution at 1/16 dilution with tap water is used for irrigation.

TABLE 4

Preparation of 20 L of Modified Hoagland's Solution Using the Following Recipe:

Component	**Amount/20 L**
10X Micronutrient Solution	16 mL
KH_2PO_4 (MW: 136.02)	22 g
$MgSO_4$ (MW: 120.36)	77 g
KNO_3 (MW: 101.2)	129.5 g

$Ca(NO_3)_2 \cdot 4H_20$ (MW: 236.15)	151 g
NH_4NO_3 (MW: 80.04)	25.6 g
Sprint 330 (Iron chelate)	32 g

TABLE 5

Preparation of 1L of 10X Micronutrient Solution Using the Following Recipe:

Component	mg/L	Concentration
H_3BO_3	1854	30 mM
$MnCl_2 \cdot 4H_20$	1980	10 mM
$ZnSO_4 \cdot 7H_20$	2874	10 mM
$CuSO_4 \cdot 5H_20$	250	1 mM
$H_2MoO_4 \cdot H_20$	242	1 mM

Fertilizer grade KNO_3 is used.

It is useful to add half a teaspoon of *Osmocote* (NPK 15:9:12) to the pot at the time of transplanting or after emergence (The Scotts Miracle-Gro Company, OH, USA).

Border plants: Place a row of border plants on bench-edges adjacent to the glass walls of the greenhouse or adjacent to other potential causes of microenvironment variability such as a cooler fan.

Automation: Watering can be done using PVC pipes with drilled holes to supply water to systematically positioned pots using a siphoning device. Irrigation scheduling can be done using timers.

Statistical analysis: Mean values for plant size, color and chlorophyll fluorescence recorded on transgenic events under different stress treatments will be exported to Spotfire (Spotfire, Inc., MA, USA). Treatment means will be evaluated for differences using Analysis of Variance.

Replications: Eight to ten individual plants are used per treatment per event.

Observations Made: Lemnatec measurements are made three times a week throughout growth to capture plant-growth rate. Leaf color determinations are made three times a week throughout the stress period using Lemnatec. Chlorophyll fluorescence is recorded as PhiPSII (which is indicative of the operating quantum efficiency of photosystem II photochemistry) and Fv'/Fm' (which is the maximum

efficiency of photosystem II) two to four times during the experimental period, starting at 11 AM on the measurement days, using the Hansatech FMS2 instrument (LemnaTec GmbH, Wurselen, Germany). Measurements are started during the stress period at the beginning of visible drought stress symptoms, namely, leaf greying and the start of leaf rolling until the end of the experiment and measurements are recorded on the youngest most fully expanded leaf. The dates of tasseling and silking on individual plants are recorded, and the ASI is computed.

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, 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: 13, 17, 19,or 21 and wherein said plant exhibits altered root architecture when compared to a control plant not comprising said recombinant DNA construct.

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:

(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: 13, 17, 19, or 21, or

(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: 13, 17, 19, or 21, 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 an RT1 or RT1-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, soil type, 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 penetration, 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: 13, 17, 19, or 21; 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: 13, 17, 19, or 21;

(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: 13, 17, 19, or 21;

(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.

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 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: 13, 17, 19, or 21;

(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.

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 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, soil type, insect or disease.

23. The method of claim 20, wherein 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.

24. The method of claim 23, wherein said varying environmental condition is at least one selected from drought, nitrogen, soil type, 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 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: 13, 17, 19, or 21;

(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.

26. The method of claim 25, wherein step (d) 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.

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: 13, 17, 19, or 21, 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 RT1 or RT1-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, soil type, 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 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.

32. The method of claim 31, wherein said varying environmental condition is at least one selected from drought, nitrogen, soil type, 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 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: 13, 17, 19, or 21, 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 RT1 or RT1-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.

34. The method of claim 33, wherein step (d) 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, soil type, insect or disease.

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:

(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: 13, 17, 19, or 21, 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 RT1 or RT1-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.

37. The method of claim 36, 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 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:

(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: 13, 17, 19, or 21, 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 RT1 or RT1-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: 13, 17, 19, or 21, 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 RT1 or RT1-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 80%, sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO:13 or 21; 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 85%, sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO:13 or 21 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 90%, sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO:13 or 21; or

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

44. 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:13 or 21; or

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

45. The polynucleotide of claim 41, wherein the polypeptide sequence comprises SEQ ID NO:13 or 21.

46. The polynucleotide of claim 41, wherein the nucleic acid sequence comprises SEQ ID NO:12 or 20.

47. A vector comprising the polynucleotide of Claim 41.

48. A recombinant DNA construct comprising the polynucleotide of Claim 41 operably linked to at least one regulatory sequence.

49. A method for transforming a cell, comprising transforming a cell with the polynucleotide of Claim 41.

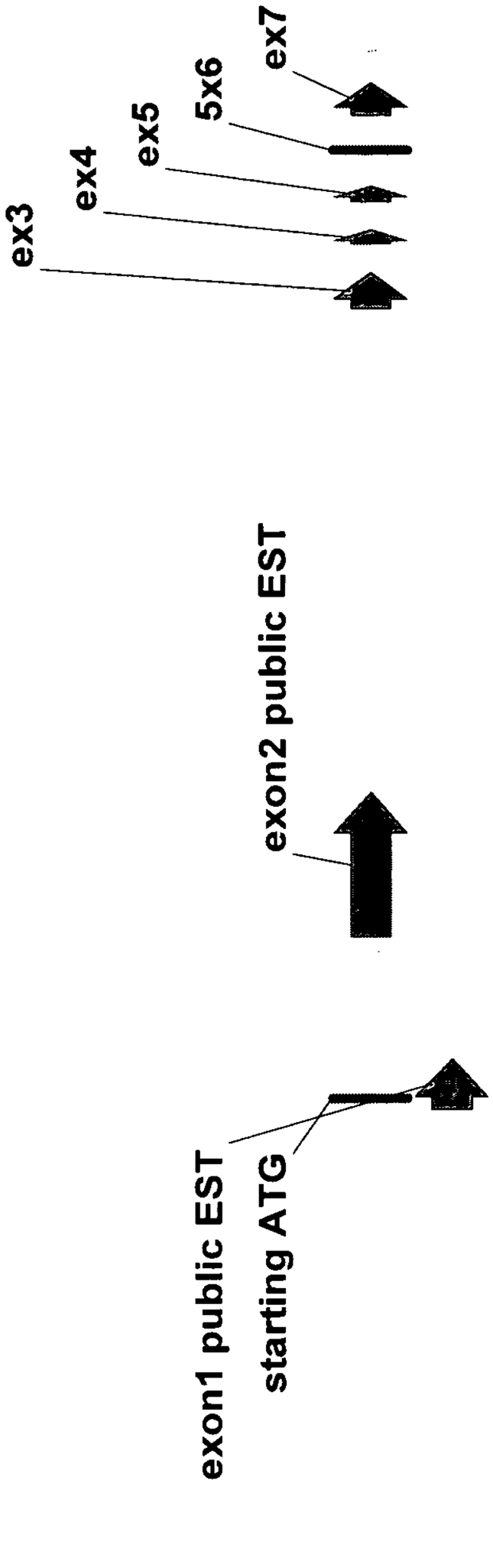
50. A cell comprising the recombinant DNA construct of Claim 48.

51. A method for producing a plant comprising transforming a plant cell with the polynucleotide of Claim 41 and regenerating a plant from the transformed plant cell.

52. The isolated polynucleotide of Claim 41, wherein the polypeptide sequence comprises at least one motif selected from the group consisting of SEQ ID NOs:22 and 23, wherein said motif is a substantially conserved subsequence.

53. An isolated polynucleotide encoding a polypeptide, wherein expression of said polypeptide results in an altered root architecture and wherein the polypeptide sequence comprises at least one motif selected from the group consisting of SEQ ID NOs:22 and 23, wherein said motif is a substantially conserved subsequence.

FIG. 1


rt1bac region around ethylen respo 7.8Kb exon annotation
7800 bp
exon1 public EST
starting ATG
exon2 public EST
ex3
ex4
ex5
5x6
ex7

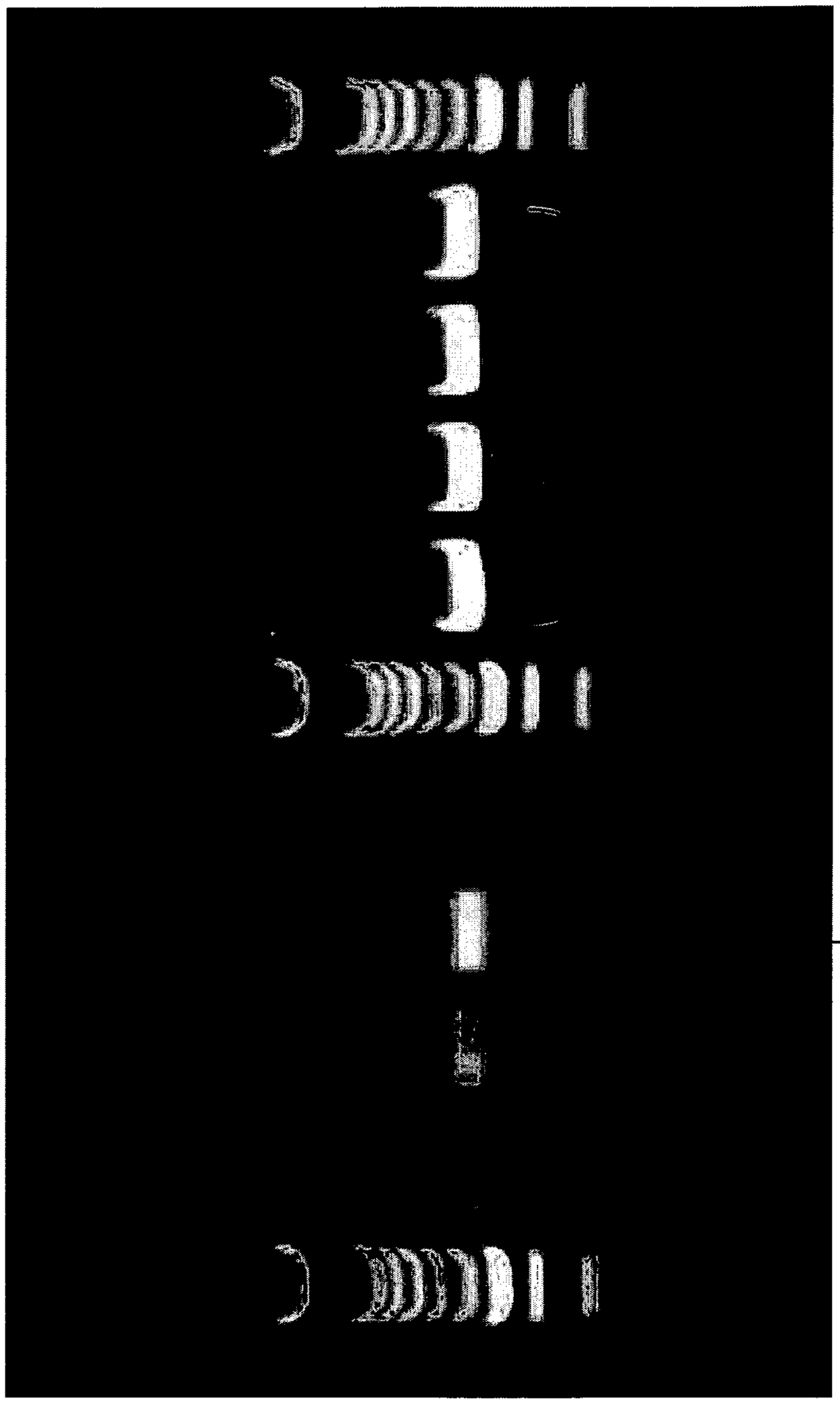

RT PCR after induction with Ethephon (1.5x10-4 M)
Induction after 2.5 Hours of treatment

FIG. 2

FIG. 3A

```
                                        **            *  ** *
SEQ ID NO:13    MMAASSSSSFNLSDHLVQDDL-PWPASSSSSSQLPFAPSHPPHGAIIGGSQQWWQQPQML
SEQ ID NO:16    MMAASSSSS-NLSDHLAQDDLLPWPSSSSSS--LPFAPAAPHHSAIIGGNSQ---QPLAL
SEQ ID NO:19    MMASSSSSSSLCDNHLLQDDLIPWPS-------MPFAPAP----NTFGLNHQWSQPPMLS
SEQ ID NO:21    MDSA--------NLHQLQDQL-QLVGSSSSSSSLDNNSDPSCYGA--SSAHQWSPGGISL
                                                                *    **
SEQ ID NO:13    NCADRHPSDELEVLLSAQGSHHH------HHHPSSSVIHPQLSS-LLMMQDLGFQWSSCG
SEQ ID NO:16    NCADRHHSDELEVLLSAQGSHHHQSHSHRHSHPASPVIPPQLSSSLLTMQDLGFQWSNCG
SEQ ID NO:19    SSTDQLSSYELESLQSVESQLAAAAPP-----TLSPHLQAHQLSTVLMMQELGFQWSSCA
SEQ ID NO:21    NSVSLSHNYNNEMLNT-----RAHNNNNNNNTSECMSLSSIHNHSLIQQQDFPLQWPHDQ

SEQ ID NO:13    S-FVDTSS----SSPMPPPLVNGQQHDGHNKIKEWDRPLTAN---NSSRSSCASTAM---
SEQ ID NO:16    GGFLDTS-------PSTPPTNGQLQHDGHDDDKIEAEGTLIT---NSSRPSCAGTATIAA
SEQ ID NO:19    A-----PA----DQHSIASSTNNNSNVMMNEEELRPRPDQSL---ISNPRSCSATTLLPP
SEQ ID NO:21    SSYQHHEGLLKIKEELSSSTISDHQEGISKFTDMLNSPVITNYLKINEHKDYTEKLLLKS

SEQ ID NO:13    ACRDVVLDGGGGGGLPAMAAVAADLDD---GTVLPASVNVPRTTTTRQRPFPAAPPPLPG
SEQ ID NO:16    SCHDVVLHGGGGGG-TVLPTINISLTT---TTTTHTQAQ------QQQRPFLAAPPPLPG
SEQ ID NO:19    P--HLHLDGA------VLPSINVS----------------------RLQKLAAGDEPL--
SEQ ID NO:21    MSSGFPINGDYGSSLPSSSSSSSPSSQSHRGNFSQIYPSVNISSLSESRKMSMDDMSNIS

SEQ ID NO:13    DAFEIL-ASSRLCKTLLLSQA-----SSSVLLHNG---TTMPLLRSE--HVPYG------
SEQ ID NO:16    DAFEILLASSRLCKTLLLSQAAASSSSSSVLLHDDGTISTVPSLRSESEHVAYGYGGGPP
SEQ ID NO:19    ----------QICCK---RQAAAAA------------VVGHSSIRD--EHVPCPYAGPP-
SEQ ID NO:21    RPFDINM---QVFDGRLF----------------EGNVLVPPFNAQEISSLGMSRGSLPS

SEQ ID NO:13    PPAPA---HPQG--PSIGNYKQCMQMGAS-CGG---------RAQHEAACQSMAAAAARP
SEQ ID NO:16    PPAPAAAHRPRGRRPSVDNYKQ--QMGAAACAAGAGRQWSAEHGGRAAACEERAAAAPAR
SEQ ID NO:19    ------AHLIQGPSNT-------LQMKRNTNAAAQGRG-----GRHGSSTEHRSSTA---
SEQ ID NO:21    FGLPFHHHLQQTLPHLSSSPTHQMEMFSNEPQTSEGK-------RHNFLMATKAGENAS-
```

FIG. 3B

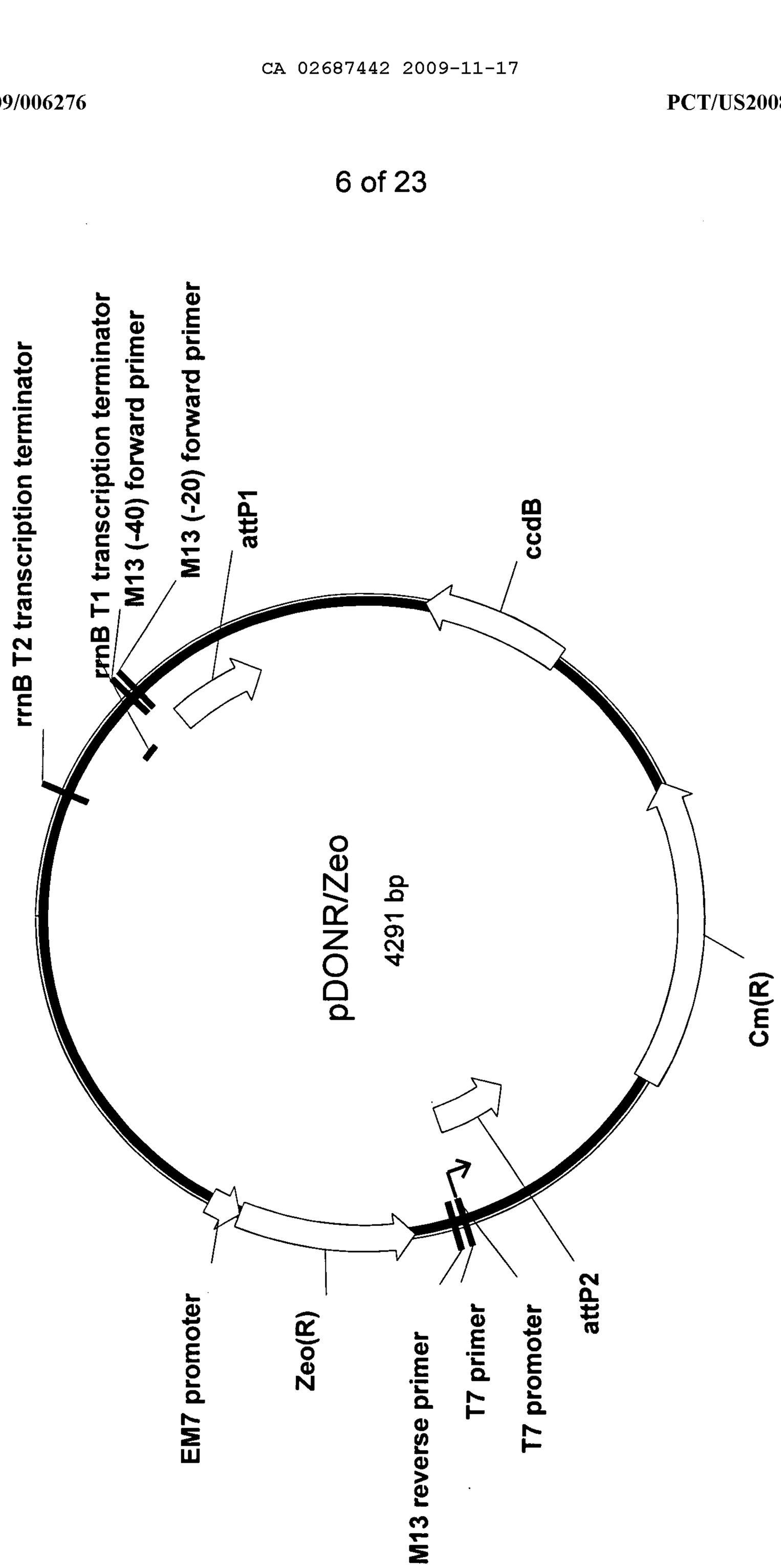
```
                        **** *  *             * * ****************************
SEQ ID NO:13    ALPVVPSSKKPRVEQRSS---------TIVPSFKVRKEKLGDRIAALQQLVSPFGKTDTA
SEQ ID NO:16    R-PVVVPWKKPRLEQRSGSGSGSGSSTTILPSFEVRKEKLGDRIAALQQLVSPFGKTDTA
SEQ ID NO:19    -LP--PSSKKPRLESHSSS---------MLPSFKVRKEKLGDRIAALQQLVSPFGKTDTA
SEQ ID NO:21    --------KKPRVESRSSC-----------PPFKVRKEKLGDRIAALQQLVSPFGKTDTA
                                                                  MOTIF I
                                ********************** **   *
SEQ ID NO:13    SVLMEAIGYIKFLQDQVETLSRPYLKSSRS-KKPRPTP-RGSSNASAGELEET-------
SEQ ID NO:16    SVLMEAIGYIKFLQDQVETLSRPYLKSSRSSKKPRPTHQRGCWNASAAGEEQEQEETTTR
SEQ ID NO:19    SVLMEAIGYIKFLQDQVETLSGPYLRSSKNSKKLACRAAQQQRKGTSNGGDAAA------
SEQ ID NO:21    SVLMEAIGYIKFLQSQIETLSVPYMRASRN---------RPGKASQLVSQSQEGDEEETR
                                ************** ***   *           *  *
SEQ ID NO:13    --RPDLRSRGLCLVPLSCTSYVTNENGV---------WV-PPNFRA-N------------
SEQ ID NO:16    RRRPDLRSRGLCLVPLSCTSYVTNENGA---------WV-PSQF.---------------
SEQ ID NO:19    --KLDLRSRGLCLVPLSCTSYVTNENGV---------WP-PPNFRGN.------------
SEQ ID NO:21    ----DLRSRGLCLVPLSCMTYVTGDGGDGGGGVGTGFWPTPPGFGGGT.-----------
                        MOTIF II
```

FIG. 4

% Identity

	SEQ ID NO:24 (B73-RT1)	SEQ ID NO:29 (cfp)	SEQ ID NO:39 (rive)	SEQ ID NO:25 (Arabidopsis
SEQ ID NO:24 (B73-RT1)		47.2	39.7	26.0
SEQ ID NO:29 (cfp..)			33.2	24.1
SEQ ID NO:39 (rice)				29.1
SEQ ID NO:25 (Arabidopsist)				

FIG. 5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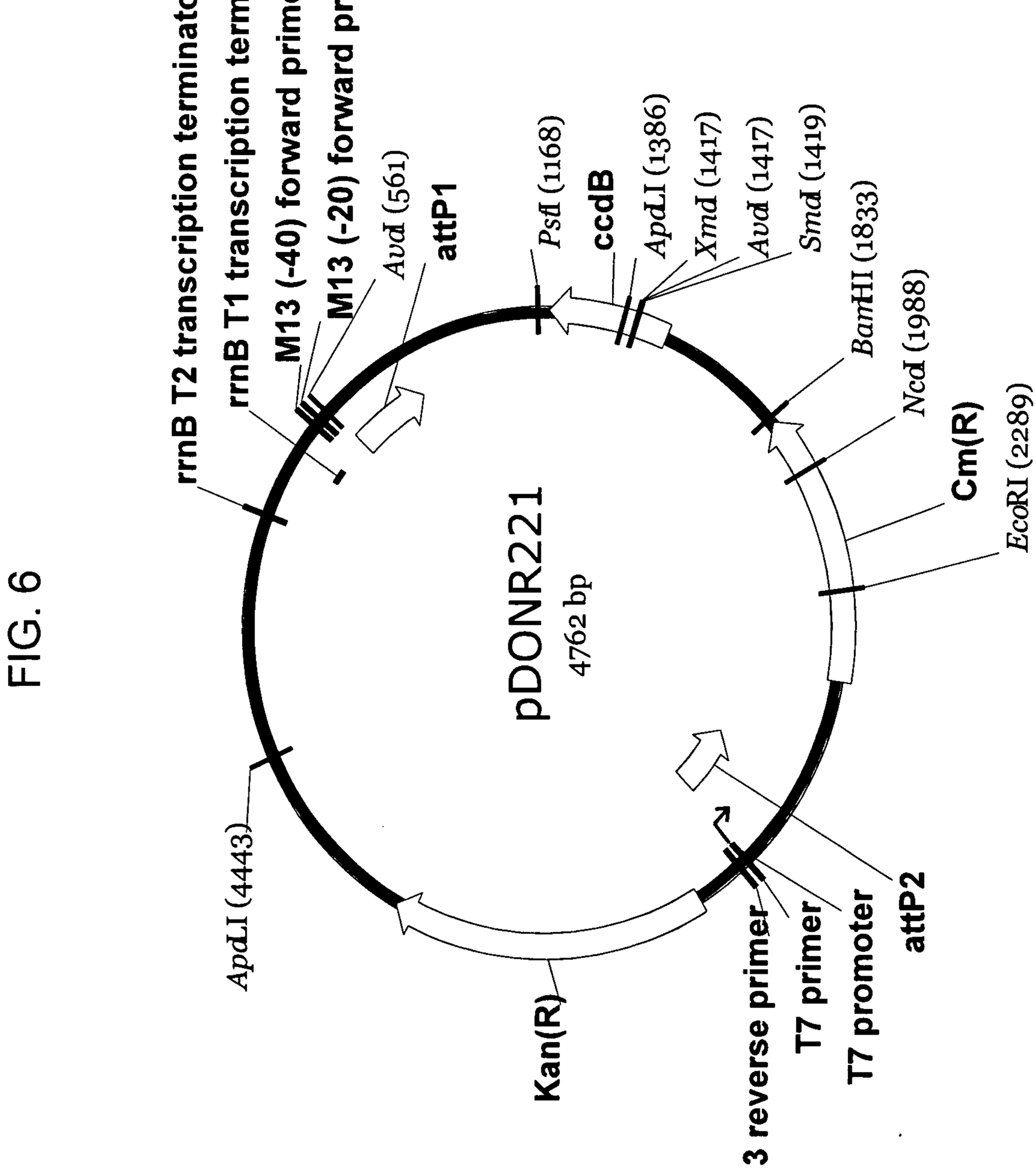
rrnB T2 transcription terminator
rrnB T1 transcription terminator
M13 (-40) forward primer
M13 (-20) forward primer
attP1
ccdB
Cm(R)
EM7 promoter
Zeo(R)
M13 reverse primer
T7 primer
T7 promoter
attP2

FIG. 6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rrnB T1 transcription terminator
M13 (-40) forward primer
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AvaI (561)
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ccdB
ApaLI (1386)
XmaI (1417)
AvaI (1417)
SmaI (1419)
BamHI (1833)
NcoI (1988)
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EcoRI (2289)
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Kan(R)
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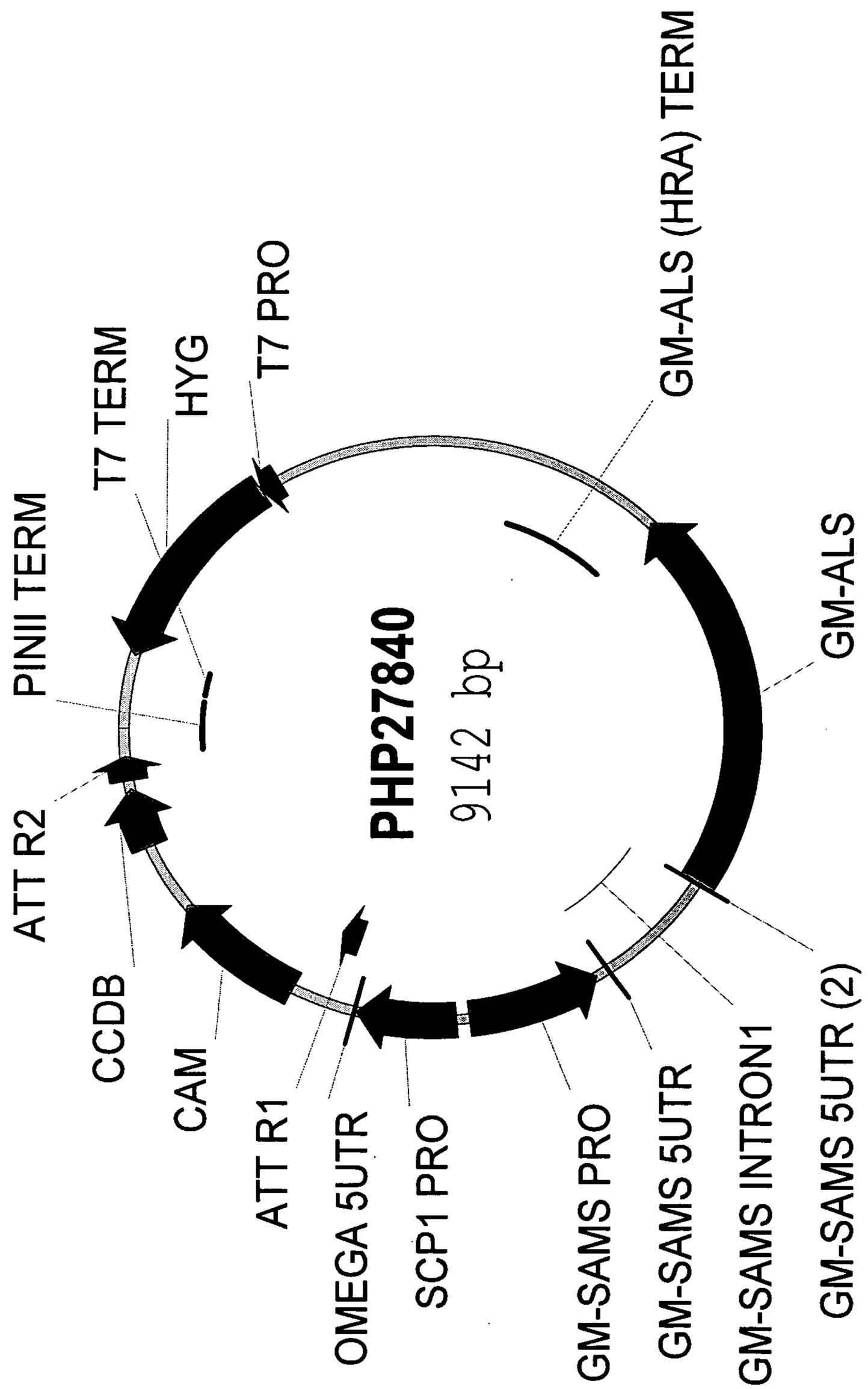
T7 primer
T7 promoter
attP2

FIG. 7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OMEGA 5UTR
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CAM
CCDB
ATT R2
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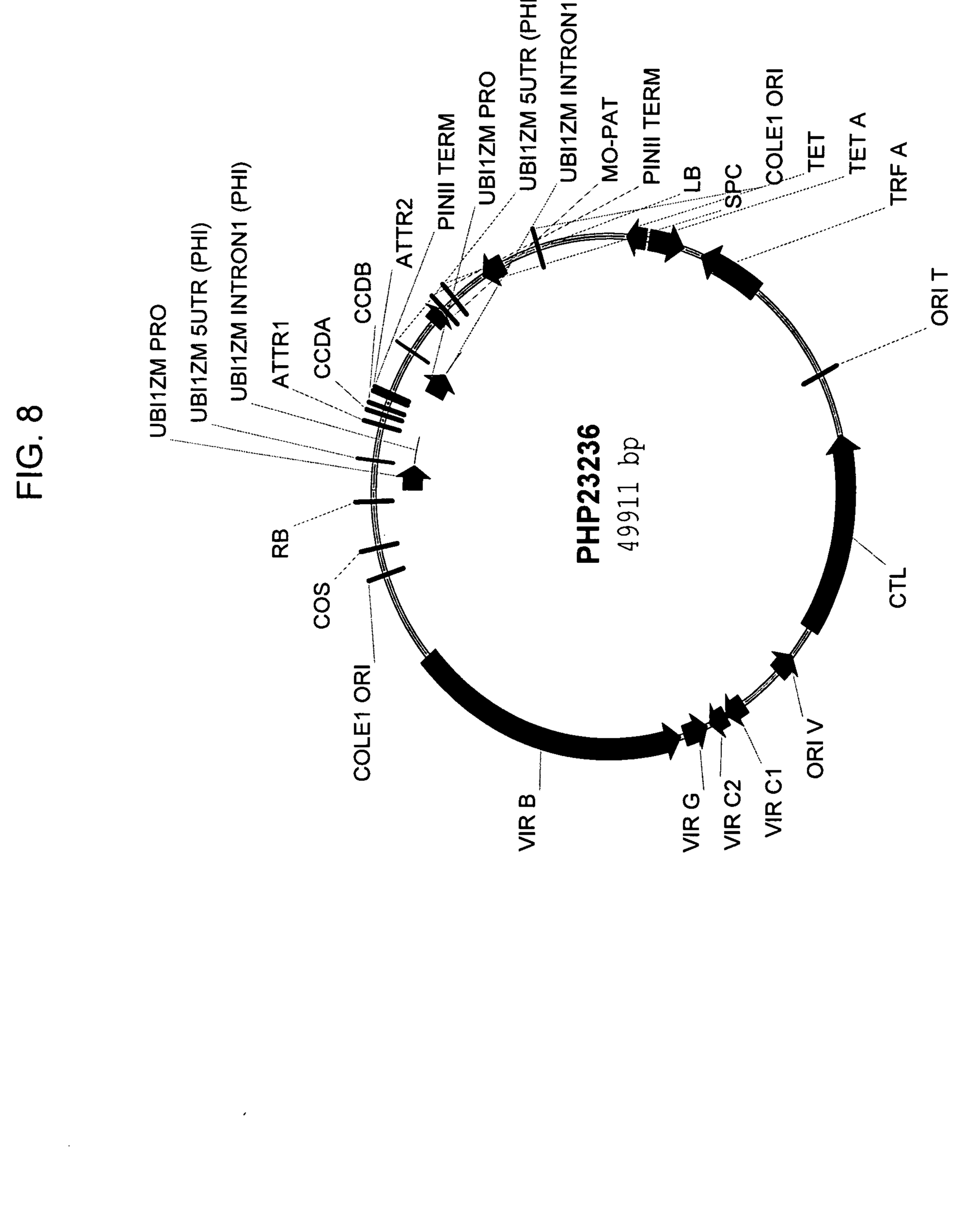
T7 TERM
HYG
T7 PRO
GM-ALS (HRA)
GM-ALS
GM-SAMS 5UTR (2)
GM-SAMS INTRON1
GM-SAMS 5UTR
GM-SAMS PRO

FIG. 8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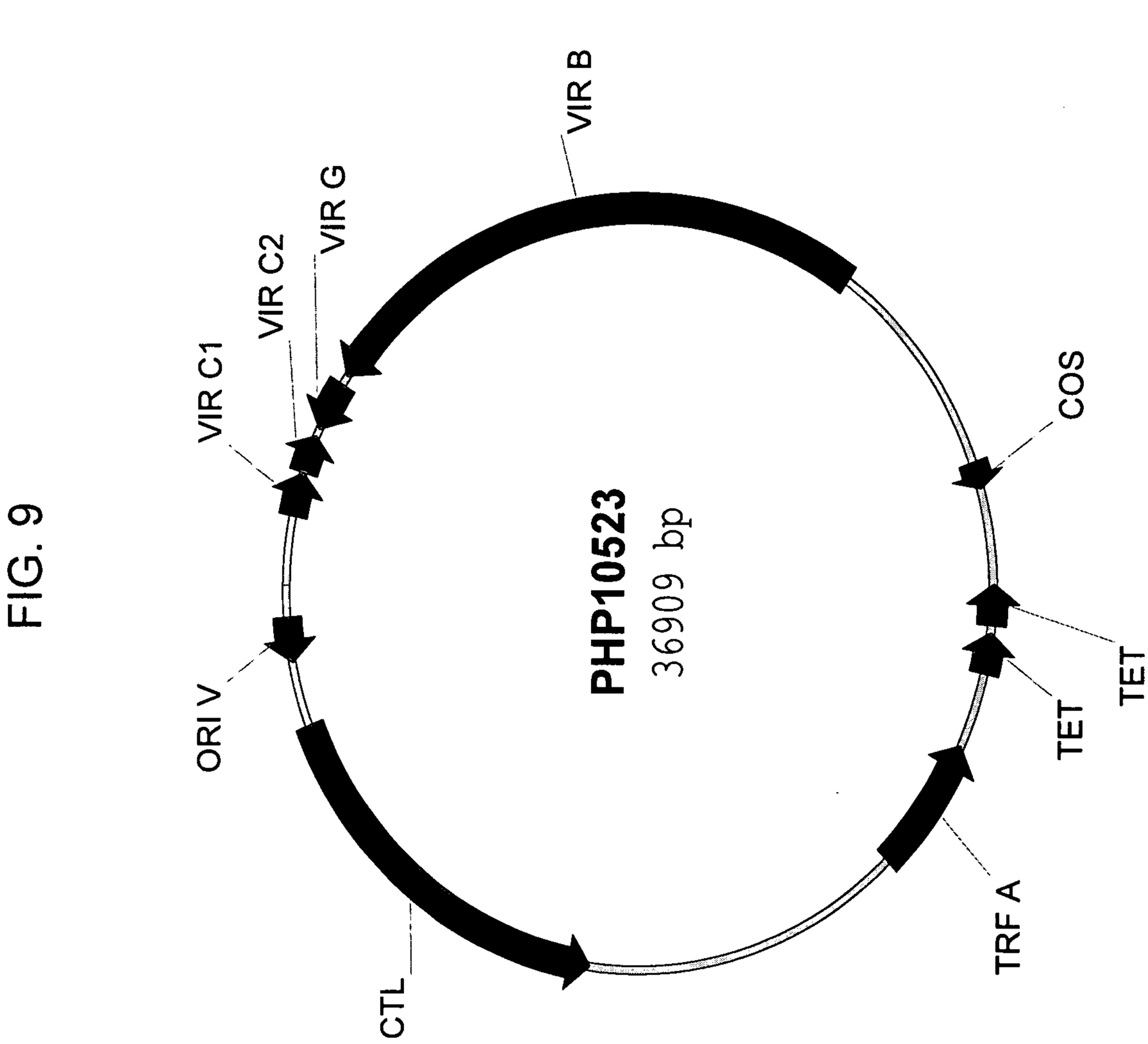
UBI1ZM 5UTR (PHI)
UBI1ZM INTRON1 (PHI)
ATTR1
CCDA
CCDB
ATTR2
PINII TERM
UBI1ZM PRO
UBI1ZM 5UTR (PHI)
UBI1ZM INTRON1 (PHI)
MO-PAT
PINII TERM
LB
SPC
COLE1 ORI
TET
TET A
TRF A
ORI T
CTL
ORI V
VIR C1
VIR C2
VIR G
VIR B
COLE1 ORI
COS
RB

FIG. 9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VIR C1
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ORI V
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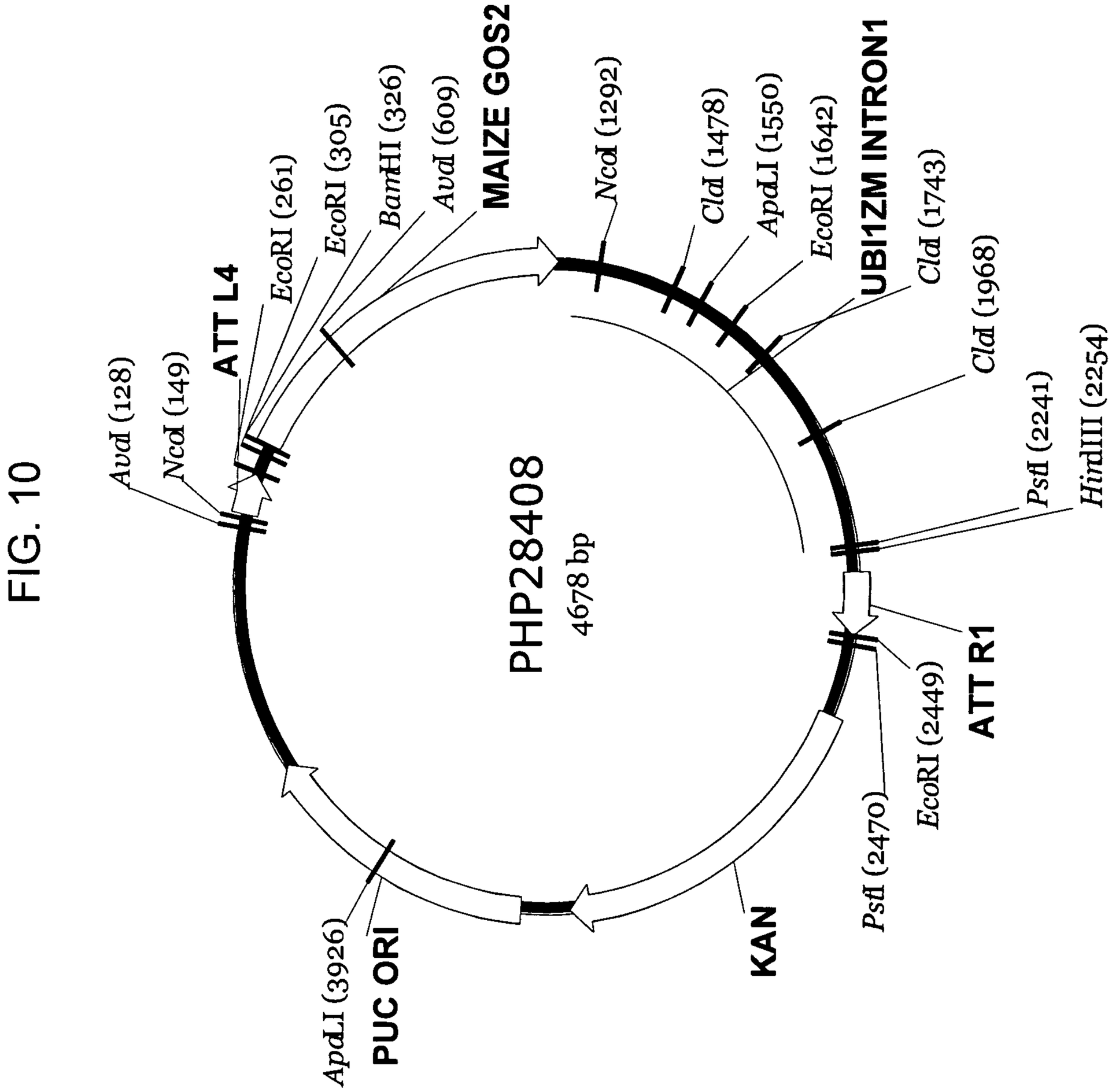
TET
TET

FIG. 10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ATT R1
KAN
PUC ORI
AvaI (128)
NcoI (149)
EcoRI (261)
EcoRI (305)
BamHI (326)
AvaI (609)
NcoI (1292)
ClaI (1478)
ApaLI (1550)
EcoRI (1642)
ClaI (1743)
ClaI (1968)
PstI (2241)
HindIII (2254)
PstI (2470)
EcoRI (2449)
ApaLI (3926)

FIG. 11

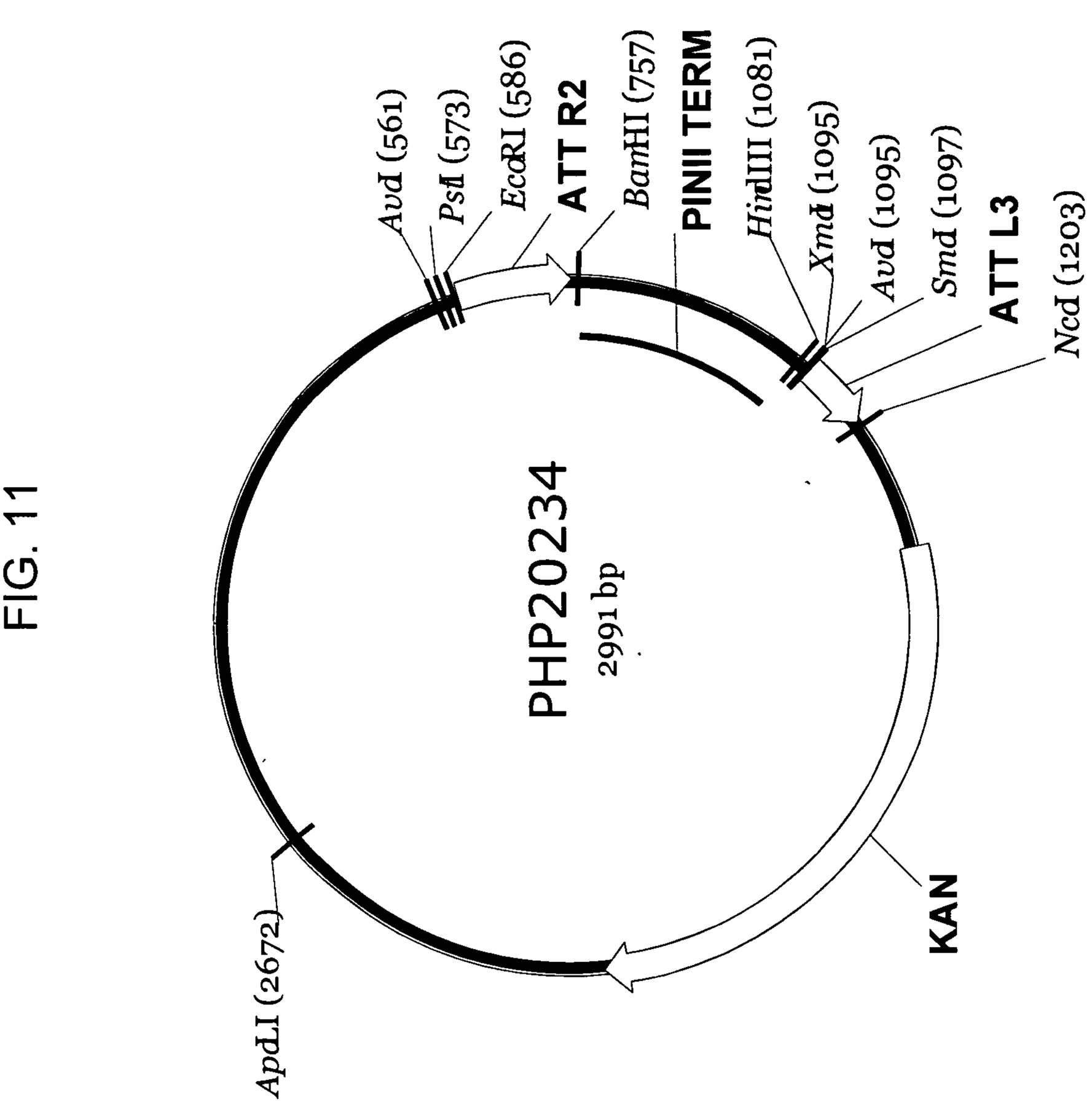

PHP20234
2991 bp
AvaI (561)
PstI (573)
EcoRI (586)
ATT R2
BamHI (757)
PINII TERM
HindIII (1081)
XmaI (1095)
AvaI (1095)
SmaI (1097)
ATT L3
NcoI (1203)
ApaLI (2672)
KAN

FIG. 12

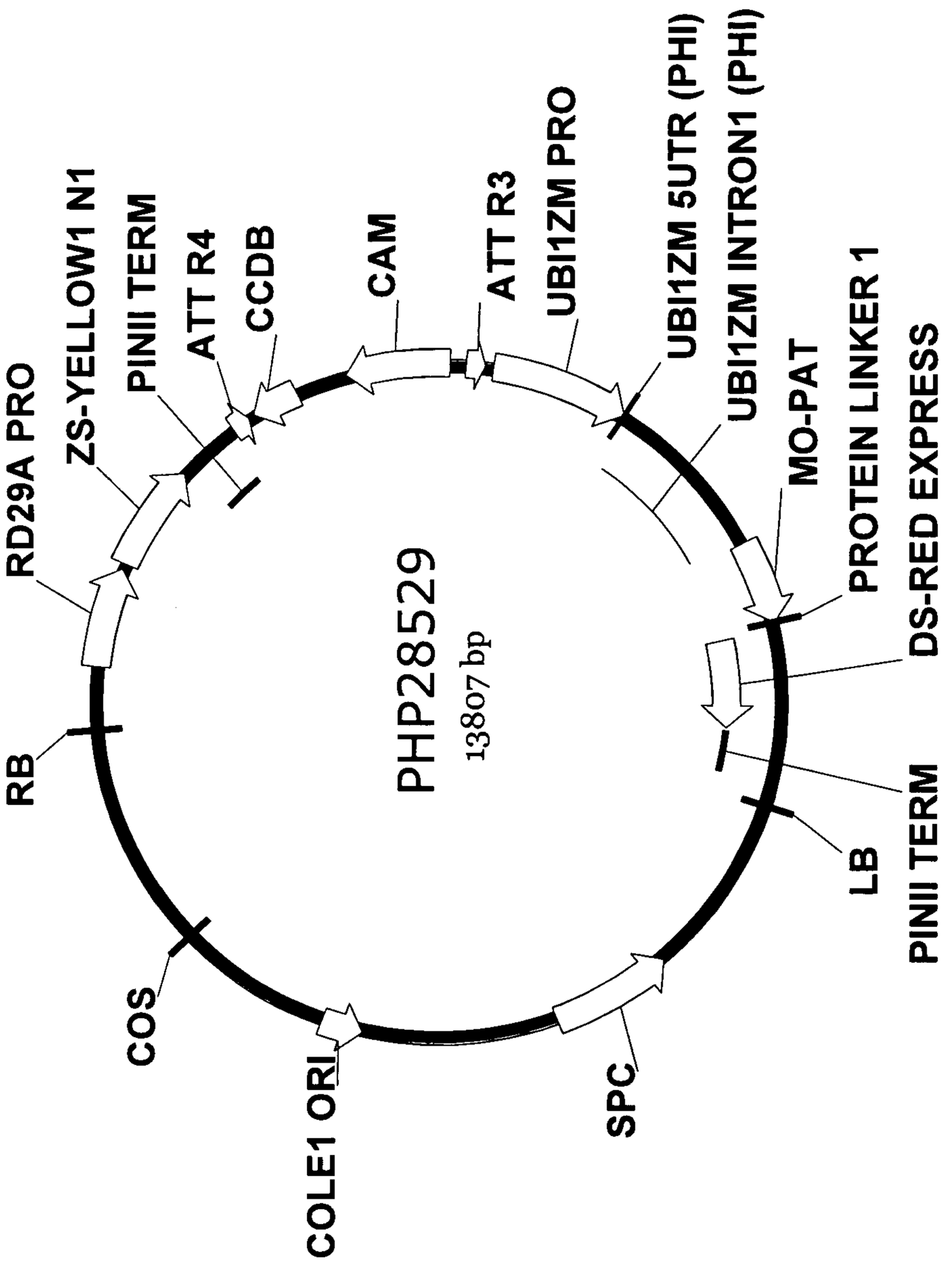

PHP28529
13807 bp
RB
RD29A PRO
ZS-YELLOW1 N1
PINII TERM
ATT R4
CCDB
CAM
ATT R3
UBI1ZM PRO
UBI1ZM 5UTR (PHI)
UBI1ZM INTRON1 (PHI)
MO-PAT
PROTEIN LINKER 1
DS-RED EXPRESS
PINII TERM
LB
SPC
COLE1 ORI
COS

FIG. 13

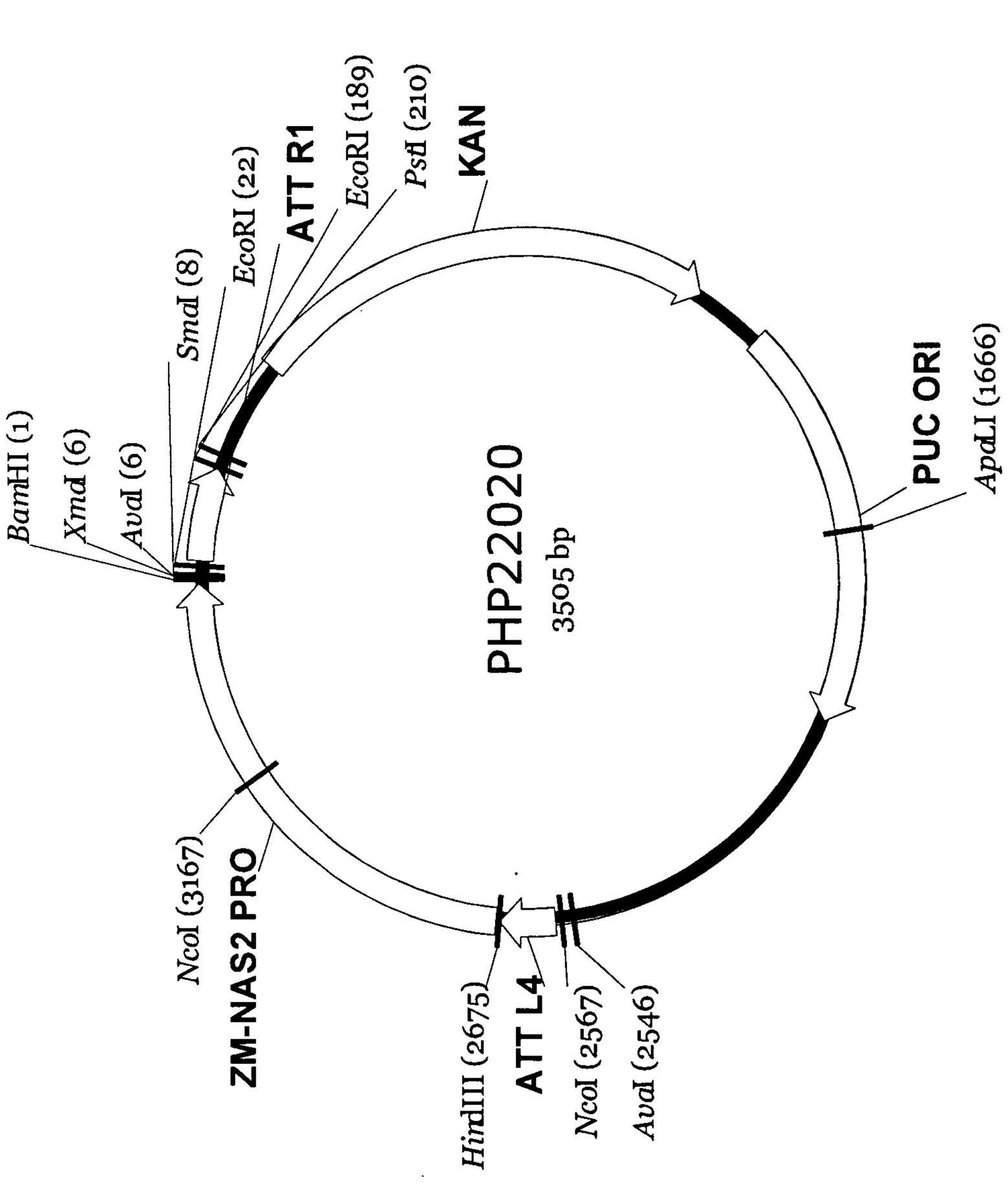

PHP22020
3505 bp
BamHI (1)
XmaI (6)
AvaI (6)
SmaI (8)
EcoRI (22)
ATT R1
EcoRI (189)
PstI (210)
KAN
PUC ORI
ApaLI (1666)
NcoI (3167)
ZM-NAS2 PRO
HindIII (2675)
ATT L4
NcoI (2567)
AvaI (2546)

FIG. 14

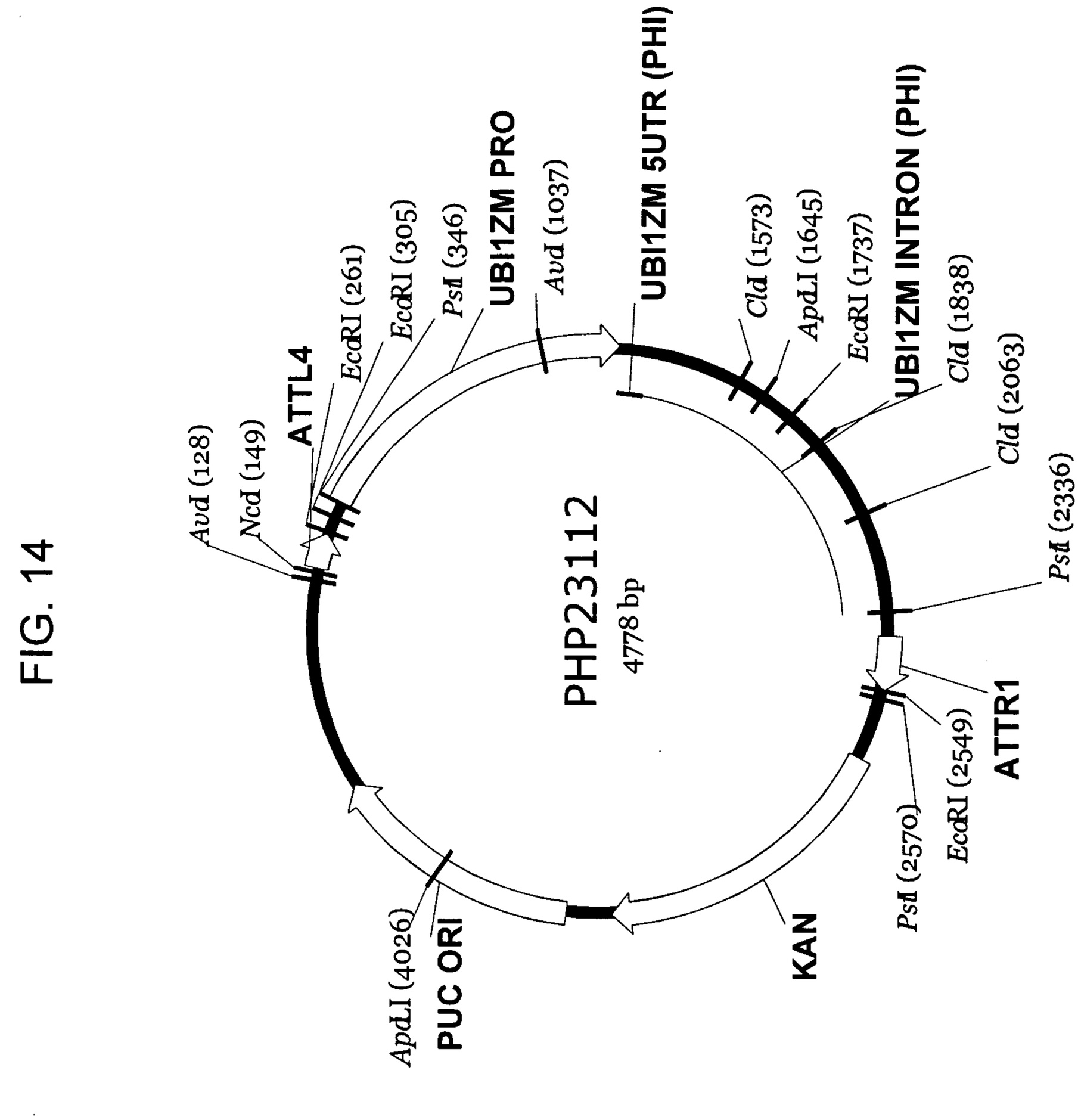

PHP23112
4778 bp
ATTL4
AvdI (128)
NcdI (149)
EcoRI (261)
EcoRI (305)
PstI (346)
UBI1ZM PRO
AvdI (1037)
UBI1ZM 5UTR (P
ClaI (1573)
ApaLI (1645)
EcoRI (1737)
UBI1ZM INTRON (PHI)
ClaI (1838)
ClaI (2063)
PstI (2336)
ATTR1
EcoRI (2549)
PstI (2570)
KAN
ApaLI (4026)
PUC ORI

FIG. 15

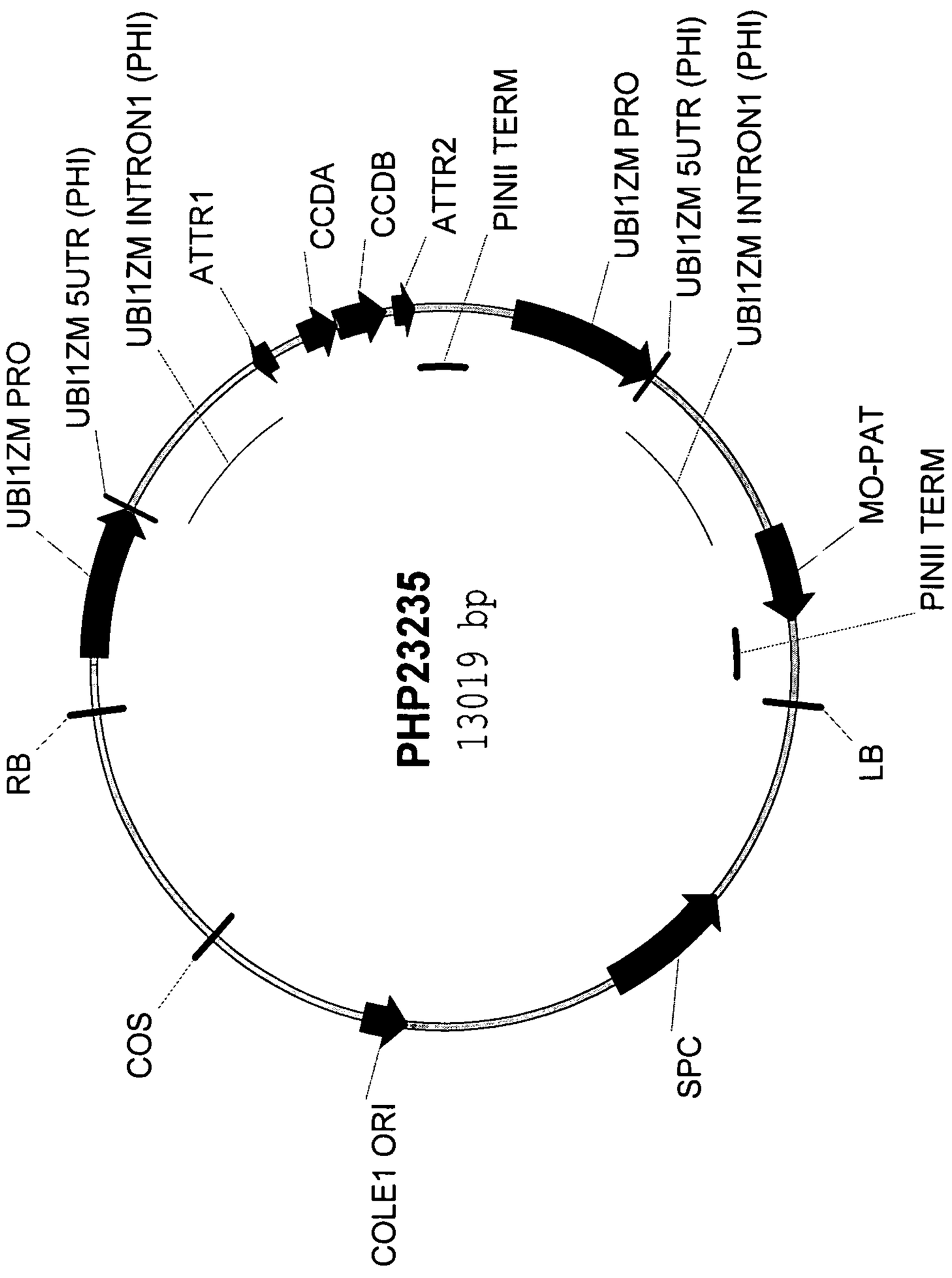

PHP23235
13019 bp
UBI1ZM PRO
UBI1ZM 5UTR (PHI)
UBI1ZM INTRON1 (PHI)
ATTR1
CCDA
CCDB
ATTR2
PINII TERM
UBI1ZM PRO
UBI1ZM 5UTR (PHI)
UBI1ZM INTRON1 (PHI)
MO-PAT
PINII TERM
LB
SPC
COLE1 ORI
COS
RB

FIG. 16

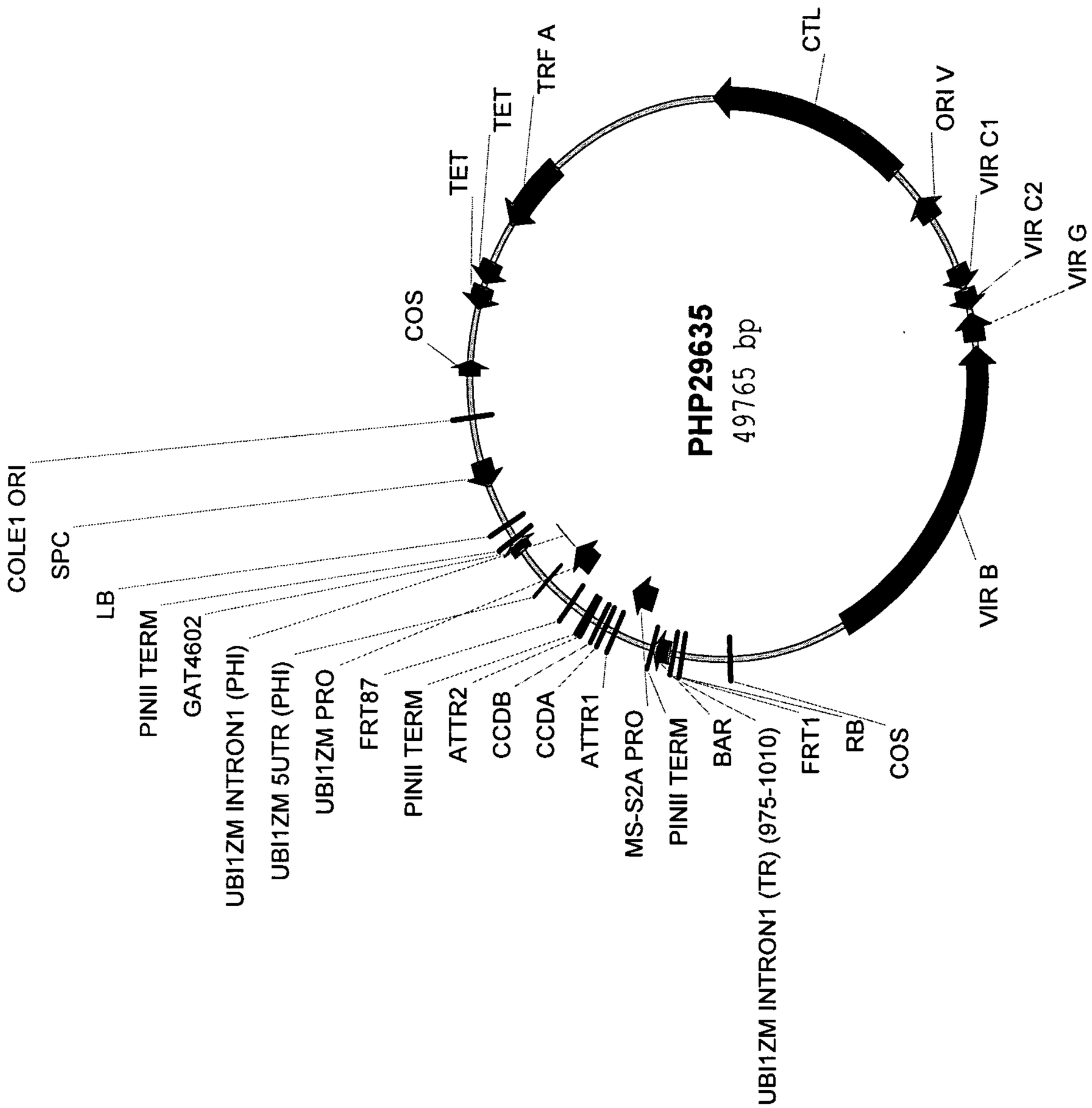

PHP29635
9765 bp
COLE1 ORI
SPC
LB
PINII TERM
GAT4602
UBI1ZM INTRON1 (PHI)
UBI1ZM 5UTR (PHI)
UBI1ZM PRO
FRT87
PINII TERM
ATTR2
CCDB
CCDA
ATTR1
MS-S2A PRO
PINII TERM
BAR
UBI1ZM INTRON1 (TR) (975-1010)
FRT1
RB
COS
VIR B
VIR G
VIR C2
VIR C1
ORI V
CTL
TRF A
TET
TET
COS

FIG. 17

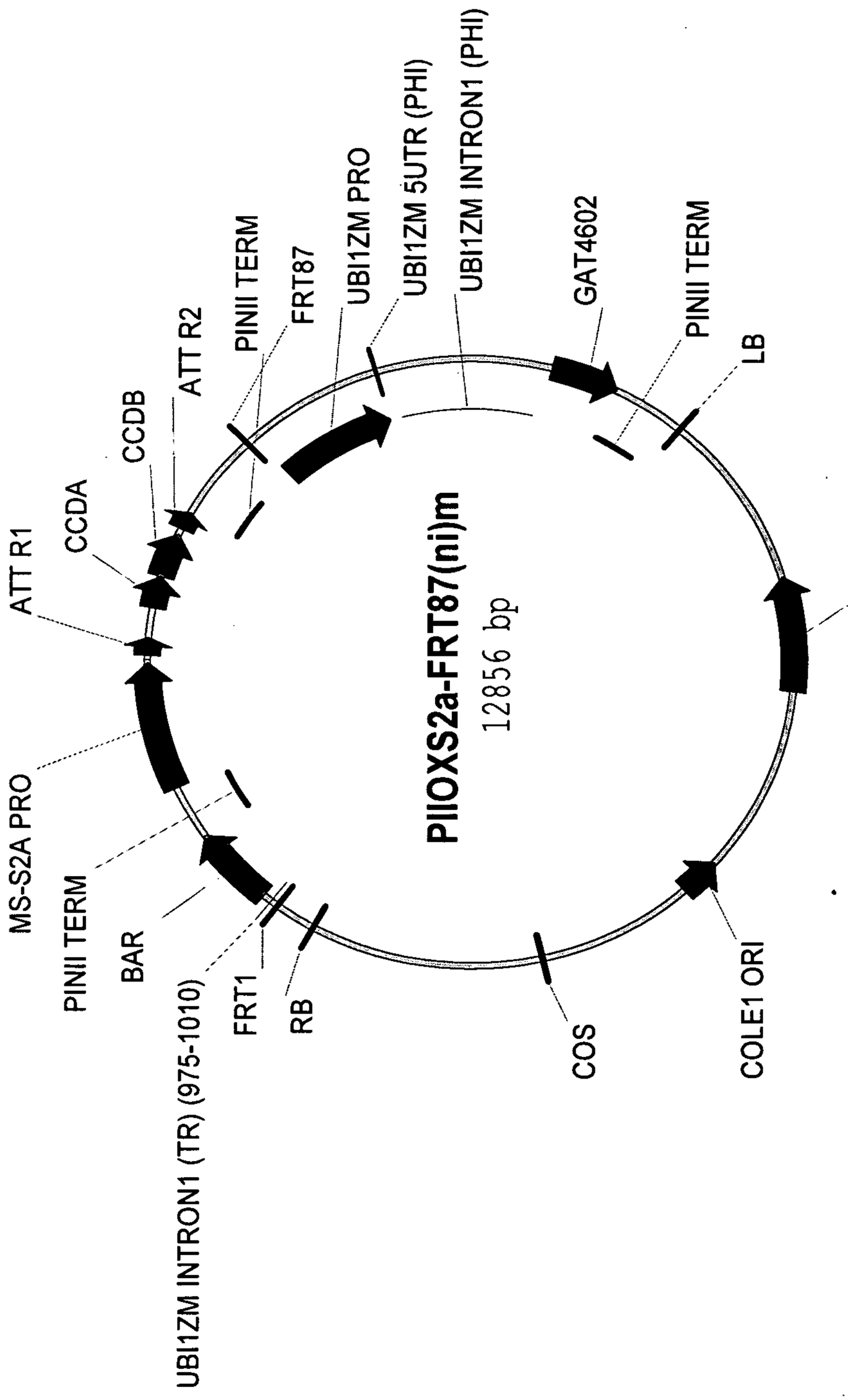

PIIOXS2a-FRT87(ni)m
12856 bp
ATT R1
CCDA
CCDB
ATT R2
PINII TERM
FRT87
UBI1ZM PRO
UBI1ZM 5UTR (PHI)
UBI1ZM INTRON1 (PHI)
GAT4602
PINII TERM
LB
SPC
COLE1 ORI
COS
RB
FRT1
TRON1 (TR) (975-1010)
BAR
PINII TERM
MS-S2A PRO
UBI1ZM INTRON1 (PHI)

FIG. 18

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_2PO_4	11 mM	11 mM	11 mM	11 mM
$MgSO_4$	16 mM	16 mM	16 mM	16 mM
$CaCl_2 \cdot 2H_2O$	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_3BO_3	24 μM	24 μM	24 μM	24 μM
5 mM $MnCl_2 \cdot 4H_2O$	8 μM	8 μM	8 μM	8 μM
5 mM $ZnSO_4 \cdot 7\ H_2O$	8 M	8 μM	8 μM	8 μM
0.5 mM $CuSO_4 \cdot 5\ H_2O$	800 nM	800 nM	800 nM	800 nM
0.5 mM $H_2MoO_4 \cdot H_2O$	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 H_2SO_4 if the pH is above 6.5.
Optimum pH is 5.0 - 5.5

FIG. 19

The effect of different nitrate concentrations on the growth and development of Gaspe Bay Flint derived maize lines.

[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	*	*

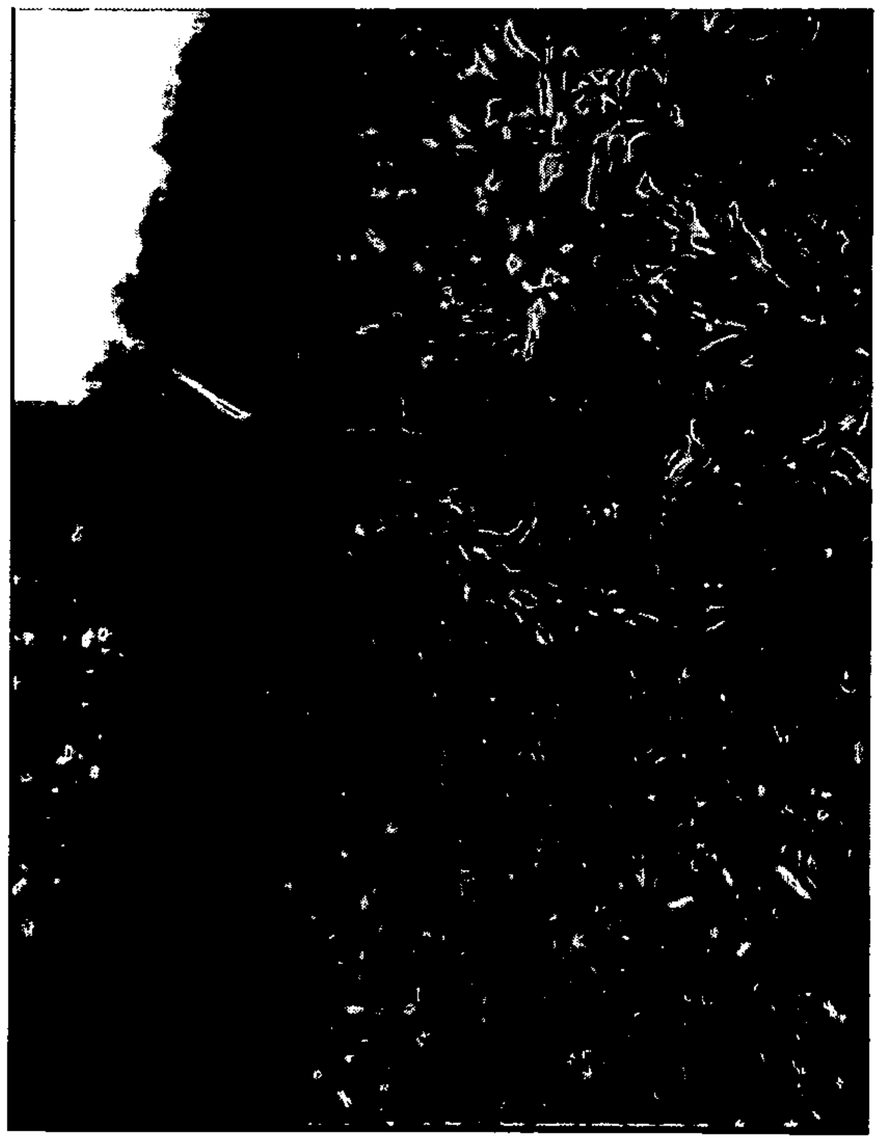
* Tillers removed 3 weeks after emergence
Means with similar letters are not different by protected Least Significant Difference (LSD) (0.05)

FIG. 20a

Phenotypic Characterization

rt1 **in greenhouse**

rt1 **in field**



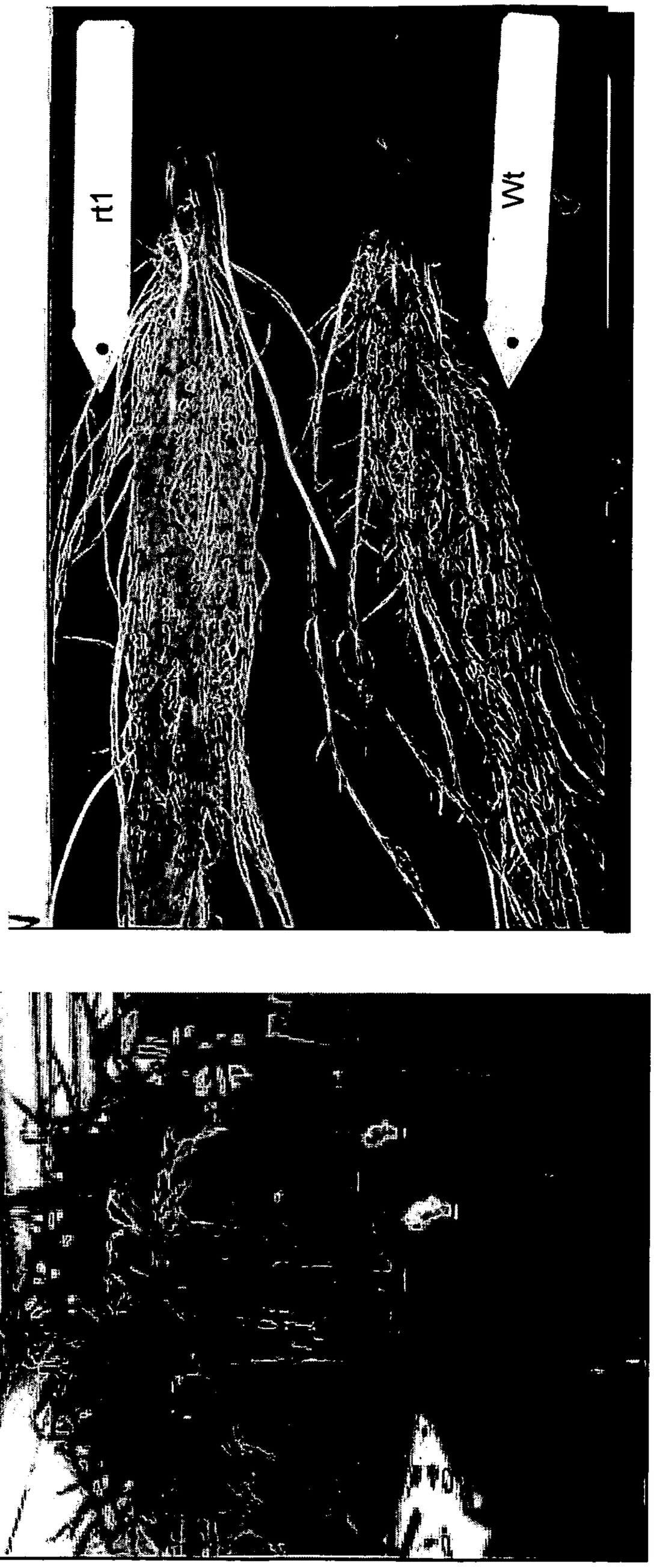


FIG. 20b

In a hydroponic system

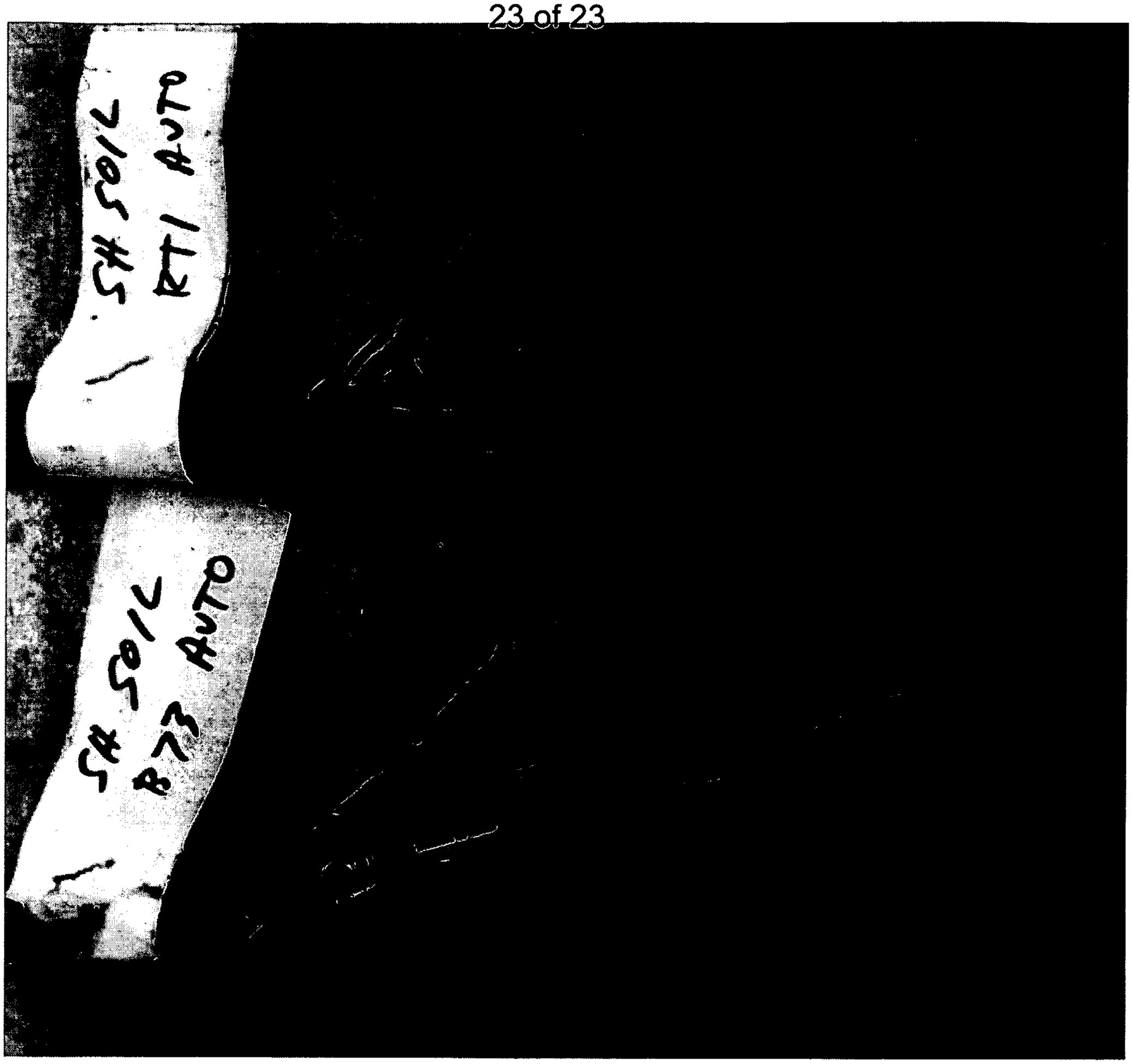


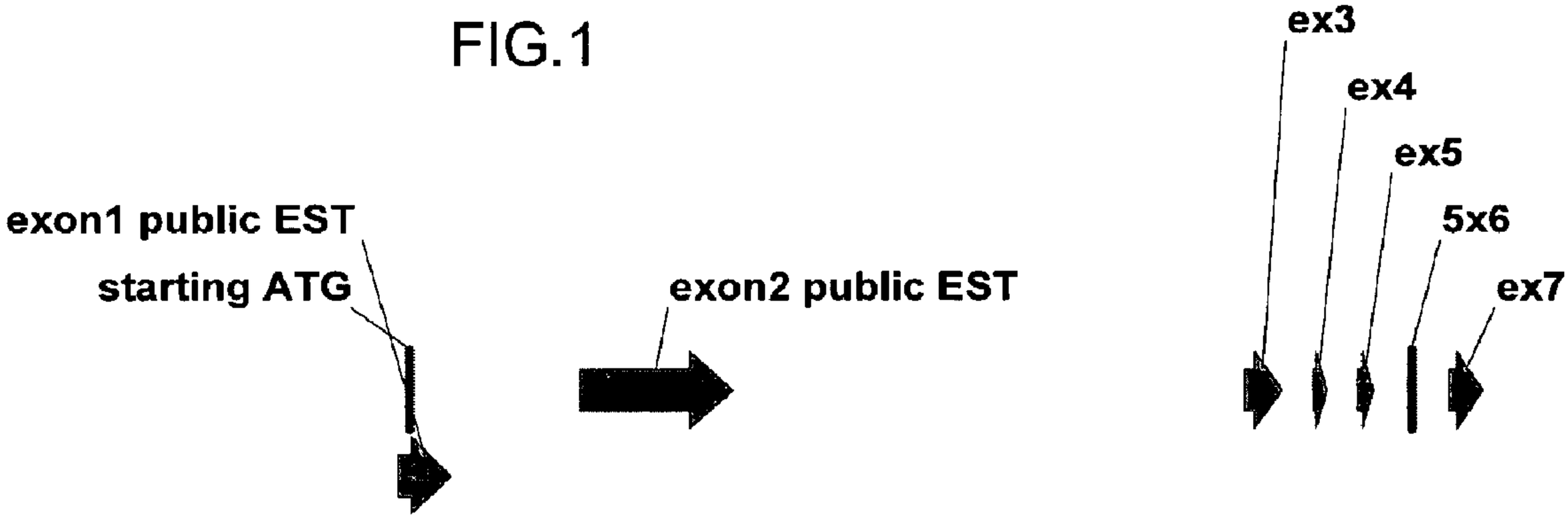
FIG. 20c

In autoclaved hard soil in the Greenhouse

Left: Wt plants

Right: rt1 mutant

FIG.1



new rt1bac region around ethylen respo 7.8Kb exon annotation

7800 bp