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(54) Title: COMPOSITIONS AND METHODS CONFERRING RESISTANCE OF MAIZE TO CORN ROOTWORM II

FIG. 1A

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1.....11.....21.....31.....41.....51.....60
Crw2      ATGAAGCAGCGGAGGGGCGGCGGAGCGGGCGGGTGGGCAACGGCGCGTGGTGGT
crw2-Mutag ATGAAGCAGCGGAGGGGCGGCGGAGCGGGCGGGTGGGCAACGGCGCGTGGTGGT
*****

61.....71.....81.....91.....101.....111.....120
Crw2      ACCATGGTCTCTCCCTCTGGCTCCTCAGGTACATCAAGGCGCGGATCTGCTCCAAACCTT
crw2-Mutag ACCATGGTCTCTCCCTCTGGCTCCTCAGGTACATCAAGGCGCGGATCTGCTCCAAACCTT
*****

121.....131.....141.....151.....161.....171.....180
Crw2      TTCCCCAAGCGGTGGCGGAGGIGGAGGTGGACGAGGACTACGACAGCAGCGGTACAAG
crw2-Mutag TTCCCCAAGCGGTGGCGGAGGIGGAGGTGGACGAGGACTACGACAGCAGCGGTACAAG
*****

181.....191.....201.....211.....221.....231.....240
Crw2      CTGACGGGCGCGGTGGCGGAGGAGGACTTCGACCGCTCCGCGCCCACTGCTACCAACCC
crw2-Mutag CTGACGGGCGCGGTGGCGGAGGAGGACTTCGACCGCTCCGCGCCCACTGCTACCAACCC
*****

241.....251.....261.....271.....281.....291.....300
Crw2      AGCAAGCGGTGCGAGCGGTGCGCGCGGTGGCGGACATCGCGTGGAGCGGCAACCACTCG
crw2-Mutag AGCAAGCGGTGCGAGCGGTGCGCGCGGTGGCGGACATCGCGTGGAGCGGCAACCACTCG
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(57) Abstract: Methods and compositions for increasing a plant's resistance to an insect pest such as the corn rootworm are provided. Methods are provided for overexpression of *Crw2*, or variants thereof, in a host plant or plant cell to increase resistance to an insect pest in a plant such as maize. Methods are also provided for identifying variants of *Crw2* that when incorporated into a plant via transgenic or traditional breeding means increase resistance to an insect pest in a plant such as maize. Also provided are methods for increasing resistance by overexpressing *Crw1* and *Crw2*.



TITLE

COMPOSITIONS AND METHODS CONFERRING RESISTANCE OF MAIZE TO CORN ROOTWORM II

CROSS REFERENCE TO RELATED APPLICATIONS

5 This application claims the benefit of U.S. Provisional Application
No. 61/703,396, filed September 20, 2012; U.S. Provisional Application
No. 61/781,057, filed March 14, 2013; U.S. Provisional Application No.
61/703,414, filed September 20, 2012; and U.S. Provisional Application
No. 61/781,124, filed March 14, 2013; the entire contents of which are
10 herein incorporated by reference.

REFERENCE TO SEQUENCE LISTING

The Sequence Listing submitted September 20, 2013 as a text file named "BB2190PCT_Sequence_Listing," created on September 20, 2013, and having a size of 151,366 bytes is hereby incorporated by reference pursuant to 37 C.F.R. § 1.52(e)(5).

FIELD

The field relates to plant breeding and genetics and, in particular, relates to recombinant DNA constructs useful in maize plants for conferring resistance to corn rootworm.

20 BACKGROUND

The larval forms of three species of *Diabrotica* beetles, the Western corn rootworm (*Diabrotica virgifera virgifera* LeConte), the Northern corn rootworm (*Diabrotica barberi* Smith and *Diabrotica barberi* Lawrence), and the Southern corn rootworm (*Diabrotica undecimpunctata howardi* Barber), represent serious insect pests of corn in the Midwestern United States . Approximately 30 million acres (120,000 km²) of corn (out of 80 million grown) are infested with corn rootworms, the larvae of which can cause losses estimated by the United States Department of Agriculture on the order of about \$1 billion per year.

30 There are many different management practices aimed at the control of corn rootworms, including corn variety selection, early planting, insecticides, crop rotation, and the use of transgenic corn varieties; however, none on its own has proven to effectively manage the pest. An

additional complication exists in that corn rootworm insects have shown a remarkable ability to evolve resistance to several control measures, including insecticides, cultural practices, and resistance genes that have been introduced into plants.

5 Thus, there is a constant need for new mechanisms of corn rootworm resistance in maize that can be incorporated into an integrated pest management strategy.

SUMMARY

10 Methods of increasing resistance to herbivory by an insect pest in a maize plant are provided in which polynucleotides encoding CRW2 are expressed in maize plants. In addition, methods of increasing resistance to herbivory by an insect pest in a maize plant are provided in which polynucleotides encoding CRW1 and CRW2 are expressed in maize plants.

15 Also provided are methods of identifying variants of ZmCw2 (or ZmCRW2) that give maize plants increased resistance to herbivory by an insect pest and then further introducing the variants into the maize plants. The variants can be identified through gene shuffling experiments or can be naturally occurring allelic variants identified through linkage mapping or
20 whole genome association analyses. Variants arising from gene shuffling can be transgenically introduced into maize plants to give them the increased resistance, while allelic variants identified using the methods presented herein can be incorporated into maize plants using molecular breeding.

25 In some embodiments, the insect pest is Coleopteran.

 In other embodiments, the Coleopteran insect pest is of the genus *Diabrotica*. In still other embodiments, the insect pest is a corn rootworm, including without limitation, the Western corn rootworm (*Diabrotica virgifera virgifera* LeConte), the Northern corn rootworm (*Diabrotica barberi* Smith and *Diabrotica barberi* Lawrence), and/or the Southern corn
30 rootworm (*Diabrotica undecimpunctata howardi* Barber).

In other embodiments, the Coleopteran insect pest is of the genus *Popillia*, and the insect pest is the Japanese beetle.

In some embodiments, the insect pest is Lepidopteran. The Lepidopteran insect pest may be European corn borer (*Ostrinia* sp.), fall webworm (*Hyphantria cunea*), or cattail caterpillar (*Simyra insularis*).

Isolated polynucleotides or cDNAs, recombinant constructs containing said polynucleotides, and plants and plant cells containing said recombinant constructs are also provided.

BRIEF DESCRIPTION OF THE DRAWINGS AND SEQUENCE LISTING

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

Figures 1A-1G shows the alignment between *ZmCrw2* and the *crw2-Mutag* mutant allele.

Figures 2A-2F shows the alignment between *ZmCrw2* and the *crw2-EMS* mutant allele.

Figure 3 shows the structure of the maize Homogalacturonan1 (HGA1) gene (also referred to as glycosyltransferase AER61 or *ZmCrw2*). The positions of the two Mutator insertions and the EMS allele are shown.

Figures 4A-4J show an alignment of the *ZmCrw2* protein (SEQ ID NO:2) and its homologs (SEQ ID NOs: 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 22, and 23).

Figure 5 shows the percent sequence identity and the divergence values for each pair of amino acids sequences of the polypeptides displayed in Figures 4A-4J.

Figure 6 (A) shows gene expression of *crw2-Mutag* mutant allele in 10 day old seedlings. Total RNA samples were collected from leaf, stalk, and root tissues, and RT-PCR analysis was performed using gene specific primers. The *crw2-Mutag* mutant showed differential expression in three tissues and has a transcript that is 145 bp longer than in the WT-sibs. Figure 6 (B) shows RT-PCR analysis of the *crw2-TUSC* mutant as compared to its WT-sibs. In this figure, "-1" and "-2" refer to two separate

samples that were tested for each allele.

Figure 7 shows expression of the *ZmCrw2* gene in different plant tissues compiled from the Lynx database.

Figure 8 shows *Crw2* transcript levels in a *Crw1* mutant in comparison to its WT at various time points after WCR beetle feeding. There is a rapid up-regulation in the levels of the *Crw2* transcript in WT plants within 45 minutes of WCR feeding (upper panel). Such up-regulation is not observed in the *Crw1* mutant upon WCR feeding (lower panel). The 18S rRNA control with the same loading scheme is represented in B.

Figure 9 shows the expression of *ZmCrw1* and *ZmCrw2* (as assessed using RT-PCR) in a doubled haploid line that is highly susceptible to herbivory by the WCR beetle. Results show that *ZmCrw1* is expressed in the doubled haploid line while *ZmCrw2* is not (Fig. 9).

Figures 10A and B shows that a *Crw2* mutant ("MT") has (A) lower levels of cell wall bound p-coumaric acid (pCA) and ferulic acid (FA) and (B) lower levels of lignin, in comparison to wild-type ("WT") during adult stages ($p < 0.05$; unpaired t test).

SEQ ID NO:1 is the nucleotide sequence of the coding region of the wild-type *Zea mays Crw2* (*ZmCrw2*) cDNA.

SEQ ID NO:2 is the amino acid sequence of the wild-type *Zea mays* CRW2 (*ZmCRW2*) protein, which is also referred to as a glycosyltransferase (UniProt entry B6TY42).

SEQ ID NO:3 is the nucleotide sequence of the cDNA of the mutant *crw2-Mutag* allele.

SEQ ID NO:4 is the amino acid sequence of the polypeptide encoded by the mutant *crw2-Mutag* allele.

SEQ ID NO:5 is the nucleotide sequence of the cDNA of the mutant *crw2-EMS* allele.

SEQ ID NO:6 is the amino acid sequence of the polypeptide encoded by the mutant *crw2-EMS* allele.

SEQ ID NO:7 is the amino acid sequence of the *Zea mays* putative uncharacterized protein (UniProt entry C0PDR7).

SEQ ID NO:8 is the amino acid sequence of the *Zea mays* putative uncharacterized protein (UniProt entry C4J6G0).

5 SEQ ID NO:9 is the amino acid sequence of the *Oryza sativa* putative HGA1 (Identifier Os06g49320; UniProt entry Q5Z8T7).

SEQ ID NO:10 is the amino acid sequence of the *Oryza sativa* putative HGA1 (Identifier Os02g0135500; UniProt entry Q6Z0Z4).

10 SEQ ID NO:11 is the amino acid sequence of the *Sorghum bicolor* putative uncharacterized protein Sb10g029380 (UniProt entry C5Z9B2).

SEQ ID NO:12 is the amino acid sequence of the *Sorghum bicolor* putative uncharacterized protein Sb04g002850 (UniProt entry C5XTX5).

SEQ ID NO:13 is the amino acid sequence of the *Glycine max* uncharacterized protein (Identifier Glyma05g34170; UniProt entry I1K5F9).

15 SEQ ID NO:14 is the amino acid sequence of the *Glycine max* uncharacterized protein (Identifier Glyma08G05490; UniProt entry I1KQG2).

20 SEQ ID NO:15 is the amino acid sequence of the *Arabidopsis thaliana* glycosyltransferase family 61 protein (TAIR Identifier: At3g18170; NCBI GI No. GI30684813).

SEQ ID NO:16 is the amino acid sequence of the *Arabidopsis thaliana* At3g18180 locus also referred to as glycosyltransferase family 61 protein (UniProt entry Q9LV22).

25 SEQ ID NO:17 is the amino acid sequence of the *Hordeum vulgare* predicted protein (UniProt entry F2DBB4).

SEQ ID NO:18 is the amino acid sequence of the *Brachypodium distachyon* uncharacterized protein also known as BRADI1G34670 (UniProt entry I1GWV1).

30 SEQ ID NO:19 is the nucleotide sequence of a homolog of ZmCrw2 from *Paspalum notatum* (identified in an internal proprietary database and referred to herein as PnCrw2).

SEQ ID NO:20 is the amino acid sequence of the polypeptide encoded by SEQ ID NO:19. The polypeptide is herein referred to as PnCRW2.

5 SEQ ID NO:21 is the nucleotide sequence of a homolog of *ZmCrw2* from *Eragrostis nindensis* (identified in an internal proprietary database and referred to herein as *EnCrw2*).

SEQ ID NO:22 is the amino acid sequence of the polypeptide encoded by SEQ ID NO:21. The polypeptide is referred to herein as EnCRW2.

10 SEQ ID NO:23 is the amino acid sequence of an uncharacterized glycosyltransferase AGO61-like from *Glycine max* (NCBI GI No. 356511269).

SEQ ID NO:24 is the nucleotide sequence of the forward gene specific primer PHN151293 used for RT-PCR analysis.

15 SEQ ID NO:25 is the nucleotide sequence of the reverse gene specific primer PHN151298 used for RT-PCR analysis.

SEQ ID NO:26 is the amino acid sequence of the wild-type *Zea mays* CRW1 (*ZmCRW1*) protein.

20 SEQ ID NO:27 is the amino acid sequence of a secondary wall NAC transcription factor 2 from *Oryza sativa* (UniProt entry G3M8D2).

SEQ ID NO:28 is the amino acid sequence of a putative NAM protein (*OsNAC7*) from *Oryza sativa* (Identifier Os06g04090.1; UniProt entry Q9SNM6).

25 SEQ ID NO:29 is the amino acid sequence of a putative uncharacterized protein from *Sorghum bicolor* (Identifier Sb07g001550.1; UniProt entry C5YM23).

SEQ ID NO:30 is the amino acid sequence of a putative NAM protein from *Sorghum bicolor* (Identifier Sb10g002120.1; UniProt entry Q5NKS7).

30 SEQ ID NO:31 is the amino acid sequence of an uncharacterized protein from *Glycine max* (Identifier Glyma16g02200.1; UniProt entry I1MKD6).

SEQ ID NO:32 is the amino acid sequence of an uncharacterized protein from *Glycine max* (Identifier Glyma07g05660.1; UniProt entry I1KHQ4).

5 SEQ ID NO:33 is the amino acid sequence of a NAC domain-containing protein 43 from *Arabidopsis thaliana* (Identifier At2g46770.1; UniProt entry Q84WP6).

SEQ ID NO:34 is the amino acid sequence of a NAC domain-containing protein 12 from *Arabidopsis thaliana* (At1g32770.1; UniProt entry Q9LPI7).

10 SEQ ID NO:35 is the amino acid sequence of a NAC domain-containing protein 66 from *Arabidopsis thaliana* (Identifier At3g61910.1; UniProt entry Q9M274).

SEQ ID NO:36 is the amino acid sequence of a secondary wall NAC transcription factor 2 from *Zea mays* (UniProt entry B4FPS5)

15 SEQ ID NO:37 is the amino acid sequence of a putative NAM protein from *Zea mays* (UniProt entry Q5NKQ3).

SEQ ID NO:38 is the amino acid sequence of a CRW1 homolog from *Brachypodium distachyon* (NCBI GI No. 357139497 and herein referred to as BdCRW1).

20 SEQ ID NO:39 is the amino acid sequence of a putative uncharacterized protein from *Vitis vinifera* (UniProt entry F6HU82).

SEQ ID NO:40 is the amino acid sequence of a CRW1 homolog from *Glycine max* (NCBI GI No. 356522480 and herein referred to as GmCRW1).

25 SEQ ID NO:41 is the amino acid sequence of a NAC domain-containing protein from *Gossypium hirsutum* (UniProt entry G4V2G0).

SEQ ID NO:42 is the amino acid sequence of a NAC domain class transcription factor (NAC12) from *Pyrus malus* (UniProt entry D9ZJ90).

30 SEQ ID NO:43 is the amino acid sequence of a predicted protein from *Hordeum vulgare* (UniProt entry F2DV83).

SEQ ID NO:44 is the amino acid sequence of a CRW1 homolog from *Arabidopsis thaliana* (NCBI GI No.3510262; UniProt entry Q84WP6).

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.

5 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
10 rules set forth in 37 C.F.R. §1.822.

DETAILED DESCRIPTION

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

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

20 As used herein:

The terms "monocot" and "monocotyledonous plant" are used interchangeably herein.

The terms "dicot" and "dicotyledonous plant" are used interchangeably herein.

25 The terms "full complement" and "full-length complement" are used interchangeably herein, and refer to a complement of a given nucleotide sequence, wherein the complement and the nucleotide sequence consist of the same number of nucleotides and are 100% complementary.

30 An "Expressed Sequence Tag" ("EST") is a DNA sequence derived from a cDNA library and therefore is a sequence which has been transcribed. An EST is typically obtained by a single sequencing pass of a cDNA insert. The sequence of an entire cDNA insert is termed the "Full-

Insert Sequence" ("FIS"). A "Contig" sequence is a sequence assembled from two or more sequences that can be selected from, but not limited to, the group consisting of an EST, FIS and PCR sequence. A sequence encoding an entire or functional protein is termed a "Complete Gene Sequence" ("CGS") and can be derived from an FIS or a contig.

A "trait" refers to a physiological, morphological, biochemical, or physical characteristic of a plant or particular plant material or cell. In some instances, this characteristic is visible to the human eye, such as seed or plant size, or can be measured by biochemical techniques, such as detecting the protein, starch, or oil content of seed or leaves, or by observation of a metabolic or physiological process, e.g. by measuring tolerance to water deprivation or particular salt or sugar concentrations, or by the observation of the expression level of a gene or genes, or by agricultural observations such as osmotic stress tolerance or yield.

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

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

"Plant" includes reference to whole plants, plant organs, plant tissues, plant propagules, 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 plant" includes reference to a plant which comprises within its genome a heterologous polynucleotide. For example, 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.

The commercial development of genetically improved germplasm has also advanced to the stage of introducing multiple traits into crop plants, often referred to as a gene stacking approach. In this approach, multiple genes conferring different characteristics of interest can be introduced into a plant. Gene stacking can be accomplished by many means including but not limited to co-transformation, retransformation, and crossing lines with different transgenes.

"Transgenic plant" also includes reference to plants which comprise more than one heterologous polynucleotide within their genome. Each heterologous polynucleotide may confer a different trait to the transgenic plant.

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

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

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

10 "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.

"Coding region" refers to the portion of a messenger RNA (or the corresponding portion of another nucleic acid molecule such as a DNA molecule) which encodes a protein or polypeptide. "Non-coding region" refers to all portions of a messenger RNA or other nucleic acid molecule that are not a coding region, including but not limited to, for example, the promoter region, 5' untranslated region ("UTR"), 3' UTR, intron and terminator. The terms "coding region" and "coding sequence" are used interchangeably herein. The terms "non-coding region" and "non-coding sequence" are used interchangeably herein.

25 "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.

30 "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
5 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
10 techniques. "Recombinant" also includes reference to a cell or vector, that has been modified by the introduction of a heterologous nucleic acid or a cell derived from a cell so modified, but does not encompass the alteration of the cell or vector by naturally occurring events (e.g., spontaneous mutation, natural transformation/transduction/transposition) such as those
15 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,
20 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 "entry clone" and "entry vector" are used interchangeably herein.

25 "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, but are not limited to, promoters, translation leader sequences, introns, and polyadenylation
30 recognition sequences. The terms "regulatory sequence" and "regulatory element" are used interchangeably herein.

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

Promoters that cause a gene to be expressed in most cell types at most times are commonly referred to as "constitutive promoters."

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

"Tissue-specific promoter" and "tissue-preferred promoter" are used interchangeably, and refer to a promoter that is expressed predominantly but not necessarily exclusively in one tissue or organ, but that may also be
10 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.
15 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
20 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.

"Overexpression" refers to the production of a gene product in transgenic organisms that exceeds levels of production in a null
25 segregating (or non-transgenic) organism from the same experiment.

"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
30 "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).

5 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.

10 "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.

15 "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.

20 The term "crossed" or "cross" means the fusion of gametes via pollination to produce progeny (e.g., cells, seeds or plants). The term encompasses both sexual crosses (the pollination of one plant by another) and selfing (self-pollination, e.g., when the pollen and ovule are from the same plant). The term "crossing" refers to the act of fusing gametes via pollination to produce progeny.

25 The term "locus" generally refers to a genetically defined region of a chromosome carrying a gene or, possibly, two or more genes so closely linked that genetically they behave as a single locus responsible for a phenotype. When used herein with respect to *ZmCrw2*, the "*ZmCrw2* locus" shall refer to the defined region of the chromosome carrying the *ZmCrw2* gene including its associated regulatory sequences. Similarly, when used herein with respect to *ZmCrw1*, the "*ZmCrw1* locus" shall refer to the defined region of the chromosome carrying the *ZmCrw1* gene
30 including its associated regulatory sequences.

A "gene" shall refer to a specific genetic coding region within a locus, including its associated regulatory sequences. One of ordinary skill in the art would understand that the associated regulatory sequences will be within a distance of about 4 kb from the *Zmcrw2* coding sequence, with
5 the promoter located upstream.

"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
10 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.

A "favorable allele" is the allele at a particular locus that confers, or
15 contributes to, a desirable phenotype, or alternatively, is an allele that allows the identification of plants that do not have the desirable phenotype so that they can be removed from a breeding program or planting. A favorable allele of a marker is a marker allele that segregates with the favorable phenotype, or alternatively, segregates with the unfavorable
20 plant phenotype, therefore providing the benefit of identifying plants.

"Germplasm" refers to genetic material of or from an individual (e.g., a plant), a group of individuals (e.g., a plant line, variety or family), or a clone derived from a line, variety, species, or culture. The germplasm can be part of an organism or cell, or can be separate from the organism
25 or cell. In general, germplasm provides genetic material with a specific molecular makeup that provides a physical foundation for some or all of the hereditary qualities of an organism or cell culture. As used herein, germplasm includes cells, seed or tissues from which new plants may be grown, or plant parts, such as leaves, stems, pollen, or cells, that can be
30 cultured into a whole plant.

Sequence alignments and percent identity calculations may be determined using a variety of comparison methods designed to detect

homologous sequences including, but not limited to, the Megalign® program of the LASERGENE® bioinformatics computing suite (DNASTAR® Inc., Madison, WI). Unless stated otherwise, multiple alignment of the sequences provided herein were performed using the
5 Clustal W method of alignment.

The Clustal W method of alignment (described by Higgins and Sharp, CABIOS. 5:151-153 (1989); Higgins, D. G. et al., Comput. Appl. Biosci. 8:189-191 (1992)) can be found in the MegAlign™ v6.1 program of the LASERGENE® bioinformatics computing suite (DNASTAR® Inc.,
10 Madison, Wis.). Default parameters for multiple alignment correspond to GAP PENALTY=10, GAP LENGTH PENALTY=0.2, Delay Divergent Sequences=30%, DNA Transition Weight=0.5, Protein Weight Matrix=Gonnet Series, DNA Weight Matrix=IUB. For pairwise alignments the default parameters are Alignment=Slow-Accurate, Gap Penalty=10.0,
15 Gap Length=0.10, Protein Weight Matrix=Gonnet 250 and DNA Weight Matrix=IUB.

After alignment of the sequences, using the Clustal W program, it is possible to obtain “percent identity” and “divergence” values by viewing the “sequence distances” table on the same program; unless stated
20 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
25 Press: Cold Spring Harbor, 1989 (hereinafter “Sambrook”).

Isolated polynucleotides, cDNAs, polypeptides, recombinant DNA constructs useful for increasing a plant's resistance to an insect pest are provided herein. Also provided are compositions (such as plants or seeds) comprising these recombinant DNA constructs and methods
30 utilizing these recombinant DNA constructs.

CRW2

***crw2-Mutag* (corn rootworm susceptible) and *crw2-EMS* are maize mutants whose leaves are devoured by the Western corn rootworm (WCR) beetle. The polypeptide encoded by *Crw2* is a glycosyltransferase. Glycosyltransferases are members of a large superfamily that can transfer single or multiple activated sugars to a wide range of small molecular acceptors in plants. Recent studies have shown that glycosyltransferases in plants may have roles in numerous processes of plant growth, development, and response to the environment (Wang, J. and Hou, B. (2009) *Front. Biol. China* 4:39-46).**

The mutant alleles of *ZmCrw2* (SEQ ID NO:1) are inherited in a recessive fashion and inheritance is controlled by a single gene. *ZmCRW2* (SEQ ID NO:2) is a glycosyltransferase from *Zea mays*. *CRW2* can refer to the *ZmCRW2* (SEQ ID NO:2) or any of its homologs (SEQ ID NOs: 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 22, and 23).

CRW1

crw1 (corn rootworm susceptible) is a maize mutant whose leaves are devoured by the Western corn rootworm (WCR) beetle (Dhillon B, Moose SP; and Johal GS. (2007). *crw1* - A novel maize mutant exceptionally susceptible to Western Corn Rootworm. *Maize Genetics Conference*. March 22-25, St. Charles, Illinois. Abstract and Presentation available online), which is unusual because the WCR beetle normally feeds on maize pollen and silks and not leaves. Thus, it appears that a mechanism that normally renders maize leaves unpalatable to the WCR beetle is compromised in the mutant.

ZmCrw1 is inherited in a recessive fashion and is controlled by a single gene. *CRW1* (SEQ ID NO:26) is a NAC transcription factor from *Zea mays*. *CRW1* can refer to the *ZmCRW1* (SEQ ID NO:26) or any of its homologs (SEQ ID NOs:27-44).

Isolated polynucleotides, cDNAs and polypeptides

The present disclosure includes the following isolated polynucleotides, cDNAs and polypeptides:

An isolated polynucleotide or cDNA 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 W method of alignment, when compared to SEQ ID NO:2, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 22, and 23, and combinations thereof; 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 of the present disclosure. The polypeptide is preferably a CRW2 polypeptide.

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 W method of alignment, when compared to SEQ ID NO:2, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 22, and 23, and combinations thereof. The polypeptide is preferably a CRW2 polypeptide.

An isolated polynucleotide or cDNA 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 W method of alignment, when compared to SEQ ID NO:1, 19, or 21; 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 of the present disclosure.

An isolated polynucleotide or cDNA comprising a nucleotide sequence, wherein the nucleotide sequence is hybridizable under stringent conditions with a DNA molecule comprising the full complement of SEQ ID NO:1, 19, or 21.

An isolated polynucleotide or cDNA comprising a nucleotide sequence, wherein the nucleotide sequence is derived from SEQ ID NO:1, 19, or 21 by alteration of one or more nucleotides by at least one method selected from the group consisting of: deletion, substitution, addition and insertion.

An isolated polynucleotide or cDNA comprising a nucleotide sequence, wherein the nucleotide sequence corresponds to an allele of SEQ ID NO:1, 19, or 21.

An isolated polynucleotide or cDNA comprising: (i) a nucleic acid sequence encoding a polypeptide having an amino acid sequence of at least 90% sequence identity, based on the Clustal W method of alignment, when compared to SEQ ID NO:20 or 22; or (ii) a full complement of the nucleic acid sequence of (i).

It is understood, as those skilled in the art will appreciate, that the disclosure 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.

The protein of the current disclosure may also be a protein which
5 comprises an amino acid sequence comprising deletion, substitution,
insertion and/or addition of one or more amino acids in an amino acid
sequence presented in SEQ ID NO: 2, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16,
17, 18, 20, 22, and 23. The substitution may be conservative, which
means the replacement of a certain amino acid residue by another residue
10 having similar physical and chemical characteristics. Non-limiting
examples of conservative substitution include replacement between
aliphatic group-containing amino acid residues such as Ile, Val, Leu or Ala,
and replacement between polar residues such as Lys-Arg, Glu-Asp or Gln-
Asn replacement.

15 Proteins derived by amino acid deletion, substitution, insertion
and/or addition can be prepared when DNAs encoding their wild-type
proteins are subjected to, for example, well-known site-directed
mutagenesis (see, e.g., *Nucleic Acid Research*, Vol. 10, No. 20, p.6487-
6500, 1982, which is hereby incorporated by reference in its entirety). As
20 used herein, the term "one or more amino acids" is intended to mean a
possible number of amino acids which may be deleted, substituted,
inserted and/or added by site-directed mutagenesis.

Site-directed mutagenesis may be accomplished, for example, as
follows using a synthetic oligonucleotide primer that is complementary to
25 single-stranded phage DNA to be mutated, except for having a specific
mismatch (i.e., a desired mutation). Namely, the above synthetic
oligonucleotide is used as a primer to cause synthesis of a complementary
strand by phages, and the resulting duplex DNA is then used to transform
host cells. The transformed bacterial culture is plated on agar, whereby
30 plaques are allowed to form from phage-containing single cells. As a
result, in theory, 50% of new colonies contain phages with the mutation as
a single strand, while the remaining 50% have the original sequence. At a

temperature which allows hybridization with DNA completely identical to one having the above desired mutation, but not with DNA having the original strand, the resulting plaques are allowed to hybridize with a synthetic probe labeled by kinase treatment. Subsequently, plaques
5 hybridized with the probe are picked up and cultured for collection of their DNA.

Techniques for allowing deletion, substitution, insertion and/or addition of one or more amino acids in the amino acid sequences of biologically active peptides such as enzymes while retaining their activity
10 include site-directed mutagenesis mentioned above, as well as other techniques such as those for treating a gene with a mutagen, and those in which a gene is selectively cleaved to remove, substitute, insert or add a selected nucleotide or nucleotides, and then ligated.

The protein of the present disclosure may also be a protein which is
15 encoded by a nucleic acid comprising a nucleotide sequence comprising deletion, substitution, insertion and/or addition of one or more nucleotides in the nucleotide sequence of SEQ ID NO:1, 19, or 21. Nucleotide deletion, substitution, insertion and/or addition may be accomplished by site-directed mutagenesis or other techniques as mentioned above.

20 The protein of the present disclosure may also be a protein which is encoded by a nucleic acid comprising a nucleotide sequence hybridizable under stringent conditions with the complementary strand of the nucleotide sequence of SEQ ID NO:1, 19, or 21.

The term "under stringent conditions" means that two sequences
25 hybridize under moderately or highly stringent conditions. More specifically, moderately stringent conditions can be readily determined by those having ordinary skill in the art, e.g., depending on the length of DNA. The basic conditions are set forth by Sambrook et al., *Molecular Cloning: A Laboratory Manual, third edition*, chapters 6 and 7, Cold Spring Harbor
30 Laboratory Press, 2001 and include the use of a prewashing solution for nitrocellulose filters 5xSSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0), hybridization conditions of about 50% formamide, 2xSSC to 6xSSC at

about 40-50 °C (or other similar hybridization solutions, such as Stark's solution, in about 50% formamide at about 42 °C) and washing conditions of, for example, about 40-60 °C, 0.5-6xSSC, 0.1% SDS. Preferably, moderately stringent conditions include hybridization (and washing) at
5 about 50 °C and 6xSSC. Highly stringent conditions can also be readily determined by those skilled in the art, e.g., depending on the length of DNA.

Generally, such conditions include hybridization and/or washing at higher temperature and/or lower salt concentration (such as hybridization
10 at about 65 °C, 6xSSC to 0.2xSSC, preferably 6xSSC, more preferably 2xSSC, most preferably 0.2xSSC), compared to the moderately stringent conditions. For example, highly stringent conditions may include hybridization as defined above, and washing at approximately 65-68 °C, 0.2xSSC, 0.1% SDS. SSPE (1xSSPE is 0.15 M NaCl, 10 mM NaH₂PO₄,
15 and 1.25 mM EDTA, pH 7.4) can be substituted for SSC (1xSSC is 0.15 M NaCl and 15 mM sodium citrate) in the hybridization and washing buffers; washing is performed for 15 minutes after hybridization is completed.

It is also possible to use a commercially available hybridization kit which uses no radioactive substance as a probe. Specific examples
20 include hybridization with an ECL direct labeling & detection system (Amersham). Stringent conditions include, for example, hybridization at 42 °C for 4 hours using the hybridization buffer included in the kit, which is supplemented with 5% (w/v) Blocking reagent and 0.5 M NaCl, and washing twice in 0.4% SDS, 0.5xSSC at 55 °C for 20 minutes and once in
25 2xSSC at room temperature for 5 minutes.

Recombinant DNA Constructs

In one aspect, the present disclosure includes recombinant DNA constructs.

In one embodiment, a recombinant DNA construct comprises a
30 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%

- 5 sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NO: 2, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 22, and 23, and combinations thereof; or (ii) a full complement of the nucleic acid sequence of (i).

- 10 In another 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%, 15 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:1, 19, or 21, and combinations thereof; or (ii) a full complement of the nucleic acid sequence of (i).

- 20 In another 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 CRW2 polypeptide. The CRW2 polypeptide may be from *Arabidopsis thaliana*, *Zea mays*, *Glycine max*, *Glycine tabacina*, *Glycine soja*, *Glycine tomentella*, *Oryza sativa*, *Brachypodium distachyon*, *Vitis vinifera*, 25 *Gossypium mexicanum*, *Pyrus malus*, *Hordeum vulgare*, *Brassica napus*, *Sorghum bicolor*, *Saccharum officinarum*, *Triticum aestivum*, *Paspalum notatum*, and *Eragrostis nindensis*.

- A recombinant DNA construct comprising an isolated polynucleotide 30 comprising: (i) a nucleic acid sequence encoding a polypeptide having an amino acid sequence of at least 90% sequence identity, based on the Clustal W method of alignment, when compared to SEQ ID NO:20 or 22;

or (ii) a full complement of the nucleic acid sequence of (i), operably linked to at least one regulatory element is provided herein. The at least one regulatory element may be a promoter. For example, a promoter may be any one disclosed herein, such as a root-specific promoter or the maize ubiquitin promoter.

Regulatory Sequences

A recombinant DNA construct of the present disclosure may comprise at least one regulatory sequence.

A regulatory sequence may be a promoter.

A number of promoters can be used in recombinant DNA constructs of the present disclosure. 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.

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), the constitutive synthetic core promoter SCP1 (International Publication No. 03/033651) 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.

Tissue-specific promoters useful in the current disclosure may 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 No. 1063664).

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.

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

 An intron sequence can be added to the 5' untranslated region, the protein-coding region or the 3' untranslated region 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
15 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).

 Any plant can be selected for the identification of regulatory
20 sequences and genes encoding CRW2 polypeptides to be used in recombinant DNA constructs and other compositions (e.g. transgenic plants, seeds and cells) and methods of the present disclosure. Examples of suitable plants for the isolation of genes and regulatory sequences and for compositions and methods of the present disclosure would include but
25 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,
30 Douglas fir, eggplant, endive, escarole, eucalyptus, fennel, figs, garlic, gourd, grape, grapefruit, honey dew, jicama, kiwifruit, lettuce, leeks, lemon, lime, Loblolly pine, linseed, mango, melon, mushroom, nectarine,

nut, oat, oil palm, oil seed rape, okra, olive, onion, orange, an ornamental plant, palm, papaya, parsley, parsnip, pea, peach, peanut, pear, pepper, persimmon, pine, pineapple, plantain, plum, pomegranate, poplar, potato, pumpkin, quince, radiata pine, radicchio, radish, rapeseed, raspberry, rice, 5 rye, sorghum, Southern pine, soybean, spinach, squash, strawberry, sugarbeet, sugarcane, sunflower, sweet potato, sweetgum, switchgrass, tangerine, tea, tobacco, tomato, triticale, turf, turnip, a vine, watermelon, wheat, yams, and zucchini.

Compositions

10 A composition of the present disclosure includes a transgenic microorganism, cell, plant, and seed comprising the recombinant DNA construct. The cell may be eukaryotic, e.g., a yeast, insect or plant cell, or prokaryotic, e.g., a bacterial cell.

A composition of the present disclosure is a plant comprising in its 15 genome any of the recombinant DNA constructs of the present disclosure. Compositions also include any progeny of the plant, and any seed obtained from the plant or its progeny, wherein the progeny or seed comprises within its genome the recombinant DNA construct. Progeny includes subsequent generations obtained by self-pollination or out- 20 crossing of a plant. Progeny also includes hybrids and inbreds. 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. These seeds can be grown to produce plants that would exhibit 25 increased resistance to herbivory by an insect pest, or used in a breeding program to produce hybrid seed, which can be grown to produce plants that would exhibit increased resistance to herbivory by an insect pest. The seeds may be maize seeds.

The plant may be a monocotyledonous or dicotyledonous plant, for 30 example, a maize or soybean 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, millet, sugar cane or switchgrass.

The recombinant DNA construct may be stably integrated into the genome of the plant.

Particular embodiments include but are not limited to the following:

1. A plant (for example, a maize 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: 2, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 22, and 23, and wherein said plant exhibits increased resistance to herbivory by an insect pest when compared to a control plant not comprising said recombinant DNA construct.
2. A plant (for example, a maize 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 CRW2 polypeptide, and wherein said plant exhibits increased resistance to herbivory by an insect pest when compared to a control plant not comprising said recombinant DNA construct.
3. A plant (for example, a maize plant) comprising in its genome a recombinant DNA construct comprising a polynucleotide operably linked to at least one regulatory element, wherein said polynucleotide comprises a nucleotide sequence, wherein the nucleotide sequence is: (a) hybridizable under stringent conditions with a DNA molecule comprising the full complement of SEQ ID NO:1, 19, or 21; or (b) derived from SEQ ID NO:1, 19, or 21 by alteration of one or more nucleotides by at least one method selected from the group consisting of: deletion, substitution, addition and insertion; and wherein said plant exhibits increased resistance to herbivory by an insect pest, when compared to a control plant not comprising said

recombinant DNA construct.

4. A plant (for example, a maize plant) comprising in its genome (A) a first recombinant DNA construct comprising a polynucleotide operably linked to at least one regulatory element, wherein said polynucleotide is
5 selected from the group consisting of: (i) a polynucleotide encoding a polypeptide having the amino acid sequence set forth in SEQ ID NO:2, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 22, and 23; (ii) a polynucleotide encoding a polypeptide having an amino acid sequence of at least 80% sequence identity, based on the Clustal W method of alignment, when
10 compared to SEQ ID NO:2, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 22, and 23, and (iii) a polynucleotide having a nucleotide sequence that is fully complementary to the nucleotide sequence of a polynucleotide of (i) or (ii); and (B) a second recombinant DNA construct comprising a polynucleotide operably linked to at least one regulatory element, wherein
15 said polynucleotide is selected from the group consisting of: (iv) a polynucleotide encoding a polypeptide having the amino acid sequence set forth in SEQ ID NO:26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, and 44; (v) a polynucleotide encoding a polypeptide having an amino acid sequence of at least 80% sequence identity, based
20 on the Clustal W method of alignment, when compared to SEQ ID NO:26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, and 44, and (vi) a polynucleotide having a nucleotide sequence that is fully complementary to the nucleotide sequence of a polynucleotide of (i) or (ii); and wherein said plant exhibits increased resistance to herbivory by an
25 insect pest when compared to a control plant not comprising said recombinant DNA construct.

5. Any progeny of the plants in the embodiments described herein, any seeds of the plants in the embodiments described herein, any seeds of progeny of the plants in embodiments described herein, and cells from
30 any of the above plants in embodiments described herein and progeny thereof.

A plant cell comprising a recombinant DNA construct comprising a recombinant DNA construct comprising 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 W method of alignment, when compared to SEQ ID NO:20 or 22; or (ii) a full complement of the nucleic acid sequence of (i), operably linked to at least one regulatory element is provided herein. The at least one regulatory element may be a promoter. For example, a promoter may be any one disclosed herein, such as a root-specific promoter or the maize ubiquitin promoter. Also provided is a plant comprising such a plant cell. Such a plant may display increased resistance to herbivory by an insect pest. The insect pest may be a Coleopteran insect pest. The Coleopteran insect pest may be of the genus *Diabrotica* or *Popillia*. The insect pest may be a Lepidoperan insect pest. The Lepidoperan insect pest may be European corn borer. The plant may be a monocot. The plant may be maize..

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 W method of alignment, when compared to SEQ ID NO:2, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 22, and 23, and wherein said plant exhibits increased resistance to herbivory by an insect pest when compared to a control plant not comprising said recombinant DNA construct is provided herein. The at least one regulatory element may be a promoter. For example, a promoter may be any one disclosed herein, such as a root-specific promoter or the maize ubiquitin promoter. Also provided is a plant comprising such a plant cell. Such a plant may display increased resistance to herbivory by an insect pest. The insect pest may be a Coleopteran insect pest. The Coleopteran insect pest may be of the genus *Diabrotica* or *Popillia*. The insect pest may be a Lepidoperan insect

pest. The Lepidopteran insect pest may be European corn borer. The plant may be a monocot. The plant may be maize.

Also provided is a plant comprising in its genome: (a) a first recombinant DNA construct comprising a polynucleotide operably linked to
5 at least one regulatory element, wherein said polynucleotide is selected from the group consisting of: (i) a polynucleotide encoding a polypeptide having the amino acid sequence set forth in SEQ ID NO:2, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 22, and 23; (ii) a polynucleotide encoding a polypeptide having an amino acid sequence of at least 80% sequence
10 identity, based on the Clustal W method of alignment, when compared to SEQ ID NO:2, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 22, and 23, and (iii) a polynucleotide having a nucleotide sequence that is fully complementary to the nucleotide sequence of a polynucleotide of (i) or (ii); and (b) a second recombinant DNA construct comprising a polynucleotide
15 operably linked to at least one regulatory element, wherein said polynucleotide is selected from the group consisting of: (iv) a polynucleotide encoding a polypeptide having the amino acid sequence set forth in SEQ ID NO:26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, and 44; (v) a polynucleotide encoding a polypeptide
20 having an amino acid sequence of at least 80% sequence identity, based on the Clustal W method of alignment, when compared to SEQ ID NO:26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, and 44, and (vi) a polynucleotide having a nucleotide sequence that is fully complementary to the nucleotide sequence of a polynucleotide of (i) or (ii);
25 and wherein said plant exhibits increased resistance to herbivory by an insect pest when compared to a control plant not comprising said recombinant DNA construct. The at least one regulatory element may be a promoter. For example, a promoter may be any one disclosed herein, such as a root-specific promoter or the maize ubiquitin promoter. A plant
30 comprising such a plant cell is further provided herein. Such a plant may display increased resistance to herbivory by an insect pest. The insect pest may be a Coleopteran insect pest. The Coleopteran insect pest may

be of the genus *Diabrotica* or *Popillia*. The insect pest may be a Lepidoperan insect pest. The Lepidopteran insect pest may be European corn borer. The plant may be a monocot. The plant may be maize.

In any of the embodiments described herein, the CRW1 and/or
5 CRW2 polypeptide may be from *Arabidopsis thaliana*, *Zea mays*, *Glycine max*, *Glycine tabacina*, *Glycine soja*, *Glycine tomentella*, *Oryza sativa*, *Brachypodium distachyon*, *Vitis vinifera*, *Gossypium mexicanum*, *Pyrus malus*, *Hordeum vulgare*, *Brassica napus*, *Sorghum bicolor*, *Saccharum officinarum*, or *Triticum aestivum*.

10 In any of the embodiments described herein, the recombinant DNA construct may comprise at least a promoter functional in a plant as a regulatory sequence.

In any of the embodiments described herein, the plant (for example, the maize plant) may exhibit less yield loss relative to the control plants,
15 for example, at least 25%, at least 20%, at least 15%, at least 10% or at least 5% less yield loss, under similar environmental conditions and pest pressure.

"Insect pest pressure" refers to the level of infestation of an insect.

"Insect" and "insect pest" are used interchangeably herein.

20 The insect may be in the adult or larval stage. The adult stage is of particular interest.

The insect may be in the order Coleoptera, and the Coleopteran insect may be of the genus *Diabrotica* or *Popillia*.

Diabrotica is a widespread genus of beetles that includes several
25 destructive agricultural pest species including, for example, corn rootworms. Corn rootworms are one of the most economically destructive insects of maize in the United States. The Western corn rootworm, *D. virgifera virgifera*, and the Northern corn rootworm, *D. barberi*, are the most devastating rootworm species in Iowa, a major corn-growing area. A
30 third species, the Southern corn rootworm, *D. undecimpunctata howardi*, causes much economic damage in other regions.

Popillia is a genus of scarab beetles, and the most familiar is the Japanese beetle (*Popillia japonica*). The Japanese beetle is a significant insect pest responsible for crop losses around the world.

The insect may be in the order Lepidoptera, and the Lepidopteran insect may be in the genus *Ostrinia*, *Hyphantria*, or *Simyra*. *Ostrinia* is a genus of moths, and one such member is the European Corn Borer, a serious pest of maize.

An insect pest as set forth in the methods of the disclosure may include but is not limited to any species of corn rootworm (such as but not limited to the Western corn rootworm), the Japanese beetle, European Corn Borer, fall webworm (*Hyphantria cunea*), or cattail caterpillar (*Simyra insularis*).

“Herbivory” as used herein is the consumption of living plant tissue by insects. The plant tissue may be tissue from any plant part including but not limited to leaves, stem, roots, reproductive parts, etc. Chronic attack by herbivores can have dramatic cumulative effects on the size, longevity, or reproductive output of individual plants.

“Susceptibility” refers to the inability of a plant variety to restrict the growth and development of a specified pest.

“Resistance” refers to the ability of a plant variety to restrict the growth and development of a specified pest and/or the damage they cause when compared to susceptible plant varieties under similar environmental conditions and pest pressure.

Typically, when a transgenic plant comprising a recombinant DNA construct in its genome exhibits increased resistance to herbivory by an insect pest relative to a reference or control plant, the reference or control plant does not comprise in its genome the recombinant DNA construct.

One of ordinary skill in the art is familiar with protocols for evaluating insect response (i.e. attractiveness or repulsiveness) to plant tissue and for evaluating a plant’s level of resistance to an insect pest.

In addition, as presented herein, one can perform a feeding choice assay as a way to assess the level of a plant’s resistance (e.g. a maize

plant) to an insect pest such as the corn rootworm beetle. In this assay, a PVC box containing a detachable lid is used to contain the insects and plant hosts, and equal weights of freshly harvested mature leaves of the plant hosts are affixed to moist filter paper in a randomized manner.

- 5 Insects are starved overnight and placed in the box with the tissue. Leaf feeding can be scored on a scale from 0 to 5, with 0 indicating no damage and 5 indicating complete decimation.

One can also evaluate a plant's resistance to an insect pest by the plant's ability to maintain sufficient yield (at least 75%, 76%, 77%, 78%,
10 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% yield) in field testing under sufficient pest pressure.

One of ordinary skill in the art would readily recognize a suitable control or reference plant to be utilized when assessing or measuring a
15 phenotype of a transgenic plant in any embodiment of the present disclosure in which a control 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
20 a recombinant DNA construct, such that the progeny are segregating into plants either comprising or not comprising the recombinant DNA construct: the progeny comprising the recombinant DNA construct would be typically measured relative to the progeny not comprising the recombinant DNA construct (i.e., the progeny not comprising the recombinant DNA construct
25 is the control or reference plant).
2. Introgression of a recombinant 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).
- 30 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: the second hybrid line would typically be measured relative to the first hybrid line (i.e., the first hybrid line is the control or reference plant).

4. A plant comprising a recombinant DNA construct: the plant may be assessed or measured relative to a control plant not comprising the recombinant 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). 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 a phenotype of a transgenic plant would not include a plant that had been previously selected, via mutagenesis or transformation, for the desired phenotype.

Methods

- Methods include but are not limited to methods for increasing resistance to herbivory by an insect pest in a plant, methods for evaluating resistance to an insect pest in a plant, methods of identifying variants and/or naturally occurring alleles of *Crw2* that give plants increased resistance to herbivory by an insect pest, and methods for producing seed. The plant may be a monocotyledonous or dicotyledonous plant, for example, a maize or soybean plant. The plant may also be sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley, millet, sugar cane or

sorghum. The seed may be a maize or soybean seed, for example, a maize hybrid seed or maize inbred seed.

Methods include but are not limited to the following:

A method for transforming a cell (or microorganism) comprising
5 transforming a cell (or microorganism) with any of the isolated
polynucleotides or recombinant DNA constructs of the present disclosure.
The cell (or microorganism) transformed by this method is also included.
In particular embodiments, the cell is eukaryotic cell, e.g., a yeast, insect
or plant cell, or prokaryotic, e.g., a bacterial cell. The microorganism may
10 be *Agrobacterium*, e.g. *Agrobacterium tumefaciens* or *Agrobacterium*
rhizogenes.

A method for producing a transgenic plant comprising transforming
a plant cell with any of the isolated polynucleotides or recombinant DNA
constructs of the present disclosure and regenerating a transgenic plant
15 from the transformed plant cell. The disclosure is also directed to the
transgenic plant produced by this method, and transgenic seed obtained
from this transgenic plant. The transgenic plant obtained by this method
may be used in other methods of the present disclosure.

A method for isolating a polypeptide of the disclosure from a cell or
20 culture medium of the cell, wherein the cell comprises a recombinant DNA
construct comprising a polynucleotide of the disclosure operably linked to
at least one regulatory sequence, and wherein the transformed host cell is
grown under conditions that are suitable for expression of the recombinant
DNA construct.

25 A method of altering the level of expression of a polypeptide of the
disclosure in a host cell comprising: (a) transforming a host cell with a
recombinant DNA construct of the present disclosure; and (b) growing the
transformed host cell under conditions that are suitable for expression of
the recombinant DNA construct wherein expression of the recombinant
30 DNA construct results in production of altered levels of the polypeptide of
the disclosure in the transformed host cell.

A method of increasing resistance to herbivory by an insect pest 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 (for example, 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: 2, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 22, and 23; 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 increased resistance to a plant pest 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, wherein said progeny plant comprises in its genome the recombinant DNA construct and exhibits increased resistance to herbivory by an insect pest when compared to a control plant not comprising the recombinant DNA construct. The at least one regulatory element may be a promoter. For example, a promoter may be a root-specific promoter or the maize ubiquitin promoter. The insect pest may be a Coleopteran insect pest. The insect pest may be a Coleopteran insect pest of the genus *Diabrotica* or *Popillia*. The insect pest may be a Lepidoperan insect pest. The Lepidoperan insect pest may be the European corn borer.

A method of increasing resistance to herbivory by an insect pest in a plant, comprising: (A) introducing into a regenerable plant cell: (i) a first recombinant DNA construct comprising a polynucleotide operably linked to at least one regulatory element, wherein said polynucleotide is selected from the group consisting of: (a) a polynucleotide encoding a polypeptide having the amino acid sequence set forth in SEQ ID NO:2, 7, 8, 9, 10, 11,

12, 13, 14, 15, 16, 17, 18, 20, 22, and 23; (b) a polynucleotide encoding a polypeptide having an amino acid sequence of at least 80% sequence identity, based on the Clustal W method of alignment, when compared to SEQ ID NO:2, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 22, and 23, and (c) a polynucleotide having a nucleotide sequence that is fully complementary to the nucleotide sequence of a polynucleotide of (i) or (ii); and (ii) a second recombinant DNA construct comprising a polynucleotide operably linked to at least one regulatory element, wherein said polynucleotide is selected from the group consisting of: (a) a polynucleotide encoding a polypeptide having the amino acid sequence set forth in SEQ ID NO:26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, and 44; (b) a polynucleotide encoding a polypeptide having an amino acid sequence of at least 80% sequence identity, based on the Clustal W method of alignment, when compared to SEQ ID NO:26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, and 44, and (c) a polynucleotide having a nucleotide sequence that is fully complementary to the nucleotide sequence of a polynucleotide of (i) or (ii); 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 increased resistance to a plant pest 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, wherein said progeny plant comprises in its genome the recombinant DNA construct and exhibits increased resistance to herbivory by an insect pest when compared to a control plant not comprising the recombinant DNA construct. The at least one regulatory element may be a promoter. For example, a promoter may be any one disclosed herein, such as a root-specific promoter or the maize ubiquitin promoter. A plant comprising such a plant cell is further provided herein. Such a plant may display increased resistance to herbivory by an insect pest. The insect pest may be a Coleopteran insect pest. The Coleopteran insect pest may be of the genus *Diabrotica* or *Popillia*. The

insect pest may be a Lepidoperan insect pest. The Lepidopteran insect pest may be European corn borer. The plant may be a monocot. The plant may be maize.

5 A method of increasing resistance to herbivory by an insect pest in a plant, the method comprising: (a) introducing into a regenerable plant cell a recombinant DNA construct comprising a polynucleotide operably linked to at least one regulatory element, wherein said polynucleotide comprises a nucleotide sequence, wherein the nucleotide sequence is: (a) hybridizable under stringent conditions with a DNA molecule comprising
10 the full complement of SEQ ID NO:1, 19, or 21; or (b) derived from SEQ ID NO:1, 19, or 21 by alteration of one or more nucleotides by at least one method selected from the group consisting of: deletion, substitution, addition and insertion; and (b) regenerating a transgenic plant from the regenerable plant cell after step (a), wherein the transgenic plant
15 comprises in its genome the recombinant DNA construct and exhibits increased resistance to herbivory by an insect pest in a plant 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, wherein said progeny plant comprises in
20 its genome the recombinant DNA construct and exhibits increased resistance to herbivory by an insect pest in a plant, when compared to a control plant not comprising the recombinant DNA construct.

A method of evaluating resistance to an insect pest in a plant, comprising (a) obtaining a transgenic plant, wherein the transgenic plant
25 comprises in its genome a recombinant DNA construct comprising a polynucleotide operably linked to at least one regulatory sequence (for example, 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%,
30 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: 2, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 22, and 23; (b) obtaining a progeny plant derived from said transgenic plant, wherein the progeny plant comprises in its genome the recombinant DNA construct; and (c) evaluating the progeny plant for resistance to an insect pest in a plant compared to a control plant not comprising the recombinant DNA construct.

In another embodiment, a method of evaluating resistance to an insect pest in a plant, comprising: (a) obtaining a transgenic plant, wherein the transgenic plant comprises 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%, 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: 2, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 22, and 23; (b) growing the transgenic plant of part (a) under conditions wherein the polynucleotide is expressed; and (c) evaluating the transgenic plant of part (b) for resistance to an insect pest in a plant compared to a control plant not comprising the recombinant DNA construct.

A method of evaluating resistance to an insect pest in a plant, the method comprising: (a) obtaining a transgenic plant, wherein the transgenic plant comprises in its genome a recombinant DNA construct comprising a polynucleotide operably linked to at least one regulatory element, wherein said polynucleotide comprises a nucleotide sequence, wherein the nucleotide sequence is: (a) hybridizable under stringent conditions with a DNA molecule comprising the full complement of SEQ ID NO:1, 19, or 21; or (b) derived from SEQ ID NO:1, 19, or 21 by alteration of one or more nucleotides by at least one method selected from the group

consisting of: deletion, substitution, addition and insertion; (b) obtaining a progeny plant derived from said transgenic plant, wherein the progeny plant comprises in its genome the recombinant DNA construct; and (c) evaluating the progeny plant for increased resistance to herbivory by an insect pest in a plant, when compared to a control plant not comprising the recombinant DNA construct.

In any of the preceding methods or any other embodiments of methods of the present disclosure, in said introducing step said regenerable plant cell may comprise a callus cell, an embryogenic callus cell, a gametic cell, a meristematic cell, or a cell of an immature embryo. The regenerable plant cells may derive from an inbred maize plant.

In any of the preceding methods or any other embodiments of methods of the present disclosure, said regenerating step may comprise the following: (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.

In any of the preceding methods or any other embodiments of methods of the present disclosure, at least one regulatory element may be a promoter. For example, a promoter may be a root-specific promoter or the maize ubiquitin promoter. An insect pest may be a Coleopteran insect pest. The insect pest may be a Coleopteran insect pest of the genus *Diabrotica* or *Popillia*. The insect pest may be a Lepidoperan insect pest. The Lepidoperan insect pest may be the European corn borer. Phenotyping may be established using a feeding choice assay.

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

Techniques for plant transformation and regeneration have been described in International Patent Publication WO 2009/006276, the contents of which are herein incorporated by reference.

The development or regeneration of plants containing the foreign,
5 exogenous isolated nucleic acid fragment that encodes a protein of interest is well known in the art. The regenerated plants may be 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
10 important lines is used to pollinate regenerated plants. A transgenic plant of the present disclosure containing a desired polypeptide is cultivated using methods well known to one skilled in the art.

In another embodiment, a method of identifying a variant of *Crw2* that gives plants increased resistance to herbivory by an insect pest is
15 provided. Such method comprises: (a) combining through gene shuffling one or more nucleotide sequences encoding one or more fragments of SEQ ID NO: 2, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 22, and 23, or a protein that is at least 70% identical to SEQ ID NO: 2, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 22, and 23, or a fragment thereof; (b)
20 transforming the shuffled sequences from step (a) into a population of regenerable plant cells; (c) regenerating a population of transformed plants from the population of transformed regenerable plant cells of step (b); (d) screening the population of transformed plants from step (c) for increased resistance to said insect pest; and (e) identifying the variant from
25 the transformed plant exhibiting the increased resistance. The method can further comprise: (f) introducing into a regenerable plant cell a recombinant construct comprising a variant of *Crw2* that gives plants increased resistance to herbivory by an insect pest; (g) regenerating a transgenic plant from the regenerable plant cell after step (f), wherein the
30 transgenic plant comprises in its genome the recombinant DNA construct; and (h) selecting a transgenic plant of (g), wherein the transgenic plant comprises the recombinant DNA construct and exhibits increased

resistance to said insect pest, when compared to a control plant not comprising the recombinant DNA construct.

The terms "gene shuffling" and "directed evolution" can be used interchangeably herein. The method of "gene shuffling" consists of
5 iterations of DNA shuffling followed by appropriate screening and/or selection to generate variants of *Crw2* nucleic acids or portions thereof having a modified biological activity (Castle et al., (2004) *Science* 304(5674): 1151-4; U.S. Pat. Nos. 5,811,238 and 6,395,547).

Methods of identifying allelic variants of *Crw2* in maize that are
10 associated with increased resistance to herbivory by an insect pest by way of traditional linkage mapping are also provided. In some embodiments, the allelic variants are identified by (a) crossing two maize plants with differing levels of resistance to said insect pest; (b) evaluating allelic variations in the progeny plants with respect to the polynucleotide
15 sequence encoding a protein comprising SEQ ID NO:2, 20, or 22 or in the genomic region that regulates the expression of the polynucleotide encoding the protein; (c) phenotyping the progeny plants for resistance to said insect pest; (d) associating allelic variations with said resistance; and (e) identifying the alleles that are associated with increased resistance
20 to said insect pest. A method comprising identifying an allelic variant of *Crw2* in a maize plant that is associated with increased resistance to herbivory by an insect pest is provided herein in which the method comprises the steps of: a) crossing two maize plants with differing levels of resistance to said insect pest; b) evaluating allelic variations in the
25 progeny plants with respect to the polynucleotide sequence encoding a protein comprising SEQ ID NO:2 or in the genomic region that regulates the expression of the polynucleotide encoding the protein; c) phenotyping the progeny plants for resistance to said insect pest; d) associating allelic variations with said resistance; and e) identifying the alleles that are
30 associated with increased resistance to said insect pest. The phenotyping step (c) could be performed using any method of assessing resistance to an insect pest that is known in the art, for example, resistance for each

plant in the population is obtained from historical data or could be performed using a feeding choice assay presented herein.

In other embodiments the allelic variants are identified through whole genome association analysis by: (a) obtaining a population of
5 maize plants, wherein said maize plants exhibit differing levels of resistance to said insect pest; (b) evaluating allelic variations with respect to the polynucleotide sequence encoding a protein comprising SEQ ID NO:2, or in the genomic region that regulates the expression of the polynucleotide encoding the protein; (c) associating allelic variations with
10 said resistance; and (d) identifying an allelic variant that is associated with increased resistance to the insect pest. Resistance could be assessed using any method known to one of ordinary skill in the art or the feeding choice assay. Alternatively, historical phenotypic data regarding the resistance could also be used.

15 Also provided are methods of identifying a maize plant that exhibits increased resistance to herbivory by an insect pest, the method comprising: (a) detecting the presence of at least one allelic variant of *Crw2* that is associated with increased resistance to said insect pest, in the genome of the maize plant (wherein the allelic variant can be identified
20 using the methods described above); and (b) identifying a maize plant that comprises said at least one allelic variant. The method can further comprise: (c) crossing said maize plant to a second maize plant; and (d) identifying and selecting progeny plants arising from said cross that have said allelic variant.

25 In any of the methods presented above, the insect pest may be in the order Coleoptera, and the Coleopteran insect may be of the genus *Diabrotica* or *Popillia*.

Alternatively, the insect pest may be in the order Lepidoptera, and the Lepidopteran insect may be of the genus *Ostrinia*, *Hyphantria*, or
30 *Simyra*.

The insect pest may be any species of corn rootworm, the Japanese beetle, European Corn Borer, fall webworm (*Hyphantria cunea*),

or cattail caterpillar (*Simyra insularis*).

In any of the methods presented above, the evaluation of resistance to an insect pest can comprise any protocol known to one of ordinary skill in the art. The feeding choice assay presented herein could
5 also be used.

In any of the methods presented above, the plant is a monocot plant and can be maize.

EXAMPLES

The present disclosure is further illustrated in the following
10 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 embodiments of the disclosure, 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
15 disclosure, and without departing from the spirit and scope thereof, can make various changes and modifications of the disclosure to adapt it to various usages and conditions. Thus, various modifications of the disclosure in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such
20 modifications are also intended to fall within the scope of the appended claims.

EXAMPLE 1

Foliar Feeding Choice Assay

A feeding choice assay was performed to assess the level of
25 resistance to corn rootworm beetles in *Crw2* mutant and wild-type plants. A PVC box containing a detachable lid was used, and equal weights of freshly harvested mature leaves from both mutant and wild-type plants are affixed to moist filter paper in a randomized manner. Western corn rootworm beetles and southern corn rootworm beetles, which had been
30 starved overnight, were placed into the box. Previous observations showed that the establishment of feeding preference is usually preceded by random scouting and within the first 45 minutes, and that preferential

feeding usually continues until the leaves of choice are completely devoured. Leaf feeding was scored on a scale from 0 to 5, with 0 indicating no damage and 5 indicating complete decimation.

EXAMPLE 2

5 Cloning and validation of the *Crw2* gene

A maize mutant showing enhanced susceptibility to WCR adults was isolated from an EMS population and named *crw2-EMS*. Independently, another WCR-susceptible mutant named *crw2-Mutag* was identified in an F2 population derived from a *Mu*-active line. The *Crw2* mutants showed enhanced susceptibility to WCR adults as well as to Japanese beetles and European Corn Borer (ECB when evaluated in field testing. Both *crw2-EMS* and *crw2-Mutag* segregated as monogenic recessive, mapped to the same region of chromosome 5, and were allelic to each other in reciprocal crosses. A candidate gene was isolated from the *crw2-Mutag* mutant using co-segregation analysis, and the gene (SEQ ID NO:1) was found to encode a putative Homogalacturonan (HGA1), which is also annotated as "Glycosyltransferase AER61" or a "putative uncharacterized protein". The candidate gene comprised two exons and one intron and encoded a product 445 amino acids (aa) in length (SEQ ID NO:2) with one transmembrane helix (TMH) of 20aa (from amino acid 13-32 of the peptide). The transmembrane helix is expected to localize the protein to ER/Golgi, with the first 12 amino acids of the peptide facing the organelle interior and the rest (from aa 33-455) hanging outside into the cytoplasm. Cloning and sequence of *crw2-Mutag* (SEQ ID NO:3 is the nucleotide sequence of the cDNA and SEQ ID NO:4 is the amino acid sequence of the encoded polypeptide) revealed that the *Mu*-element added 145bp (See alignment Figs. 1A-1G).

To validate the candidate gene, a set of nested primers was used to amplify the full length gene from the *crw2-EMS* mutant allele (SEQ ID NO:5 is the nucleotide sequence of the cDNA and SEQ ID NO:6 is the amino acid sequence of the encoded polypeptide) and its progenitor MO20W, a public maize inbred line. A single amino acid change R292C

was detected in the *crw2-EMS* allele as compared to the MO20W HGA1 gene (Figs. 2A-2F). This amino acid change is in the conserved region of HGA1 and is likely the causative allele of the *crw2-EMS* mutation. An independent TUSC allele with a *Mutator* insertion located between the positions of the EMS allele and the Mu-tag allele was isolated and subjected to phenotypic and molecular analyses. The TUSC allele was found to be allelic to *crw2-Mutag* allele and showed similar enhanced susceptibility to WCR adults and to Japanese beetles. Figure 3 shows a diagram of the maize *Crw2* gene structure and the positions of each of the *Crw2* mutations. Additional validation for the function of this gene was identified in a doubled haploid line that is highly susceptible to WCR beetle. The expression of *ZmCrw1* and *ZmCrw2* was examined in the doubled haploid line using RT-PCR. Results show that *ZmCrw1* is expressed in the doubled haploid line while *ZmCrw2* is not (Fig. 9).

EXAMPLE 3

Identification of Homologs of the Maize CRW2 polypeptide

The maize CRW2 polypeptide can be analyzed for similarity to all publicly available amino acid sequences contained in the "nr" database using the BLASTP algorithm provided by the National Center for Biotechnology Information (NCBI) as well as to the DUPONT™ proprietary internal databases.

A BLAST search using the sequence of the maize CRW2 (SEQ ID NO:2) polypeptide revealed similarity of the maize CRW2 polypeptide to homogalacturonans from various organisms. Shown in Table 1 (non-patent literature are the BLASTP results for the amino acid sequence of the maize CRW2. Also shown in Table 1 are the percent sequence identity values for each pair of amino acid sequences using the Clustal W method of alignment with default parameters:

Table 1. BLASTP Results for Maize CRW2 Polypeptide (Non-patent)

<u>UniProt Identifier</u>	<u>% Seq Identity</u>
C0PDR7	
(SEQ ID NO:7)	64.5
C4J6G0	60.7

(SEQ ID NO:8)	
Q5Z8T7	
(SEQ ID NO:9)	86.3
Q6Z0Z4	
(SEQ ID NO:10)	63.5
C5Z9B2	
(SEQ ID NO:11)	93.8
C5XTX5	
(SEQ ID NO:12)	64.7
I1K5F9	
(SEQ ID NO:13)	40.5
I1KQG2	
(SEQ ID NO:14)	38.6
Q9LV23	
(SEQ ID NO:15)	41.9
Q9LV22	
(SEQ ID NO:16)	36.7
F2DBB4	
(SEQ ID NO:17)	83.0
I1GWV1	
(SEQ ID NO:18)	82.4
PnCRW2*	
(SEQ ID NO:20)	89.4
EnCRW2*	
(SEQ ID NO:22)	86.8
NCBI GI No. 356511269	
(SEQ ID NO:23)	40.0

*indicates that the sequence was discovered in an internal proprietary database

Figures 4A-4J present an alignment of the amino acid sequences of the polypeptides set forth in SEQ ID NOs:2, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 22, and 23. Figure 5 presents the percent sequence identities and divergence values for each sequence pair presented in Figures 4A-4J.

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 W method of alignment (Thompson et al. (1994) *Nucleic Acids Research*. 22:4673-80) with the default parameters (GAP PENALTY=10, GAP LENGTH

PENALTY=0.20). Default parameters for pairwise alignments using the Clustal method were GAP PENALTY=10.00 and GAP LENGTH = 0.10. The Protein Weight Matrix used was the Gonnet series.

EXAMPLE 4

5 RT-PCR Analysis of the ZmCrw2 Gene in Mutants and Wild-type Sibs

Total RNA samples were collected from leaf, stalk, and root tissue of 10-day-old seedlings of *crw2*-Mutag mutants and their WT siblings, and RT-PCR analysis was completed using gene specific primers (SEQ ID NO:24 and SEQ ID NO:25; FIG. 6A). The *crw2*-Mutag mutant showed
10 differential expression in three tissues (more in stalk as compared to leaf and root samples) and has a transcript that is 145bp longer than in WT-sibs. Cloning and sequencing of the *crw2*-Mutag transcript revealed that the Mu-element interferes with intron-splicing. The Mu-element added
141bp of the MuTIR (Terminal Inverted Repeat) end and 4bp from a 9bp
15 direct duplication, resulting in a frame shift (changing the last 73 amino acids as compared to the WT). RT-PCR of the *crw2*-TUSC mutant showed complete absence of *Crw2* transcript in leaves as compared to its WT-sibs (FIG. 6B).

EXAMPLE 5

20 Expression of ZmCrw2 Gene in Different Tissues Using Lynx Database

The Lynx database shows that the expression of ZmCrw2 is relatively low in different tissues such as elongating internodes (370 PPM), roots (367 PPM), inner husks at silking stage (324 PPM), tassel (310 PPM), and vascular bundles (220 PPM) (Fig. 7). The expression of the
25 ZmCrw2 gene in leaf tissue is highest in V5 leaves (300 PPM); however, expression in V5 leaf tissue goes down significantly in response to European Corn Borer (ECB) infection. When V5 leaf whorls were infested with ECB larvae, the expression of ZmCrw2 was 300, 200, 150, and 137 RPMs at 0h, 3h, 6h, and 24h, respectively, after infestation.

EXAMPLE 6

Crw1 and Crw2 Pathway Analysis

TBO staining of *crw2-EMS* resulted in a pattern that was identical to that of *crw1-Ac*, suggesting that both of these mutants have defects in a single genetic pathway or network.

To investigate this link further, differences in the *Crw2* transcript levels at various time points post - insect feeding were compared between the *Crw1* mutant and WT plants. Seven week old plants of *crw1-Ac* mutants and their WT-sibs were enclosed in a tent in the field and then infested with adult beetles that had been fasting for 16 hours. RNA was collected at 0 min, 45 min, 1 hr, 6 hr, 12 hr, 24 hr, 36 hr, and 48 hr after infestation. RNA samples were then pooled from different time points for the mutants (pool 1) and WT-sibs (pool 2). *Crw2* transcript levels were evaluated in each of the two pools at the various time points. A significant upregulation in *Crw2* transcript levels was observed immediately (45 min) in the wild-type sibs ("WT-sibs") as compared to *crw1-Ac* mutant plants (Fig. 8). These results were also confirmed in RNA Seq experiments where the *Crw2* transcript was 2.8 times higher (at log2 scale) in *Crw1* WT-sibs. These results indicated that *Crw2* is insect-inducible and that responsiveness to insect feeding is dependent on having a functional *Crw1* product.

These results also suggested that *Crw1* and *Crw2* belong to the same genetic or biochemical network and *Crw2* may be acting downstream of the *Crw1*.

EXAMPLE 7

Overexpressing Crw2 in Plants

The maize *Crw2* gene or any of its homologs can be inserted into a vector, which can further be transformed into plants (including but not limited to maize) using methods known to one of ordinary skill in the art. Phenotypic analysis can then be performed similarly to that in previously described Examples or using any known method of assessment to determine the plant's resistance to an insect pest such as but not limited to

the Western corn rootworm, the Japanese beetle, and the European Corn Borer.

Maize transformation vectors were built by constructing Gateway-competent entry vectors in which a fragment of DNA containing ZM-CRW2 was ligated into a vector containing the maize ubiquitin promoter, thereby resulting in a cassette in which expression of ZM-Crw2 was driven by the maize ubiquitin promoter. The resulting entry vector was combined with another vector via an LR reaction (methods were followed according to Invitrogen for Gateway LR reactions) to produce a construct appropriate for *Agrobacterium*-mediated transformation of maize.

A second vector was built in a similar manner using a vector with a backbone having the SB-RCC3 promoter as the recipient entry vector, thereby resulting in a cassette in which expression of ZM-Crw2 was driven by the SB-RCC3 promoter, a root-specific promoter from sorghum (US20120210463). The resulting entry vector was combined with another vector via an LR reaction to produce a construct appropriate for *Agrobacterium*-mediated transformation.

T0 maize plants were produced, and the plants were placed in the greenhouse for phenotypic analysis.

20

EXAMPLE 8

Overexpressing *Crw1* and *Crw2* in Plants

Both *Crw1* and *Crw2* can be overexpressed in a plant using methods known to one of ordinary skill in the art. The *Crw1* gene can be from maize (such as SEQ ID NO:26) or can be a homolog of the *ZmCrw1* gene (SEQ ID NOs:27-44). Similarly, the *Crw2* gene can be from maize (SEQ ID NO:1) or can be a homolog of the *ZmCrw2* gene. Phenotypic analysis can then be performed as described in previous Examples or using any method known in the art to determine the plant's resistance to an insect pest such as but not limited to the Western corn rootworm, the Japanese beetle, and the European Corn Borer.

30

EXAMPLE 9

Further Assessment of *Crw2* Mutant Phenotype

Crw2 mutants and corresponding wild-type (WT) siblings were planted in the field at bi-weekly intervals from the beginning of May to the end of June during the summers of 2012 and 2013. Plants were assessed for insect damage by the Western corn rootworm (WCR) beetle in the middle of July when the WCR pressure is at its maximum. Irrespective of the availability of young mutant seedlings, the foliar susceptibility phenotype was not observed until the *Crw2* mutant plants reached the age of 5 weeks or more. Thereafter, the foliar damage continued to occur steadily and resulted in complete defoliation of the mutant plant under heavy WCR infestation. In addition, *Crw2* mutants fell prey to diverse insect herbivores that included Japanese beetles, European corn borer, fall webworm, and cattail caterpillar.

EXAMPLE 10

Biochemical Characteristics of the Maize *Crw2* gene

Staining of *Crw2* mutant leaves with toluidine blue O (TBO), which reacts with free hydroxyl groups in the cell wall, shows reduced staining in intercostal cells, presumably resulting in compromised tensile strength. To test if this was due to reduction in the levels of cell wall bound p-coumaric acid (pCA) and ferulic acid (FA), quantification of these hydroxycinnamates was performed with the juvenile (V3 stage) and adult (V8 stage) epidermal cell walls of both *Crw2* and wild-type leaves. A significant reduction in pCA and FA levels was observed in *Crw2* mutants as compared to WT siblings, but only in the adult leaves (Fig. 10A).

To address if the reduced levels of hydroxycinnamates also resulted in reduced lignin levels, lignin was extracted from isolated cell walls of adult (V8 stage) *Crw2* mutant and WT-sib leaves as acetyl bromide soluble (ABS) fraction and analyzed by UV spectroscopy. Sure enough the levels of ABS lignin are significantly lower ($p < 0.05$; unpaired t test) in the adult leaves of *Crw2* in comparison to wild-type leaves (Fig.10B).

CLAIMS

What is claimed is:

1. A method of increasing resistance to herbivory by an insect pest 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 80% sequence identity, based on the Clustal W method of alignment, when compared to SEQ ID NO:2, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 22, and 23 ; 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 increased resistance to a plant pest when compared to a control plant not comprising the recombinant DNA construct.

2. The method of claim 1, 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 increased resistance to herbivory by an insect pest when compared to a control plant not comprising the recombinant DNA construct.

3. The method of claim 1, wherein the insect pest is Coleopteran.

4. The method of claim 1, wherein the insect pest is Lepidopteran.

5. The method of claim 1, wherein said plant is a monocot.

6. The method of claim 5, wherein said monocot is maize.

7. A method of identifying a variant of *Crw2* that gives plants increased resistance to herbivory by an insect pest, the method comprising the steps of:
- a. combining through gene shuffling one or more nucleotide sequences encoding one or more fragments of SEQ ID NO:2 or a protein that is at least 70% identical to SEQ ID NO:2, or a fragment thereof;
 - b. transforming the shuffled sequences from step (a) into a population of regenerable plant cells;
 - c. regenerating a population of transformed plants from the population of transformed regenerable plant cells of step (b);
 - d. screening the population of transformed plants from step (c) for increased resistance to said insect pest; and
 - e. identifying the variant from the transformed plant exhibiting the increased resistance.
8. The method of claim 7 wherein the method further comprises the steps of:
- f. introducing into a regenerable plant cell a recombinant construct comprising the variant of *Crw2* identified by the method of claim 7;
 - g. regenerating a transgenic plant from the regenerable plant cell after step (f), wherein the transgenic plant comprises in its genome the recombinant DNA construct; and
 - h. selecting a transgenic plant of (g), wherein the transgenic plant comprises the recombinant DNA construct and exhibits increased resistance to said insect pest, when compared to a control plant not comprising the recombinant DNA construct.
9. The method of claim 7 or 8, wherein the insect pest is Coleopteran.
10. The method of claim 7 or 8, wherein the insect pest is Lepidopteran.

11. The method of claim 7 or 8, wherein said plant is a monocot.
12. The method of claim 11, wherein said monocot is maize.
13. A method of identifying an allelic variant of *Crw2* in a maize plant wherein said allelic variant is associated with increased resistance to herbivory by an insect pest, the method comprising the steps of:
 - a. obtaining a population of maize plants, wherein said maize plants exhibit differing levels of resistance to said insect pest;
 - b. evaluating allelic variations with respect to the polynucleotide sequence encoding a protein comprising SEQ ID NO:2, or in the genomic region that regulates the expression of the polynucleotide encoding the protein;
 - c. associating allelic variations with said resistance; and
 - d. identifying an allelic variant that is associated with increased resistance to the insect pest.
14. The method of claim 13, wherein the insect pest is Coleopteran.
15. The method of claim 13, wherein the insect pest is Lepidopteran.

FIG. 1A

```
1.....11.....21.....31.....41.....51.....60
ATGAAGCAGCGAGGGCGGAGAGCCGCGCGGTGGCAAGCCCATGGTCGTC
ATGAAGCAGCGAGGGCGGAGAGCCGCGCGGTGGCAAGCCCATGGTCGTC
*****
61.....71.....81.....91.....101.....111.....120
ACCATGTCGTCCTCCCTCTGCGTCTCAGTACATCAAGCGCGGATACTGCTCCAACCCP
ACCATGTCGTCCTCCCTCTGCGTCTCAGTACATCAAGCGCGGATACTGCTCCAACCCP
*****
121.....131.....141.....151.....161.....171.....180
TTCCCCAAGCGGTGCGGAGGTGGAGGTGGACGAGGACTACGACAGCAGCGGTACAAG
TTCCCCAAGCGGTGCGGAGGTGGAGGTGGACGAGGACTACGACAGCAGCGGTACAAG
*****
181.....191.....201.....211.....221.....231.....240
CTGACGGGCCCGGTGGGCGAGGAGGACTTCGACCCGTCGCCGCCCACTGCTACAACACC
CTGACGGGCCCGGTGGGCGAGGAGGACTTCGACCCGTCGCCGCCCACTGCTACAACACC
*****
241.....251.....261.....271.....281.....291.....300
AGCAAGCGGTGCGAGCGGTGCGCGCGGTGGCGGACATCCGCGTGGACGGCAACCACTCG
AGCAAGCGGTGCGAGCGGTGCGCGCGGTGGCGGACATCCGCGTGGACGGCAACCACTCG
*****
```

Crw2

crw2-Mutag

Crw2

crw2-Mutag

Crw2

crw2-Mutag

Crw2

crw2-Mutag

FIG. 1B

```
301.....311.....321.....331.....341.....351.....360
CGGATCTACATCAGCCCGCTGTCCCGGAGTGGGGACCAAGCCGTACGGCGGGCGGCAC
CGGATCTACATCAGCCCGCTGTCCCGGAGTGGGGACCAAGCCGTACGGCGGGCGGCAC
*****
361.....371.....381.....391.....401.....411.....420
GACGCCGTGGCCATGGACGACGTGCGGAGTTCACGCTGGTCCCTTCGGCGGCCCAAC
GACGCCGTGGCCATGGACGACGTGCGGAGTTCACGCTGGTCCCTTCGGCGGCCCAAC
*****
421.....431.....441.....451.....461.....471.....480
GACAGGCCCGTGGCCCGCTCTGCAAGCGCACCCACTCCGTCGCCGGCTTCCTCTTCTCC
GACAGGCCCGTGGCCCGCTCTGCAAGCGCACCCACTCCGTCGCCGGCTTCCTCTTCTCC
*****
481.....491.....501.....511.....521.....531.....540
AGCGCGGGTTCGGGGCAACCTGTACCAAGACTAAGCCGAGGTGCTGGTGCCGCTCTTC
AGCGCGGGTTCGGGGCAACCTGTACCAAGACTAAGCCGAGGTGCTGGTGCCGCTCTTC
*****
541.....551.....561.....571.....581.....591.....600
GCCAGCACCAACCACTGGGCGGGAGGTCCAGTTCCTGCTGGCCGACATCAAGGACTGG
GCCAGCACCAACCACTGGGCGGGAGGTCCAGTTCCTGCTGGCCGACATCAAGGACTGG
*****
```

crw2

crw2-Mutag

crw2

crw2-Mutag

crw2

crw2-Mutag

crw2

crw2-Mutag

crw2

crw2-Mutag

FIG. 1C

```

601.....611.....621.....631.....641.....651.....660
TGGCCGACAAAGTTCGGCCCGCTCTTCCGCCAGCTCTCCGGCTACGACGTCATCGACGTG
TGGCCCGACAAAGTTCGGCCCGCTCTTCCGCCAGCTCTCCGGCTACGACGTCATCGACGTG
*****
661.....671.....681.....691.....701.....711.....720
AACAAACGACCGGAGGTGCACTGCTTCCCGCGGATCATCATCGGCTCCACCTTCCACCGC
AACAAACGACCGGAGGTGCACTGCTTCCCGCGGATCATCATCGGCTCCACCTTCCACCGC
*****
721.....731.....741.....751.....761.....771.....780
GCCATGGGCATCGACCCCTCGCGCTCGCCCGCGCGGTACGGTGGCCGACTTCAAGCGC
GCCATGGGCATCGACCCCTCGCGCTCGCCCGCGCGGTACGGTGGCCGACTTCAAGCGC
*****
781.....791.....801.....811.....821.....831.....840
CTGCTCCGCGCGCGGTTCCGGCTGGAGCGCGCGCTCGCGTCCGGTGGGGGGGCCCCCGG
CTGCTCCGCGCGCGGTTCCGGCTGGAGCGCGCGCTCGCGTCCGGTGGGGGGGCCCCCGG
*****
841.....851.....861.....871.....881.....891.....900
CGCCGGGACCGGCCCGCTCCTCATCATCTCGCGCAAGAGCTCGCGCGCTTCTGTCAC
CGCCGGGACCGGCCCGCTCCTCATCATCTCGCGCAAGAGCTCGCGCGCTTCTGTCAC
*****

```

crw2
 crw2-Mutag

crw2
 crw2-Mutag

crw2
 crw2-Mutag

crw2
 crw2-Mutag

crw2
 crw2-Mutag

FIG. 1D

```

901.....911.....921.....931.....941.....951.....960
GAGCGCGCCATGGCGCGCGCGCGCGCGCGCGGTTTCGACGTCCGGATCCCGAGCCCC
crw2-Mutag
GAGCGCGCCATGGCGCGCGCGCGCGCGCGCGGTTTCGACGTCCGGATCCCGAGCCCC
*****
961.....971.....981.....991.....1001.....1011.....1020
GACAACCAACACGGACATGCCCAACTTCGCGAGGCTGGTGAACCTCGCGGACGTGATGATG
crw2-Mutag
GACAACCAACACGGACATGCCCAACTTCGCGAGGCTGGTGAACCTCGCGGACGTGATGATG
*****
1021.....1031.....1041.....1051.....1061.....1071.....1080
GGCGTGCACGGCGCGCGGCTCACCACATGGTGTTCCTGCCAGCGCGCGGTGCTGGTG
crw2-Mutag
GGCGTGCACGGCGCGCGGCTCACCACATGGTGTTCCTGCCAGCGCGCGGTGCTGGTG
*****
1081.....1091.....1101.....1111.....1121.....1131.....1140
CAGGTGGTGCCGTTTCGGCGGGCTGGAGTGGCTCACCCGGTCACTTCAAGGACCCCGCA
crw2-Mutag
CAGGTGGTGCCGTTTCGGCGGGCTGGAGTGGCTCACCCGGTCACTTCAAGGACCCCGCA
*****
1141.....1151.....1161.....1171.....1181.....1191.....1200
AGGGAC-----
crw2-Mutag
AGGGACGAGATAATTGCCATTATGGACCAAGAGGGAAGGGATTCCAGCAAAATGGAGGCG
*****

```

FIG. 1E

```
1201.....1211.....1221.....1231.....1241.....1251.....1260
-----
TTGGCGTTGGCTTCTCTGTTTGGAGACGCGACGCGACAGCCAAACTCCAAACGGATACG
-----

1261.....1271.....1281.....1291.....1301.....1311.....1320
-----
AGACAGCTCTTGGGGCTGCGTAACACAGGGACATGGACGTCACGTACATGGAGTACAAACGT
*****
1321.....1331.....1341.....1351.....1361.....1371.....1380
GTCGCTGGAGGAGAGCTCGCTCAGGGACCTCTACCCGGAGGACCACTTCTACCTGAAGCA
GTCGCTGGAGGAGAGCTCGCTCAGGGACCTCTACCCGGAGGACCACTTCTACCTGAAGCA
*****
1381.....1391.....1401.....1411.....1421.....1431.....1440
CCCTACGACGTGCACAAGAGGGGTGGGACGCCCATCAAGACGGTGTACCTGGACAAGCA
CCCTACGACGTGCACAAGAGGGGTGGGACGCCCATCAAGACGGTGTACCTGGACAAGCA
*****
1441.....1451.....1461.....1471.....1481.....1491.....1500
GAACGTCAGGCTCAACCTCACCGAGGTTCAACAGGACCGCTGGAGCAGGCGGAGATCTCTT
GAACGTCAGGCTCAACCTCACCGAGGTTCAACAGGACCGCTGGAGCAGGCGGAGATCTCTT
*****
```

Crw2

crw2-Mutag

Crw2

crw2-Mutag

Crw2

crw2-Mutag

Crw2

crw2-Mutag

Crw2

crw2-Mutag

FIG. 1F

```

1501.....1511.....1521.....1531.....1541.....1551.....1560
GCCGACGCCCATGACTGATGATGACCTCCCCCTTTCCCTCTGCTCTGCTGCGAGGTTTCAT
GCCGACGCCCATGACTGATGATGACCTCCCCCTTTCCCTCTGCTCTGCTGCGAGGTTTCAT
*****
1561.....1571.....1581.....1591.....1601.....1611.....1620
TCACTTCAGATCAGCTGCTCACCCTCACCCTCAGCCGCTCTCTCTCTCTCTTTTCTCTCT
TCACTTCAGATCAGCTGCTCACCCTCACCCTCAGCCGCTCTCTCTCTCTCTTTTCTCTCT
*****
1621.....1631.....1641.....1651.....1661.....1671.....1680
GTTGTTGTTCTATACATATACTTGTGTTTCCCTCTTCTTCCCTTCCCTCTCTCTCTCTCTCT
GTTGTTGTTCTATACATATACTTGTGTTTCCCTCTTCTTCCCTCTCTCTCTCTCTCTCTCTCT
*****
1681.....1691.....1701.....1711.....1721.....1731.....1740
CCCTCTCCACTCTTGTGGTGGCAAGATTCAATTCTTTCAATTGTTTCTTTTGTGTTGTTG
CCCTCTCCACTCTTGTGGTGGCAAGATTCAATTCTTTCAATTGTTTCTTTTGTGTTGTTG
*****
1741.....1751.....1761.....1771.....1781.....1791.....1800
TTGTTGAGGAAGGATAGGAACAACAAAGGATTGTCGTGTCCTCAAGGTTAATCTACACA
TTGTTGAGGAAGGATAGGAACAACAAAGGATTGTCGTGTCCTCAAGGTTAATCTACACA
*****

```

Crw2
 crw2-Mutag

 Crw2
 crw2-Mutag

 Crw2
 crw2-Mutag

 Crw2
 crw2-Mutag

 Crw2
 crw2-Mutag

FIG. 1G

1801.....1811.....1821.....1831.....1841.....1851.....1860
AACACACACTGTAAATGATTGATTGCTGTCAGTAGAGCGGAACACAAAGGAATAGGT
crw2
AACACACACTGTAAATGATTGATTGCTGTCAGTAGAGCGGAACACAAAGGAATAGGT
crw2-Mutag

1861.....1870
AAAAAAAAAAA
crw2
AAAAAAAAAAA
crw2-Mutag

FIG. 2A

```

1.....11.....21.....31.....41.....51.....60
ATGAAGCAGCCGAGGGCCGCGAGAGCCGCGCGGGTGGCAACCGCCCATGGTCGTC
ATGAAGCAGCCGAGGGCCGCGAGAGCCGCGCGGGTGGCAACCGCCCATGGTCGTC
*****
61.....71.....81.....91.....101.....111.....120
ACCATGGTCGTCCTCCTCGGTCCTCAGGTACATCAAGCGGATACCTGCTCAACCT
ACCATGGTCGTCCTCCTCGGTCCTCAGGTACATCAAGCGGATACCTGCTCAACCT
*****
121.....131.....141.....151.....161.....171.....180
TTCCCCAAAGCGGTGGCGGAGGTGGAGGTGGACGAGGACTACGACAGCAGCGGTACAAG
TTCCCCAAAGCGGTGGCGGAGGTGGAGGTGGACGAGGACTACGACAGCAGCGGTACAAG
*****
181.....191.....201.....211.....221.....231.....240
CTGACGGGCCCGGTGGCGGAGGAGGACTTCGACCCGTCGCGCCACCTGCTACAACACC
CTGACGGGCCCGGTGGCGGAGGAGGACTTCGACCCGTCGCGCCACCTGCTACAACACC
*****
241.....251.....261.....271.....281.....291.....300
AGCAAGCGGTCCGAGCGGTCCGCCCGCGGTGGCGGACATCCGCGTGGACGGCAACCTCG
AGCAAGCGGTCCGAGCGGTCCGCCCGCGGTGGCGGACATCCGCGTGGACGGCAACCTCG
*****

```

crw2
 crw2-EMS

 crw2
 crw2-EMS

 crw2
 crw2-EMS

 crw2
 crw2-EMS

FIG. 2B

```

301.....311.....321.....331.....341.....351.....360
CGGATCTACATCAGCCCGCTGTCCCGGAGTGGGGACCAAGCCGTACGGCGGGCGGCAC
crw2
CGGATCTACATCAGCCCGCTGTCCCGGAGTGGGGACCAAGCCGTACGGCGGGCGGCAC
crw2-EMS
*****
361.....371.....381.....391.....401.....411.....420
GACGCCGTGGCCATGAGACGACGTGGGAGTTCACGCTGGTCCCTTCGGGSCCCCAAC
crw2
GACGCCGTGGCCATGAGACGACGACGTGGGAGTTCACGCTGGTCCCTTCGGGSCCCCAAC
crw2-EMS
*****
421.....431.....441.....451.....461.....471.....480
GACACGGCCGTGCCCGCTGTGCACGGGCACCCACTCCGTCCGGGCTTCCTCTCTCC
crw2
GACACGGCCGTGCCCGCTGTGCACGGGCACCCACTCCGTCCGGGCTTCCTCTCTCC
crw2-EMS
*****
481.....491.....501.....511.....521.....531.....540
AGCGCGGGTTCCGGGGCAACCTGTACCACGACTACCGGACGTGCTGGTCCGCTCTTC
crw2
AGCGCGGGTTCCGGGGCAACCTGTACCACGACTACCGGACGTGCTGGTCCGCTCTTC
crw2-EMS
*****
541.....551.....561.....571.....581.....591.....600
GCCAGCACCAACCACTGGGGGGAGGTCCAGTTCCTGCTGGCCGACATCAAGGACTGG
crw2
GCCAGCACCAACCACTGGGGGGAGGTCCAGTTCCTGCTGGCCGACATCAAGGACTGG
crw2-EMS
*****

```

FIG. 2C

```
601.....611.....621.....631.....641.....651.....660
TGGGCCGACAAAGTTCCGCCGCTCTTCGCCAGCTCTCCCGCTACGACGTCATCGACGTC
TGGGCCGACAAAGTTCCGCCGCTCTTCGCCAGCTCTCCCGCTACGACGTCATCGACGTC
*****
661.....671.....681.....691.....701.....711.....720
AACAAACGACCGGAGGTGCACGTCTTCGCCGGATCATCATCGCTCCACCTTCCACCGC
AACAAACGACCGGAGGTGCACGTCTTCGCCGGATCATCATCGCTCCACCTTCCACCGC
*****
721.....731.....741.....751.....761.....771.....780
GCCATGGGCATCGACCCCTCGCGCTCGCCGGCGGCTCACGGTGGCCGACTTCAAGCGC
*****
781.....791.....801.....811.....821.....831.....840
CTGCTCCGCCGCGCTTCGGCTGGAGCGGCCGCTCGCTCGCGTGGGGGCGGCCCGG
CTGCTCCGCCGCGCTTCGGCTGGAGCGGCCGCTCGCTCGCGTGGGGGCGGCCCGG
*****
841.....851.....861.....871.....881.....891.....900
CGCCGGGACCGGCCGCTCTCATCATCTCGGCCAAGAGCTCGGCCGCTTCGTCAAC
CGCCGGGACCGGCCGCTCTCATCATCTCGGCCAAGAGCTCGGCCGCTTCGTCAAC
*****
```

crw2
crw2-EMS

crw2
crw2-EMS

crw2
crw2-EMS

crw2
crw2-EMS

crw2
crw2-EMS

FIG. 2F

```
1501.....1511.....1521.....1531.....1541.....1551.....1560
TTCCTCTTCCTCCTTCCCTCTCTCTAGTCTCTCCCTCTCCACTCTTGTGGTGGCAAG
crw2
TTCCTCTTCCTCCTTCCCTCTCTCTAGTCTCTCCCTCTCCACTCTTGTGGTGGCAAG
crw2-EMS
*****
1561.....1571.....1581.....1591.....1601.....1611.....1620
ATTCAATTTCTTTTCATTGTTTGTGTTTGTGTTGTTGAGGAAGGATAGGAACAAAA
crw2
ATTCAATTTCTTTTCATTGTTTGTGTTTGTGTTGTTGAGGAAGGATAGGAACAAAA
crw2-EMS
*****
1621.....1631.....1641.....1651.....1661.....1671.....1680
ACAAGGTATTGTCGTGTCCTCAAGGTTAATCTACACAAACACACACTGTAAATGATTG
crw2
ACAAGGTATTGTCGTGTCCTCAAGGTTAATCTACACAAACACACACTGTAAATGATTG
crw2-EMS
*****
1681.....1691.....1701.....1711.....17211725
ATTGCTGTCAGTAGAGGCCGAACACAAAGGAATAGGTAAAAA
crw2
ATTGCTGTCAGTAGAGGCCGAACACAAAGGAATAGGTAAAAA
crw2-EMS
*****
```

FIG. 3

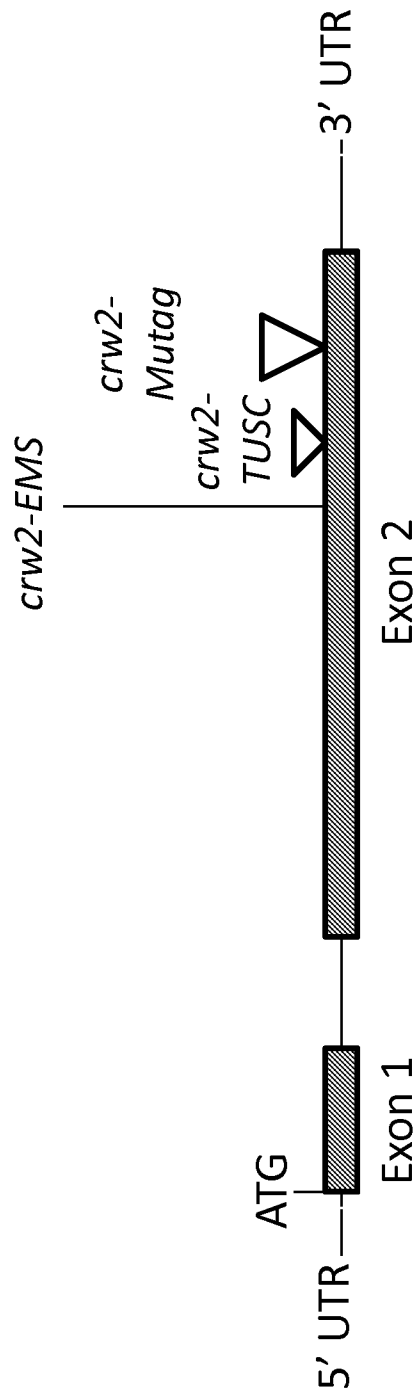
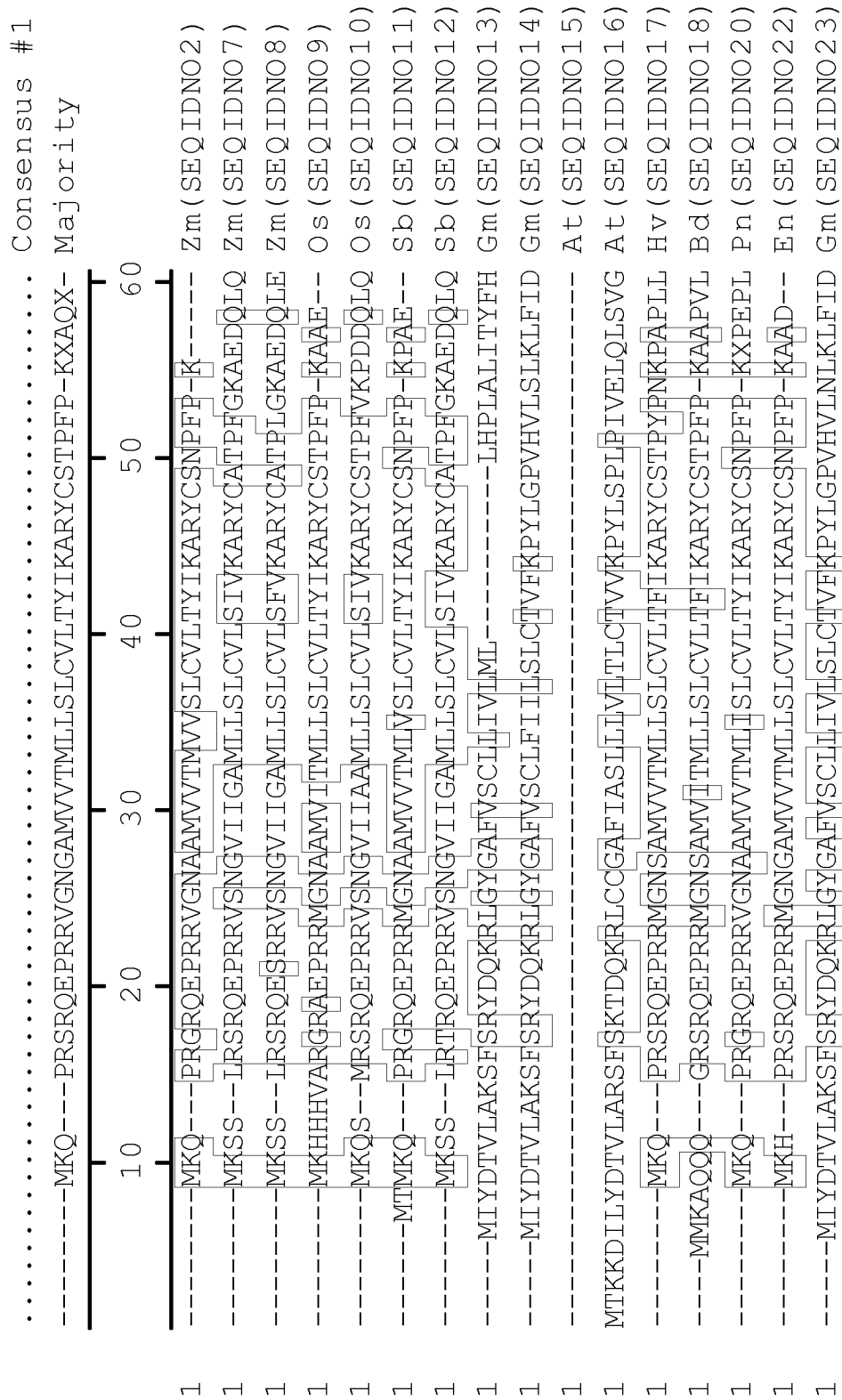


FIG. 4A



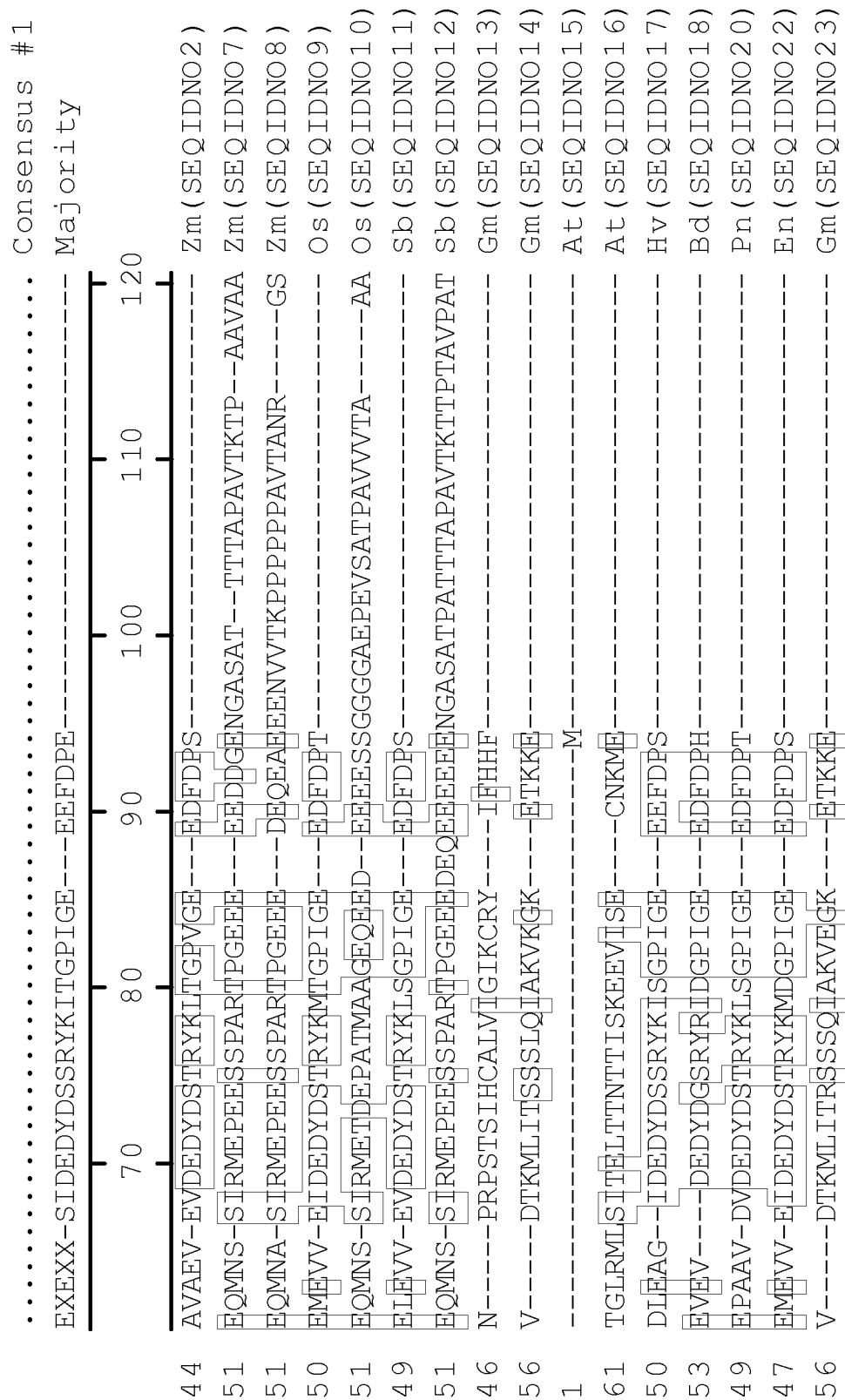


FIG. 4D

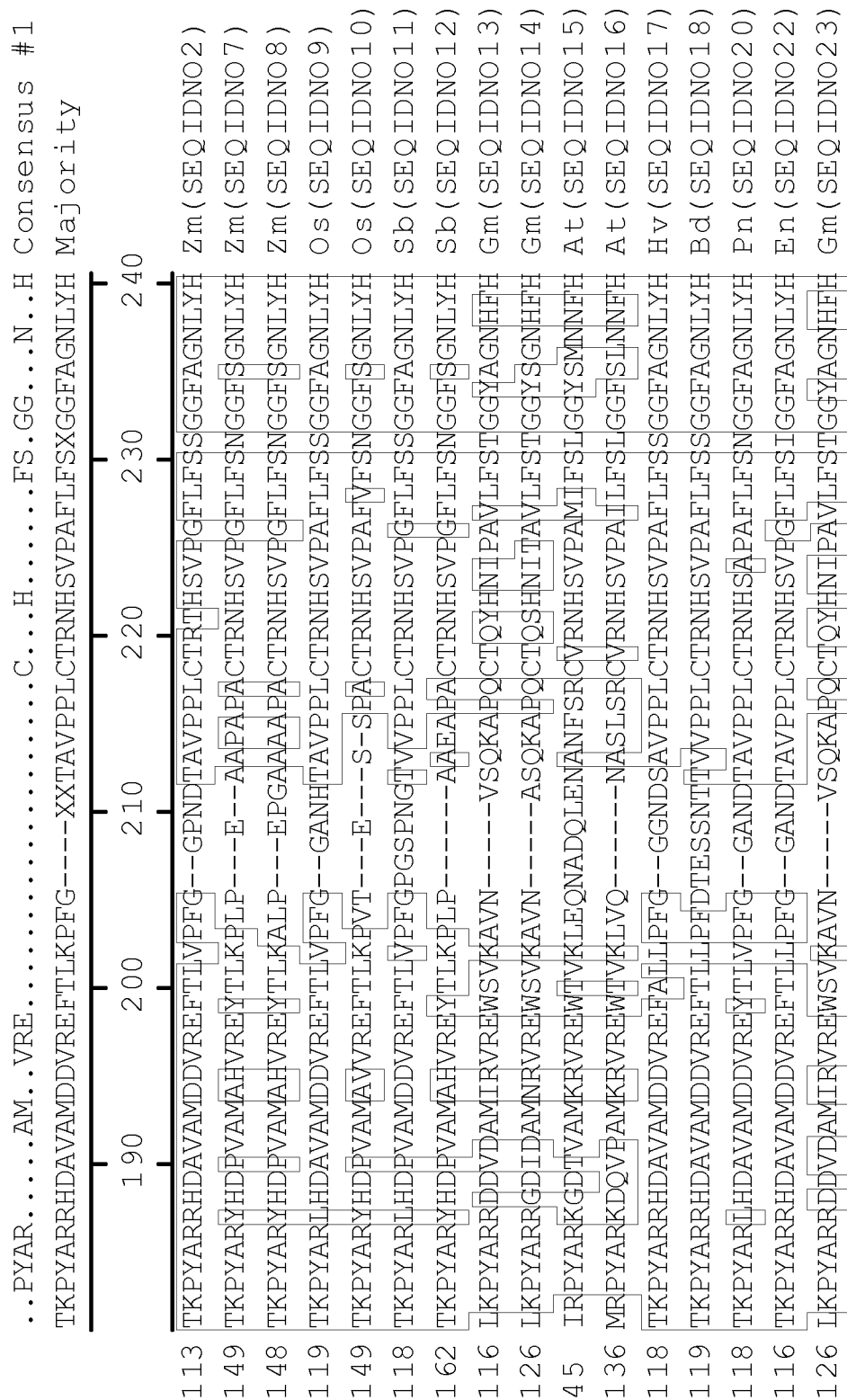


FIG. 4E

	...D...PL.....G.V.....W.K.....L.....	Consensus #1
	DYTDVLVPLFTSTHXFGGEVQFLIXDI-----KPWWXDKFTPLFRQLSNYDVVDNDQE	Majority
	250 260 270 280 290 300	
171	DYADVLVPLFASTNHLGGEVQFLIADI-----KDWWADKFRPVPFRQLSRYDVVDVNDRE	Zm (SEQ ID NO 2)
204	DYTDVLVPLFISTHQFRGRVQFLISGM-----KPWWVAKFTPFERQLTRYDVVDNDQE	Zm (SEQ ID NO 7)
205	DYTDVLVPLFISTHQFRGRVQFLISGM-----KPWWVGKFTPFERQLTRHDVIDVDKDE	Zm (SEQ ID NO 8)
177	DYTDVLVPLFTSTNHFGEVQFLISGI-----KDWWLDKFTPLFRQLSRYDVVDNDQE	Os (SEQ ID NO 9)
202	DYTDVLVPLFISTHQFRGQVQFLISGL-----KPWWVNKFNLFERQLTKYDILIDNDKD	Os (SEQ ID NO 10)
178	DYADVLVPLFASTHFGGEVQFLIADI-----KDWWADKFKPLFRQLSRYDVVDVNDRE	Sb (SEQ ID NO 11)
216	DYTDVLVPLFISTHQFRGRVQFLISGM-----KPWWVAKFTPFERQLTKYDVVDNDQE	Sb (SEQ ID NO 12)
171	EFTDIVIPLFLTARQFNGEVQFIITDK-----RPWWISKHKPPLKKLSNYETMDIDGDE	Gm (SEQ ID NO 13)
181	EFTDIVIPLFLTARQFNGEVQFIITDK-----RPWWISKHKPPLKKLSNYETMDIDGDDQ	Gm (SEQ ID NO 14)
105	DFTDIVIPLYTARRFNGEVQFLVTNK-----SPSWINKFKELVRKLSNYEVIYIDEED	At (SEQ ID NO 15)
190	DFTDIVIPLYTARRFSGEVQFLVTNK-----NLLWINKFKELVRKLSNYEVIYIDEED	At (SEQ ID NO 16)
176	DYTDVLVPLFTSTHFGGEVQFLITDI-----KDWWLDKFTPLFRQLSNYDVVDNDQE	Hv (SEQ ID NO 17)
179	DYTDVLVPLFTSTHFGGEVQFLITDI-----KDWWLDKFTPLFRQLSNYDVVDNDQ	Bd (SEQ ID NO 18)
176	DYADVLVPLFTSTHFGGEVQFLISGM-----KDWWNEKFTPLFRQLSRYDVVDNDLE	Pn (SEQ ID NO 20)
174	DYTDVLVPLFTSTHFGGEVQFLIMISDIWGKEDKDWWVDKFTPLFRQLSKYDVVDNDQE	En (SEQ ID NO 22)
181	EFTDIVIPLFLTARQFNGEVQFIITDK-----RPWWISKHKPPLKKLSNYETMDIDGDE	Gm (SEQ ID NO 23)

FIG. 4F

.HCF.....G.....DF....LR....L....A.....										Consensus #1	
VHCFPRIVGAT----										A Majority	
310	320	330	340	350	360						
226	VHCFPRTIIGST	---- <td>FHRAMGIDPSRSPGGVT</td> <td>VADF</td> <td>KRLLRRAFRLE</td> <td>AVASRSG</td> <td>----<td>A</td><td>Zm (SEQ IDNO 2)</td></td>	FHRAMGIDPSRSPGGVT	VADF	KRLLRRAFRLE	AVASRSG	---- <td>A</td> <td>Zm (SEQ IDNO 2)</td>	A	Zm (SEQ IDNO 2)		
259	VHCFPRIVGAT	---- <td>FHKDMGVDPRRSPGHVS</td> <td>VDF</td> <td>KRALRRAFGLPRE</td> <td>AASRGG</td> <td>----<td>A</td><td>Zm (SEQ IDNO 7)</td></td>	FHKDMGVDPRRSPGHVS	VDF	KRALRRAFGLPRE	AASRGG	---- <td>A</td> <td>Zm (SEQ IDNO 7)</td>	A	Zm (SEQ IDNO 7)		
260	VHCFPRIVGAT	---- <td>FHRDMGVDPRA</td> <td>PGHVS</td> <td>AVDF</td> <td>KRALRAAFGLKRE</td> <td>AASRGGGGA</td> <td></td> <td>Zm (SEQ IDNO 8)</td>	FHRDMGVDPRA	PGHVS	AVDF	KRALRAAFGLKRE	AASRGGGGA		Zm (SEQ IDNO 8)		
232	VHCFPRIIFIGAT	---- <td>FHRAMGIDPARSPGGVT</td> <td>VADF</td> <td>KRLLRRTFRLE</td> <td>RAVASRTG</td> <td>----<td>A</td><td>Os (SEQ IDNO 9)</td></td>	FHRAMGIDPARSPGGVT	VADF	KRLLRRTFRLE	RAVASRTG	---- <td>A</td> <td>Os (SEQ IDNO 9)</td>	A	Os (SEQ IDNO 9)		
257	VHCFPRIVGAT	---- <td>FHKDMGVDPKRS</td> <td>PGHVS</td> <td>VDF</td> <td>KRALRRAFGLER</td> <td>VAAASRGG</td> <td>----<td>A</td><td>Os (SEQ IDNO 10)</td></td>	FHKDMGVDPKRS	PGHVS	VDF	KRALRRAFGLER	VAAASRGG	---- <td>A</td> <td>Os (SEQ IDNO 10)</td>	A	Os (SEQ IDNO 10)	
233	VHCFPRIVIGST	---- <td>FHRAMGIDASRSPGGET</td> <td>VADF</td> <td>KRVLRRAFKLE</td> <td>RAVASRSG</td> <td>----<td>A</td><td>Sb (SEQ IDNO 11)</td></td>	FHRAMGIDASRSPGGET	VADF	KRVLRRAFKLE	RAVASRSG	---- <td>A</td> <td>Sb (SEQ IDNO 11)</td>	A	Sb (SEQ IDNO 11)		
271	VHCFPRIVAGAT	---- <td>FHKDMGVDPRRSPGHVS</td> <td>VDF</td> <td>KRALRRAFGLE</td> <td>EAASRGG</td> <td>----<td>A</td><td>Sb (SEQ IDNO 12)</td></td>	FHKDMGVDPRRSPGHVS	VDF	KRALRRAFGLE	EAASRGG	---- <td>A</td> <td>Sb (SEQ IDNO 12)</td>	A	Sb (SEQ IDNO 12)		
226	VHCFPRVTVGLKR	---- <td>YQKELSI</td> <td>EPQKYS</td> <td>----<td>YSMKDFRDLR</td><td>SSYALKRVEAIKTR</td><td>----<td>D</td><td>Gm (SEQ IDNO 13)</td></td></td>	YQKELSI	EPQKYS	---- <td>YSMKDFRDLR</td> <td>SSYALKRVEAIKTR</td> <td>----<td>D</td><td>Gm (SEQ IDNO 13)</td></td>	YSMKDFRDLR	SSYALKRVEAIKTR	---- <td>D</td> <td>Gm (SEQ IDNO 13)</td>	D	Gm (SEQ IDNO 13)	
236	VHCFPSVTVGLKR	---- <td>YQKELSID</td> <td>PQKYS</td> <td>----<td>YSMKDFRDLR</td><td>SSYALKRVEAMKIR</td><td>----<td>D</td><td>Gm (SEQ IDNO 14)</td></td></td>	YQKELSID	PQKYS	---- <td>YSMKDFRDLR</td> <td>SSYALKRVEAMKIR</td> <td>----<td>D</td><td>Gm (SEQ IDNO 14)</td></td>	YSMKDFRDLR	SSYALKRVEAMKIR	---- <td>D</td> <td>Gm (SEQ IDNO 14)</td>	D	Gm (SEQ IDNO 14)	
160	THCFSSVTVGLTRHREYFKELTID	PSNSE	---- <td>YSMSDFRS</td> <td>FLRDTYS</td> <td>SLR</td> <td>NDAVAT</td> <td>TR</td> <td>----<td>Q</td><td>At (SEQ IDNO 15)</td></td>	YSMSDFRS	FLRDTYS	SLR	NDAVAT	TR	---- <td>Q</td> <td>At (SEQ IDNO 15)</td>	Q	At (SEQ IDNO 15)
245	THCFSSVIVGLNRHRDYDKELTTD	PSNSE	---- <td>YSMSDFRK</td> <td>FLRDTYS</td> <td>SLR</td> <td>NSAVTT</td> <td>----<td>----<td>At (SEQ IDNO 16)</td></td></td>	YSMSDFRK	FLRDTYS	SLR	NSAVTT	---- <td>----<td>At (SEQ IDNO 16)</td></td>	---- <td>At (SEQ IDNO 16)</td>	At (SEQ IDNO 16)	
231	VHCFPRIVIGST	---- <td>FHRPMGIDGTRSPGGET</td> <td>VADF</td> <td>KRLLRRAFR</td> <td>LD</td> <td>RVVASHDG</td> <td>----<td>S</td><td>Hv (SEQ IDNO 17)</td></td>	FHRPMGIDGTRSPGGET	VADF	KRLLRRAFR	LD	RVVASHDG	---- <td>S</td> <td>Hv (SEQ IDNO 17)</td>	S	Hv (SEQ IDNO 17)	
234	VHCFRRIIIGAT	---- <td>FHRAMGIDPKRS</td> <td>PGGET</td> <td>VADF</td> <td>KRLLRHAF</td> <td>HLTR</td> <td>VPASRD</td> <td>----<td>----<td>Bd (SEQ IDNO 18)</td></td></td>	FHRAMGIDPKRS	PGGET	VADF	KRLLRHAF	HLTR	VPASRD	---- <td>----<td>Bd (SEQ IDNO 18)</td></td>	---- <td>Bd (SEQ IDNO 18)</td>	Bd (SEQ IDNO 18)
231	VHCFHRIVIGAT	---- <td>FHRAMGIDPTRSPGGIT</td> <td>VADF</td> <td>KRTLRAFR</td> <td>LE</td> <td>RAVASRTG</td> <td>----<td>A</td><td>Pn (SEQ IDNO 20)</td></td>	FHRAMGIDPTRSPGGIT	VADF	KRTLRAFR	LE	RAVASRTG	---- <td>A</td> <td>Pn (SEQ IDNO 20)</td>	A	Pn (SEQ IDNO 20)	
234	VHCFPRIVIGPT	---- <td>FHRAMGIDPTRSPGGIN</td> <td>IADF</td> <td>KRLLRRTFRLE</td> <td>RAVASRTG</td> <td>----<td>A</td><td>En (SEQ IDNO 22)</td></td>	FHRAMGIDPTRSPGGIN	IADF	KRLLRRTFRLE	RAVASRTG	---- <td>A</td> <td>En (SEQ IDNO 22)</td>	A	En (SEQ IDNO 22)		
236	VHCFPRVTVGLKR	---- <td>YQKELSI</td> <td>EPQKYS</td> <td>----<td>YSMKDFRDLR</td><td>SSYALKRVEAIKTR</td><td>----<td>D</td><td>Gm (SEQ IDNO 23)</td></td></td>	YQKELSI	EPQKYS	---- <td>YSMKDFRDLR</td> <td>SSYALKRVEAIKTR</td> <td>----<td>D</td><td>Gm (SEQ IDNO 23)</td></td>	YSMKDFRDLR	SSYALKRVEAIKTR	---- <td>D</td> <td>Gm (SEQ IDNO 23)</td>	D	Gm (SEQ IDNO 23)	

FIG. 4G

	PR...I...R...SR.F.N.....A..A.....F.V.....E.....FA...VNS.D.	Consensus #1
	PRR-GKPRLLIIISRKSSRRFLNEREMARAAAXAGFDVRIAEPDNHTDMPNFARLVNSADV	Majority
	370 380 390 400 410 420	
279	PRRRDRPRLLIIISRKSSRRFLNERAMARAAAAAFEDVRIAEPDNHTDMPNFARLVNSADV	Zm (SEQ IDNO 2)
312	TGR-GKPRLLIIISRGSSRRFLNEREMARAAAGAGFEVRIAEPDQHTDTAAFAALVNSADV	Zm (SEQ IDNO 7)
316	TGD-GKPRLLIIISRGSSRRFLNSREMAVAAAGDAGFEVRIAEPDQRTDMAAFALVNSADA	Zm (SEQ IDNO 8)
285	PRR-DKPRLLIIISRKSSRRFLNERAMAHAAALAFEDVRIAEPDNHTDMPNFARLVNSADV	Os (SEQ IDNO 9)
310	TGN-GKPRLLIIISRKSSRRFLNEREMAQAAAAGFEVRIAEPDQHTDMSTFAQLVNSADV	Os (SEQ IDNO 10)
286	PRRKDRPRLLIIISRKSSRRFLNERAMARAAAAAFEDVRIAEPDNHTDMPNFARLVNSADV	Sb (SEQ IDNO 11)
324	TGH-GKPRLLIIISRGSSRRFLNEREMARAAADAGFEVRIAEPDQHTDMATFAALVNSADV	Sb (SEQ IDNO 12)
278	GLR-GKPRIMILSRKRSRFFTTNTDEIAKMAESIGFDVIAKEAG--WSMWGFANVNSQDV	Gm (SEQ IDNO 13)
288	GLR-GKPRIMILSRKRSRFFTTNTDEIAKMAASIGFDVIVKEAG--WSMWGFANVNSQDV	Gm (SEQ IDNO 14)
214	IRR-RRPRIILARGRSRAFVNTGEIARAARQIGFKVVVAEAN--IGIAKFAQTVNSQDV	At (SEQ IDNO 15)
297	--R-RKPRIILISRSRSRAFVNAGEIARAARQIGFKVVVAEAN--TEIASFAITVNSQDV	At (SEQ IDNO 16)
284	ASL-GKPRLLIIISRKSSRRFLNERAMAHAAALAQFDVRIAEPDNHTDMPNFARLVNSADV	Hv (SEQ IDNO 17)
285	----NPRLLIIISRKSSRRFLNERAMAHAAALAKFDVRIAEPDNHTDMPNFARLVNSADI	Bd (SEQ IDNO 18)
284	PRR-DRPRLLIIISRRSSRRFLNERAMAHAAARAFEDVRIAEPDNHTDMPNFARLVNSADV	Pn (SEQ IDNO 20)
287	PRR-DKPRLLIIISRKSSRRFLNERAVAHAAALAKFDVRIAEPDNHTDMPNFARLVNSADV	En (SEQ IDNO 22)
288	GLR-GKPRIMILSRKRSRFFTTNTDEIAKMAESIGFDVIAKEAG--WSMWGFANVNSQDV	Gm (SEQ IDNO 23)

FIG. 4H

	430	440	450	460	470	480	
	..GVHGAG.TN..FLP...A...Q...P.GG....WL.....F...P.....Y...Y.....E						Consensus #1
	MMGVHGAGLTNMVFLPSRAVLIQVVPFGG--LEWLTTRVTFKDPKADMDVNYMEYNVSLEE						Majority
339	MMGVHGAGLTNMVFLPSRAVLIQVVPFGG--LEWLTTRVTFKDPKADMDVNYMEYNVSLEE						Zm (SEQ ID NO 2)
371	MMGVHGAGLTNMVFLPRGAVLIQVVPFGG--LEWLTGVTFKDPAADMEVSYMGYDVTLEE						Zm (SEQ ID NO 7)
375	MMGVHGAGLTNMVFLPRGAVLIQVVPFGG--LEWLTGVTFKDPAADMEVSYMDYHVRLEE						Zm (SEQ ID NO 8)
344	MMGVHGAGLTNMVFLPSRAVLIQVVPFGG--LEWLTTRVTFKDPKADMDVNYMEYNVSFDE						Os (SEQ ID NO 9)
369	MMGVHGAGLTNMVFLPRGAVLIQVVPFGG--LEWLTGVTFKDPKADMEVTTYMDYNVQLEE						Os (SEQ ID NO 10)
346	MMGVHGAGLTNMVFLPSRAVLIQVVPFGG--LEWLTTRVTFKDPKADMDVNYMEYNVSLEE						Sb (SEQ ID NO 11)
383	MMGVHGAGLTNMVFLPRGAVLIQVVPFGG--LEWLTSTVTFKDPAADMEVNYMDYNVQLEE						Sb (SEQ ID NO 12)
335	LLGVHGAGLTNIIFLPENAVFIQVVPYGGVTTLDWLATNDFGNPSKDMNIIKYLEYKISLEE						Gm (SEQ ID NO 13)
345	LLGVHGAGLTNIIFLPENAVFIQVVPYGGFTLDWLATNDFGKPSKDMNIIKYLEYKIGLEE						Gm (SEQ ID NO 14)
271	MLGVHGAGLTNMVFLPENAVFIQVLPYIGG--FEWLAKTDFFKPSKDMNIIKYLEYKIAVEE						At (SEQ ID NO 15)
352	MLGVHGAGLTNMVFLPDNAIVIQILPYIGG--FEWLAKMDFFYPSKGMNIIKYLEYKITAE						At (SEQ ID NO 16)
343	MMGVHGAGLTNMVFLPSRAVLIIQVVPFGG--LEWLSRVTFKDPKADFDVTTYMEYNVSLEE						Hv (SEQ ID NO 17)
340	MMGVHGAGLTNMVFLPSRAVLIIQVVPFGG--LEWLSRVTFKDPKADMDVNYMEYNVSLEE						Bd (SEQ ID NO 18)
343	MMGVHGAGLTNMVFLPSRAVLIIQVVPFGG--LEWLSRVTFKDPKADMDVNYMEYNVSLEE						Pn (SEQ ID NO 20)
346	MMGVHGAGLTNMVFLPSRAVLIIQVVPFGG--LEWLSRVTFKDPKADMDVNYMEYNVSLEE						En (SEQ ID NO 22)
345	LLGVHGAGLTNIIFLPENAVFIQVVPYGGVTTLDWLATNDFGNPSKDMNIIKYLEYKISLEE						Gm (SEQ ID NO 23)

FIG. 4I

		S.L....Y....H.....P.....GW....K...YL...Q.....L.....L.....L.....	Consensus #1
		SSLRDQYPRDHFY L K D P Y D V H K K G W D A I K T V Y L D K Q N V R L D L D R F X P T L E K A R D L L P S P -	Majority
		490 500 510 520 530 540	
397	SSLRDLYPEDHFY L K H P Y D V H K K G W D A I K T V Y L D K Q N V R I N L T R F T R T L E Q A R D L L P T P		Zm (SEQ ID NO 2)
429	SSLTDQYPRNHQV L T D P Y A V H K Q G W D A L K A A Y L D K Q N I R M D L D R F R A T L R E A M S R L P P A P		Zm (SEQ ID NO 7)
433	SSLVDQYPRGHQV L T D P Y A V H R Q G W D A L K T A Y L D K Q N I R M D L D R F R A T L R E V M A R L P S P		Zm (SEQ ID NO 8)
402	SSLRELYPRDHFY I Q H P Y D V H K K G W D A I K T V Y L D K Q N V E I N L T K L T N T L E R A R D F L P E P		Os (SEQ ID NO 9)
427	SSLTDQYPRNHQV L T D P Y A V H K Q G W D A L K T A Y L D K Q N I K M D M D R F K K T L Q E A L D R L P P A		Os (SEQ ID NO 10)
404	SSLRDLYPEDHFY L K H P Y D V H K K G W D A I K T V Y L D K Q N V R I N L T R F T R T L E Q A R D L L P S P		Sb (SEQ ID NO 11)
441	SSLVDQYPRNHQV L T D P Y A V H K Q G W D A L K T A Y L D K Q N I R M D L D R F R A T L R E A M S R L P S P		Sb (SEQ ID NO 12)
395	STLIQQYPLDHMF I K D P P L I E K I G W E E F K S V Y L D K Q N V K L D V D R F R P T L Q K A L E L L H Q		Gm (SEQ ID NO 13)
405	STLIQQYPLDHI F I K D P P L V E K I G W E E F K S V Y L D K Q N V K L D V D R F R P T L Q K A F E L L H Q		Gm (SEQ ID NO 14)
329	STLVKKYGRDHE I V R D P S A V A K H G W E M F K S V Y I V Q Q N V S I D I N R F K P V L V K A L E L L		At (SEQ ID NO 15)
410	STLVKQYGRDHE F V R D P L A V A K R G W G T F K S V Y I V Q Q N V S V D I N R F K L V L V K A L E L L H N Q S		At (SEQ ID NO 16)
401	SSLKNLYPKDHFY L Q H P Y D V H K K G W N A I K T V Y L D K Q S V R L D L A K L T R T L E H A R S L L P S S		Hv (SEQ ID NO 17)
398	SSLRNLYPEGHFY L K H P Y D V H K K G W D A I K T V Y L D K Q S V R I N L T K F V Q T L E L A R S R L P A		Bd (SEQ ID NO 18)
401	SSLRDLYPEGHFY L K H P Y D V H K K G W D A I K T V Y L D K Q N V R I N L T R F T E T L E Q A R D L L P L P		Pn (SEQ ID NO 20)
404	SSLRDLYPEDHFY L K H P Y D V H K K G W D A I K T T Y L D K Q N V R I N L T R F T K T L Q Q A R D L L P S P		En (SEQ ID NO 22)
405	STLIQQYPLDHMF I K D P P L I E K I G W E E F K S V Y L D K Q N V K L D V D R F R P T L Q K A L E L L H Q		Gm (SEQ ID NO 23)

FIG. 4J



FIG. 5

Percent Identity

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
1		64.5	60.7	86.3	63.5	93.8	64.7	40.5	38.6	41.9	36.7	83.0	82.4	89.4	86.8	40.0	1
2	47.8		87.2	64.8	80.1	65.0	94.0	40.1	39.9	44.4	40.0	64.1	64.1	65.4	65.0	40.8	2
3	55.1	14.1		61.1	74.0	61.2	86.4	38.9	38.7	42.6	38.6	60.7	60.9	62.0	61.4	39.6	3
4	15.1	47.2	54.4		64.7	86.2	66.1	39.9	38.6	42.7	38.0	84.4	85.9	87.5	88.8	39.5	4
5	49.7	23.2	32.0	47.5		64.4	80.6	42.2	41.2	44.7	39.9	63.7	64.2	64.1	65.5	41.9	5
6	6.4	47.0	54.2	15.3	48.1		65.3	40.2	38.2	42.2	36.9	84.2	83.1	89.3	88.0	39.8	6
7	47.5	6.2	15.1	45.0	22.5	46.3		39.0	39.2	44.0	39.2	64.6	64.9	66.2	66.7	39.7	7
8	109.7	111.0	115.7	111.8	103.6	110.6	115.4		85.8	55.6	50.9	41.8	40.7	39.8	42.0	92.7	8
9	116.8	111.8	116.5	117.1	107.0	118.5	114.4	15.7		56.4	52.0	40.4	39.5	38.2	40.2	92.2	9
10	104.4	96.0	102.2	101.7	95.3	103.4	97.4	66.1	64.3		83.4	41.4	41.0	42.2	42.2	55.6	10
11	125.0	111.3	117.1	119.5	112.0	123.8	114.4	77.5	74.7	18.8		37.4	37.1	36.7	38.5	52.7	11
12	19.3	48.6	55.1	17.5	49.4	17.8	47.6	104.8	109.8	106.3	121.7		87.1	82.8	85.1	42.0	12
13	20.1	48.5	54.7	15.7	48.4	19.1	47.0	108.9	113.2	107.7	123.1	14.2		83.1	85.7	40.5	13
14	11.4	46.3	52.6	13.7	48.6	11.6	44.8	112.3	118.5	103.5	124.8	19.7	19.2		88.0	39.6	14
15	14.6	47.0	53.7	12.1	46.0	13.2	43.9	104.1	110.8	103.5	117.5	16.7	15.9	13.2		41.5	15
16	111.5	108.5	113.0	113.5	104.6	112.3	112.7	7.7	8.2	66.1	73.0	104.1	109.8	113.2	105.8		16
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	

Zm(SEQIDNO2)

Zm(SEQIDNO7)

Zm(SEQIDNO8)

Os(SEQIDNO9)

Os(SEQIDNO10)

Sb(SEQIDNO11)

Sb(SEQIDNO12)

Gm(SEQIDNO13)

Gm(SEQIDNO14)

At(SEQIDNO15)

At(SEQIDNO16)

Hv(SEQIDNO17)

Bd(SEQIDNO18)

Pn(SEQIDNO20)

En(SEQIDNO22)

Gm(SEQIDNO23)

FIG. 6 A and B

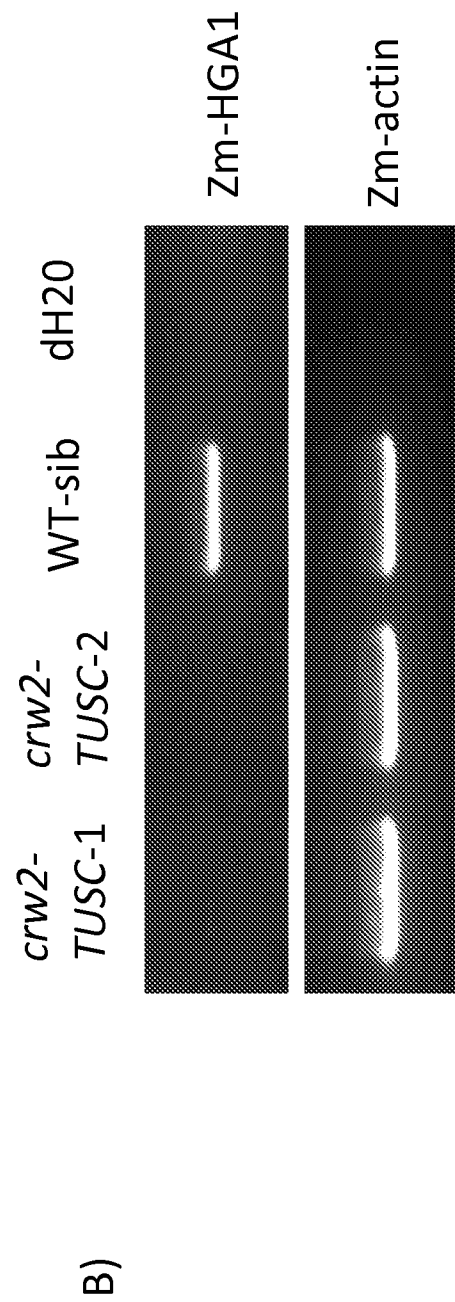
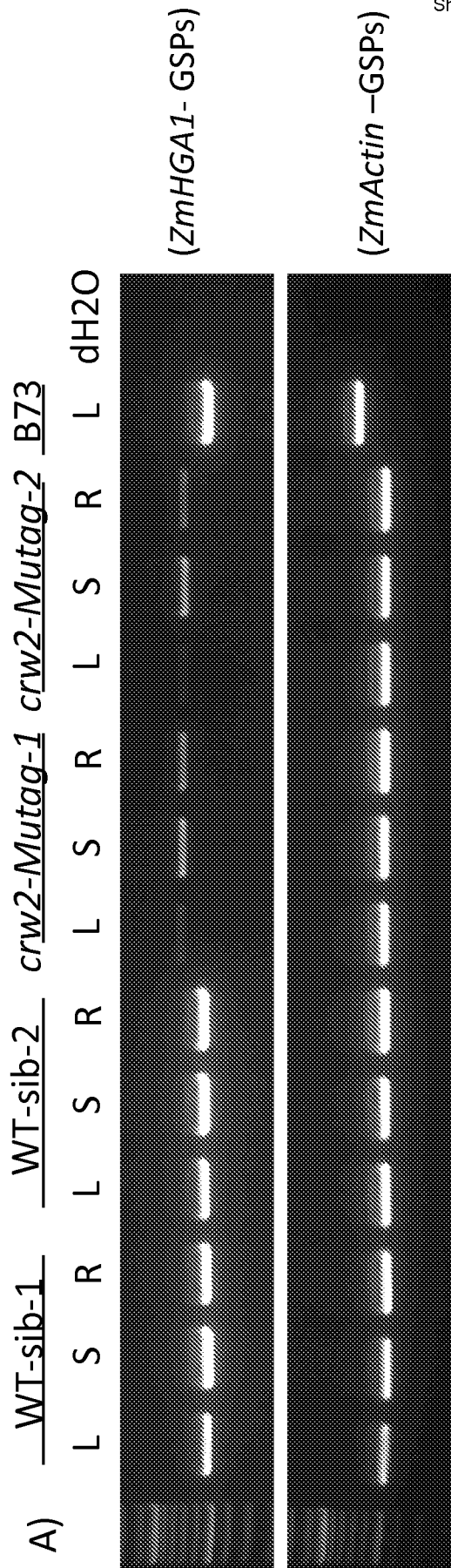


FIG. 7

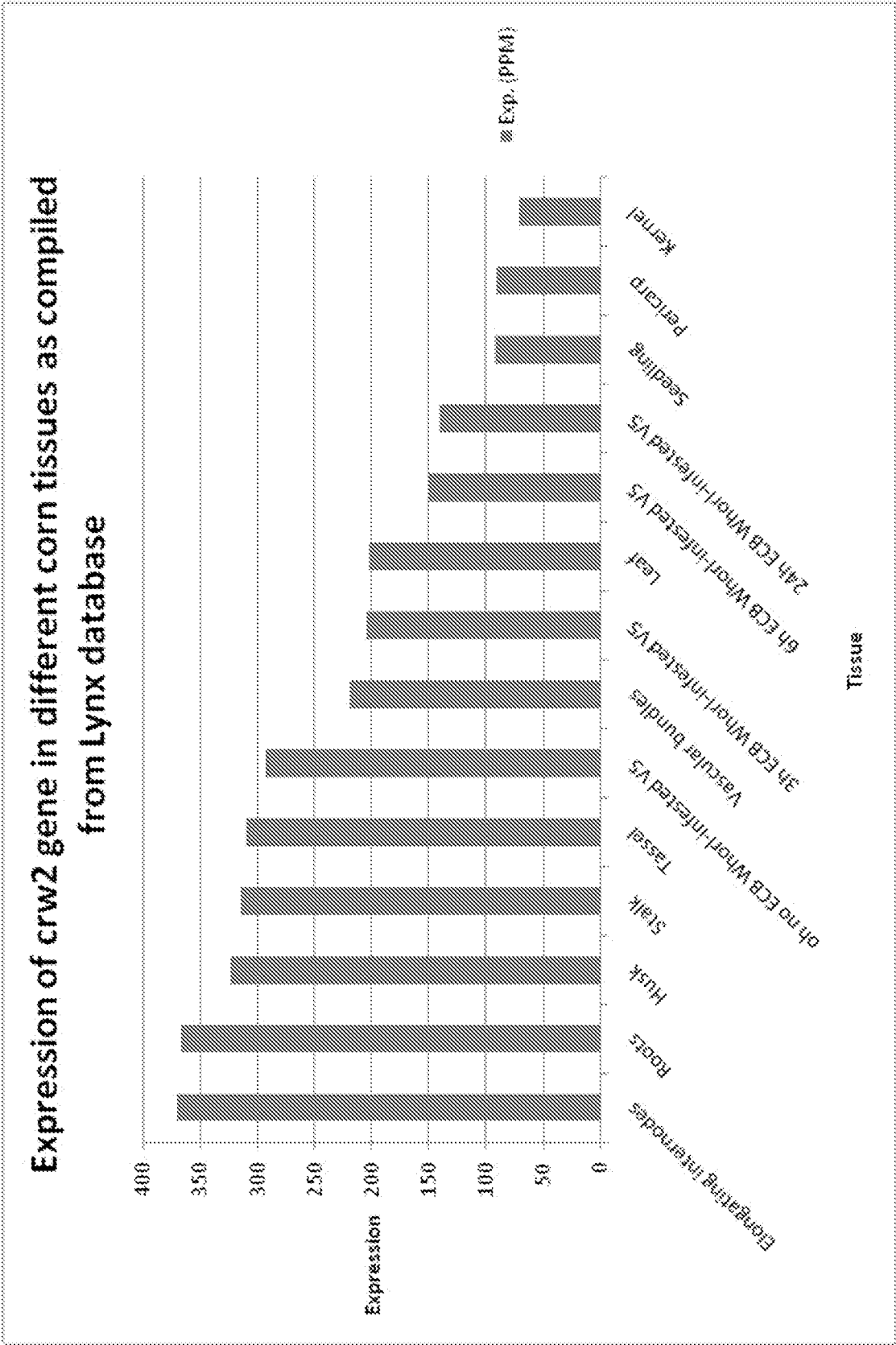


FIG. 8

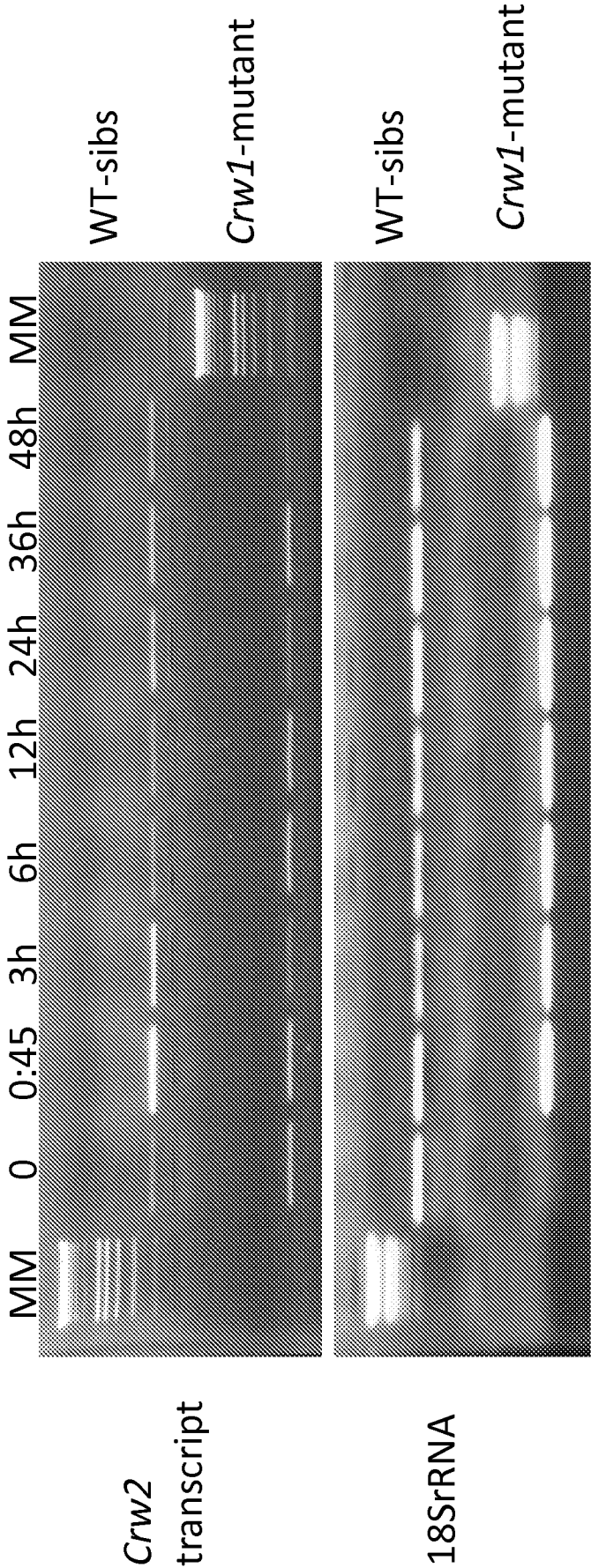
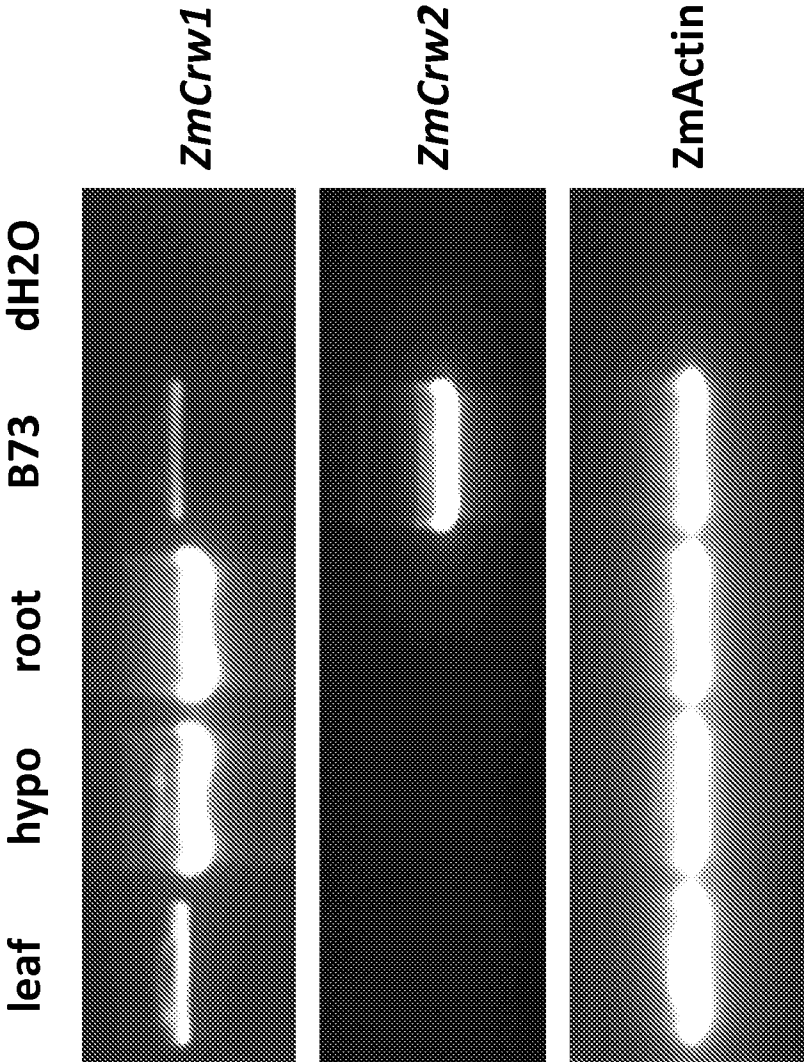
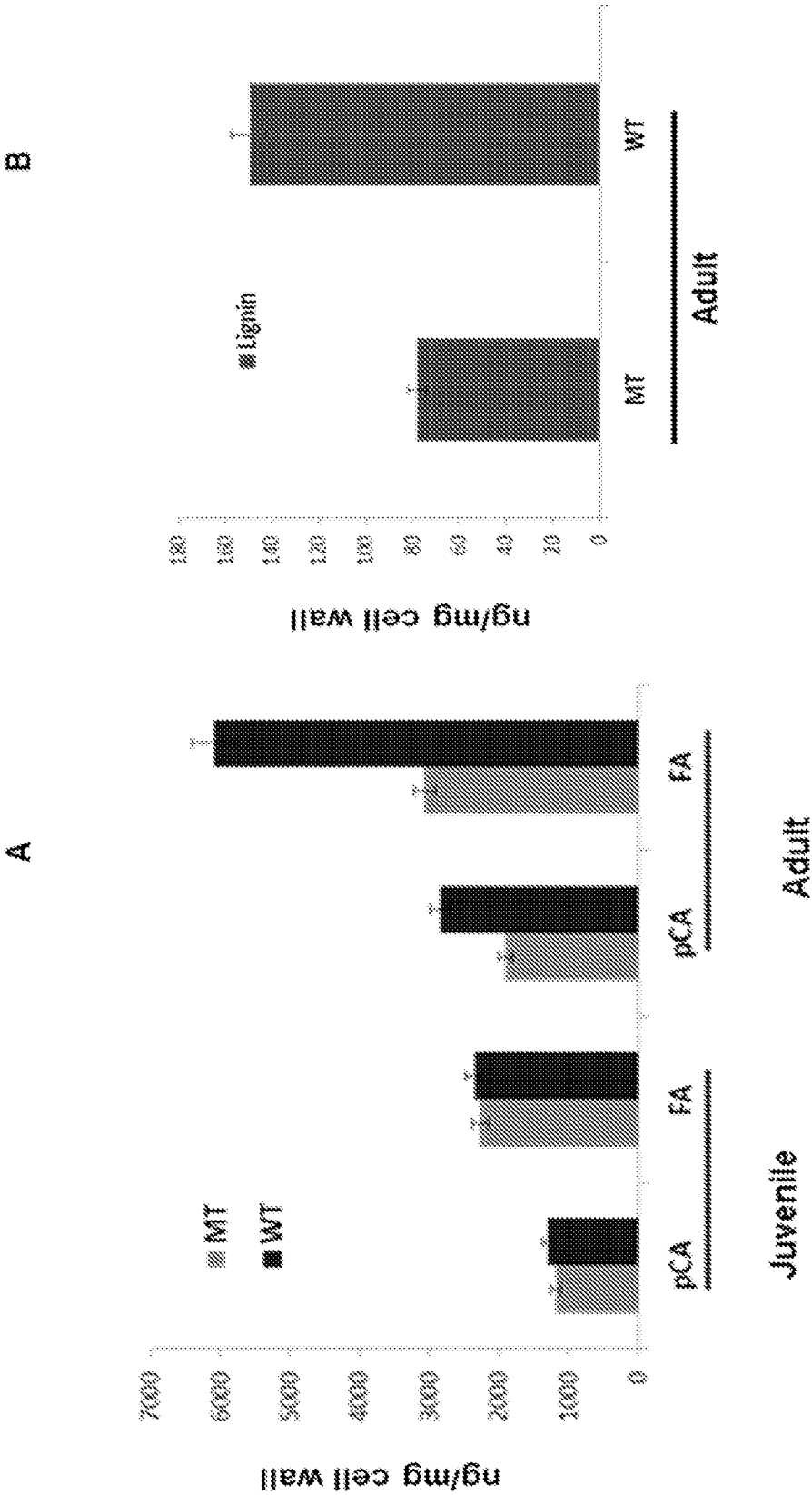


FIG. 9

DH -line



FIGS. 10A and B



Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

a. (means)

☐

on paper

☒

in electronic form

b. (time)

☒

in the international application as filed

☐

together with the international application in electronic form

☐

subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 15/82, C12N 15/87, A01H 5/00, C12N 5/04, C12N 5/00 (2013.01)

USPC - 800/279, 800/287, 800/302, 435/418, 435/412, 435/419, 435/430

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - C12N 15/82, C12N 15/87, A01H 5/00, C12N 5/04, C12N 5/00 (2013.01)

USPC - 800/279, 800/287, 800/302, 435/418, 435/412, 435/419, 435/430

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
800/300.1

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

pubWEST; Google Scholar; PatBase; GenCore 6.3

search terms - corn rootworm 2, crw-2, crw 2, crw, crw2, corn rootworm resistance mechanism 2, MON863, MON863CRW2, Lepidopteran, Lepidoptera, moth, butterfly, Coleopteran, rootworm, shuffling, maize, corn, recombinant, insect, pest, varia\$, mutat\$,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2010/0257621 A1 (KETKAR et al.) 07 October 2010 (07.10.2010) para [0059]-[0063]; [0071]; [0074]; [0083]-[0087]; [0098]; [0099]; [0113]; [0119]; Table 14.	1-6
Y	WO 2012/058223 A1 (APUYA N.) 03 May 2012 (03.05.2012) SEQ ID NO: 205 and 242; full document, especially pg 4, 6, 9, 11, 59, 85, 86, 93, 95, 96, 99.	1-15
Y	US 2012/0210462 A1 (BERMUDEZ et al.) 16 August 2012 (16.08.2012) para [0034]-[0037]; [0079]; [0125]; [0196]-[0197].	7-15

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

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

05 December 2013 (05.12.2013)

Date of mailing of the international search report

23 DEC 2013

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

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