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(54) **IDENTIFICATION AND THE USE OF KRP MUTANTS IN PLANTS**

(57) The invention provides a plant cell, part, tissue culture or whole plant comprising at least one disrupted *KRP* gene of the present invention. The present invention also provides methods of increasing weight, size, and/or number of one or more organs, and/or yield of a plant by utilizing the disrupted *KRP* genes of the present invention. Furthermore, methods of breeding plants to produce new plants having increased weight, size, and/or number of one or more organs, and/or yield are provided. The present invention provides isolated *Kinase Inhibitor Protein (KIP) Related Protein (KRP)* polynucleotide sequences and isolated KRP polypeptide sequences and methods of their use. Exemplary plants include wheat, rice and soybean.

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Description

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application Serial No. 61/474,201, filed April 11, 2011, which is hereby incorporated by reference in its entirety for all purposes.

TECHNICAL FIELD

[0002] The invention generally relates to compositions and methods for improving traits of agronomic and horticultural crops, including by increasing crop yield. More specifically, the present invention relates to compositions and methods for improving one or more agronomic or horticultural traits by disturbing one or more Kinase Inhibitor Protein (KIP) Related Proteins (KRP) in monocotyledonous and dicotyledonous plants.

BACKGROUND

[0003] The most important trait as a target for crop improvement is yield. Efforts to improve crop yields by developing new plant varieties can be divided into two approaches. One is to reduce crop yield losses by breeding or engineering crop varieties with increased resistance to abiotic stress conditions such as drought, cold, or salt or to biotic stress conditions resulting from pests or disease-causing pathogens. While this approach has value, it does not provide fundamentally improved crop yield in the absence of stress conditions and in fact, such resistance may direct plant resources that otherwise would be available for increased yield in the plant. The second approach is to breed or engineer new crop varieties in which the basic yield capacity is increased.

[0004] Classical breeding programs have initially produced substantial gains in improved yield in a variety of crops. A commonly experienced pattern though has been substantial gains in yield initially followed by incremental further improvements that become smaller and more difficult to obtain. More recently developed approaches based on molecular biology technologies have in principle offered the potential to achieve substantial improvement in crop yield by altering the timing, location, or level of expression of plant genes or heterologous genes that play a role in plant growth and/or development. Substantial progress has been made over the past twenty years in identifying plant genes and or heterologous genes that have a role in plant growth and/or development. Despite these gains in using molecular approaches, there continues to be a large unmet need for improved agronomic and horticultural plants produced through more conventional plant breeding. Because of the complexity of plant growth regulation and how it relates in the end to yield traits, it is still not obvious which, if any, of particular genes would be clear candidates to improve crop yield through either plant breeding and/or molecular techniques.

[0005] KRP proteins belong to a class of cell cycle inhibitors that bind to and inhibit cyclin/CDK kinase complexes. Mutation of conserved residues within KRP family members are expected to modify KRP's ability to function as an inhibitor of cyclin-CDK kinase complexes. Specifically, some mutations in KRP genes would lead to expression of a nonfunctional KRP cell cycle inhibitor or a cell cycle inhibitor with reduced activity. This loss of or reduced cyclin/CDK kinase inhibitory activity would lead to increased cyclin-CDK kinase activity in cells when normally these cells would have reduced cyclin-CDK activity. This loss of or reduced cyclin/CDK kinase inhibitory activity would lead to increased cell divisions in tissue where the normal wild-type KRP version is expressed. This increased cell division would result in positive agronomic traits such as increased yield, increased weight, size, and/or number of one or more organs, for example, increased seed size, larger plants, larger leaves, larger roots etc. For background on KRP-related technologies, see, for example, WO/2007/016319 and US20070056058, each of which is incorporated by reference in its entirety for all purposes. The present invention identifies new KRP genes and proteins and provides methods for their use in producing improved agronomic and horticultural plants through conventional plant breeding and/or molecular methodologies.

SUMMARY OF THE INVENTION

[0006] The inventors of the present invention have used Targeting Induced Local Lesions in Genomes (TILLING®) methods in plants to identify KRP mutants, for example, in monocot plants, such as plants in the *Triticeae* tribe (e.g., plants in the *Triticum* genus), and plants in the tribe of *Oryzeae* (e.g., plants in *Oryza* genus), or in dicot plants, for example, in *Glycine spp.* The identified KRP mutants can be used for increasing weight, size, and/or number of one or more organs in a plant. The organ can be any part of a plant, for example, organs that contribute to yield in a plant. In some embodiments, the organ is seed, leaf, branch, root, shoot, stigma, ovule, pollen, seed pods, seed heads, or tiller. For example, in some embodiments, the present invention provides methods for increasing plant seed weight, seed size, seed number and/or yield.

[0007] This invention describes the search for mutations in plant KRPs that are expressed in cells within the developing seed. The strategy was to identify KRP family member(s) with seed expression and TILL® for mutants in these KRPs. Certain mutants that affect KRP function can then be characterized for positive agronomical and horticultural traits such as increased yields, early emergence, accelerated growth etc. In addition, this same methodology can be used for KRPs expressed in other tissues such as leaves or stalks since increased cell divisions in these other tissues specific to the particular KRP expression pattern could also lead to positive agronomic traits.

[0008] To date, KRP family members in agriculturally, horticulturally and/or industrially important plant species and their expression patterns in developing seed have yet to be studied. The inventors' strategy was to identify KRPs that are expressed in the developing seed. KRPs with expression pattern during development in a tissue specific manner are identified. For example, the rice *KRP4* gene (OsKRP4) showed almost exclusive expression in the developing seed. Equivalents to this sequence in rice and other plant species are also identified.

[0009] The present invention provides mutated *KRP* genes compared to a wild type *KRP* having nucleic acid sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 60-86, 100-107, 111-128, fragments and variations derived from thereof, which encode a KRP gene. In some embodiments, the KRP has an expression pattern in a tissue specific manner during development. In some embodiments, the expression is embryonic specific, pollen specific, or seed specific.

[0010] In some embodiments, the present invention provides mutated KRP genes compared to a wild type KRP comprising a sequence comprising a nucleic acid sequence that shares at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.1%, at least 99.2%, at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, or at least 99.9% identity to SEQ ID NOs: 60-86, 100-107, 111-128, 138-139, 141-142, and 144-145.

[0011] In some embodiments, the present invention provides mutated KRP genes compared to a wild type KRP encoding an amino acid sequence having at least 90%, at least 91 %, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.1%, at least 99.2%, at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, or at least 99.9% identity to SEQ ID NO: 87-99, 108-110, 129-137, 140, 143, and 146.

[0012] For example, the present invention provides mutants in wheat (Ta) *KRP1A, KRP1B, KRP1D, KRP2A, KRP2B, KRP2D, KRP4B, KRP4D, KRP5A, KRP5D, KRP6A, KRP6B, KRP6D.* It appears that hexaploid wheat has been naturally selected to have what appear to be knock-out mutations in *KRP4A* and *KRP5B.* This was discovered during the course of TILLING®. For *KRP4A,* the gene appears to be completely missing from the hexaploid wheat genome, although it is still present in the tetraploid genome. For *KRP5B,* there appears to be one missing nucleotide in the gene, which would shift the translational frame and produce a predicted truncation a few amino acids further on.

[0013] The present invention in another aspect provides plant cells, plant parts, tissue culture, or whole plants comprising one or more disrupted *KRP* genes as described herein. In some embodiments, the present invention provides plant cells, plant parts, tissue culture or whole plants comprising at least one *KRP* gene, wherein the genome of the plant has one or more copies of the gene, and wherein the function of one or more copies of the *KRP* gene is disrupted.

[0014] In some embodiments, the plant is a monocot. In some embodiments, the monocot is a species in the *Triticeae* tribe or the *Oryzeae* tribe. In some embodiments, the plant in the *Triticeae* tribe is a plant in the *Triticum* genus, and wherein the plant in the *Oryzeae* tribe is a plant in the *Oryza* genus.

[0015] In some further embodiments, the plant in the *Triticum* genus is wheat, and wherein the plant in the Oryza genus is rice.

[0016] In some further embodiments, the wheat plant is tetraploid or hexaploid.

[0017] In some embodiments, the plant is a dicot. In some embodiments, the plant is a species in the *Fabaceae* family, for example, *Glycine spp.,* such as soybean.

[0018] In some embodiments, the *KRP* in the wheat is *TaKRP1, TaKRP2, TaKRP4, TaKRP5, TaKRP6,* for example, SEQ ID NOs. 60-86, 138-139, 141-142, 144-145 or functional variants thereof, and the *KRP* in the rice is *OsKRP1, OsKRP2, OsKRP4, or OsKRP5,* for example, SEQ ID NOs. 100-107, or functional variants thereof; and the *KRP* in the *Glycine spp.* is Gm0003x00821, Gm0013x00399, Gm0043, Gm0053x00526, Gm0087x00306, Gm0102x00087, Gm0119x00131, Gm0151x00019, Gm0067x00001, for example, SEQ ID NOs. 111-128, or functional variants thereof. For example, in a tetraploid wheat plant, the *KRP* is *TaKRP1A, TaKRP1B, TaKRP2A, TaKRP2B, TaKRP4A*, *TaKRP4B, TaKRP5A, TaKRP5B, TaKRP6A* or *TaKRP6B.* In a hexaploid wheat, the *KRP* is *TaKRP1A, TaKRP1B, TaKRP1D, TaKRP2A, TaKRP2B, TaKRP2D, TaKRP4A, TaKRP4B, TaKRP4D, TaKRP5A, TaKRP5B, TaKRP5D, TaKRP6A, TaKRP6B,* or *TaKRP6D.*

[0019] In some embodiments, the *KRP* genes function is disrupted by nucleotide substitution, deletion, insertion, homologous recombination, T-DNA, transposon, double strand oligonucleotide, antisense oligonucleotide, inverted repeat, or combination thereof.

[0020] In some embodiments, the disrupted KRP in the plant cell, plant part, tissue culture or whole plant comprises one or more mutations selected from any one of mutations listed in Tables 2 - 12, 25, 28, 29 - 37 for a particular *KRP* gene.

[0021] In another aspect, the present invention provides methods for increasing weight, size, and/or number of one or more organs, for example, for increasing seed weight, seed size, seed number and/or yield in a plant comprising disrupting one or more KRPs in the plant. In one embodiment, the plant is a dicotyledon plant or a monocotyledon plant. In some embodiments, the plant can be a plant selected from the *Triticeae* tribe, the *Oryzeae* tribe, or the *Fabaceae* family, for example, wheat, rice, and soybean. In some embodiments, methods of disrupting a gene function include but are not limited to mutagenesis (e.g., chemical mutagenesis, radiation mutagenesis, transposon mutagenesis, insertional mutagenesis, signature tagged mutagenesis, site-directed mutagenesis, and natural mutagenesis), antisense, knock-outs, and/or RNA interference. In some embodiment, the plant with increased weight, size, and/or number of one or more organs, for example, a plant with increased seed weight, seed size, seed number and/or yield is not a genetically modified organism, or a transgenic plant. For example, the disruption of the KRP in the genome of the plant is simply due to natural mutation, or mutations induced by chemical mutagenesis or radiation mutagenesis.

[0022] In some embodiments, mutations described in the Tables 2 - 12, 25, 28, 29 - 37 can be integrated into species closely related to the plants in the *Triticeae* tribe, the *Oryzeae* tribe, the *Fabaceae* family, or plants closely related to wheat, rice, or soybean. In some embodiments, amino acids in conserved domains or sites compared to KRP orthologs in other species can be substituted or deleted to make mutants with reduced or abolished activity, and/or mutants that lead to loss-of-function (e.g., protein instability). In some embodiments, one or more KRPs in a plant are knocked down or knocked out by one or more methods available to one skilled in the art.

[0023] In some embodiments, one or more copies of one or more *KRP* genes are disrupted. For example, in a tetraploid wheat plant, one or two copies of a KRP gene are disrupted (e.g., K*RP1A, KRP1B; KRP2A, KRP2B; KRP4A, KRP4B; KRP5A, KRP5B; and KRP6A, KRP6B);* in a hexaploid wheat plant, one or more copies of one, two, or three copies of a KRP gene are disrupted (e.g., *KRP1A, KRP1B, KRP1D; KRP2A, KRP2B, KRP2D; KRP4A, KRP4B, KRP4D; KRP5A, KRP5B, KRP5D; and KRP6A, KRP6B, KRP6D)*

[0024] The present invention also provides a plant having increased weight, size, and/or number of one or more organs, for example, a plant with increased seed size, seed number, and/or seed yield compared to a wild type reference plant, wherein the plant has one or more mutations in one or more KRP genes. In some embodiments, said plant is a monocot plant. In some embodiments, said monocot plant is a plant from the *Triticeae* tribe or the *Oryzeae* tribe. In some embodiments, said plant is a wheat or a rice plant. In some embodiments, said plant is a dicot plant. In some embodiments, said dicot plant is from the Fabaceae family, such as a soybean plant.

[0025] The present invention further provides a seed, a fruit, a plant cell or a plant part of the transgenic plants as described herein. For example, the present invention provides a pollen of the plant, an ovule of the plant, a genetically related plant population comprising the plant, a tissue culture of regenerable cells of the plant. In some embodiments, the regenerable cells are derived from embryos, protoplasts, meristematic cells, callus, pollen, leaves, anthers, stems, petioles, roots, root tips, fruits, seeds, flowers, cotyledons, and/or hypocotyls.

[0026] The present invention also provides methods of decreasing the activity of one or more KRP proteins in a plant cell, plant part, tissue culture or whole plant comprising contacting the plant cell, plant part, tissue culture or whole plant with an inhibitory nucleic acid having complementarity to a gene encoding said KRP protein. In some embodiments, the plant is a plant from the *Triticeae* tribe or the *Oryzeae* tribe. In some embodiments, said plant is a wheat or a rice plant. In some embodiments, said plant is a dicot plant. In some embodiments, said dicot plant is from the Fabaceae family, such as a soybean plant.

[0027] The present invention also provides methods of breeding a crop species having increased weight, size, and/or number of one or more organs, for example, a crop species with increased seed size, seed number, seed weight and/or seed yield compared to a wild type reference plant, comprising incorporating the genetic materials of a plant with disrupted KRP(s) into a recipient plant.

[0028] In some embodiments, such methods comprise making a cross between a *Triticum sp.* mutant with one or more mutations listed in Tables 2 - 12 with a second *Triticum sp.* to produce an F1 plant, or with a species in the *Triticeae* tribe which can intercross with said first *Triticum sp.* The method may further comprise backcrossing the F1 plant to the second *Triticum sp.* or species in the *Triticeae* tribe; and repeating the backcrossing step to generate an near isogenic line, wherein the one or more mutations are integrated into the genome of said second *Triticum sp.* or the species in the *Triticeae* tribe; wherein the near isogenic line derived from the second *Triticum sp.* or the species in the *Triticeae* tribe with the integrated mutations has altered weight, size, and/or number of one or more organs, for example, altered seed weight, seed size, seed number, and/or seed yield. Optionally, such methods can be facilitated by molecular markers or TILLING®.

[0029] In some embodiments, such methods comprise making a cross between an *Oryza sp.* mutant with one or more mutations listed in Table 25 with a second *Oryza sp.* to produce an F1 plant, or with a species in the *Oryzeae* tribe which can intercross with said *Oryza sp.* The method may further comprise backcrossing the F1 plant to the second *Oryza sp.* or species in the *Oryzeae* tribe; and repeating the backcrossing step to generate an near isogenic line, wherein the one or more mutations are integrated into the genome of said second *Oryza sp.* or the species in the *Oryzeae* tribe; wherein the near isogenic line derived from the second *Oryza sp.* or the species in the *Oryzeae* tribe with the integrated mutations

has altered weight, size, and/or number of one or more organs, for example, altered seed weight, seed size, seed number, and/or seed yield. Optionally, such methods can be facilitated by molecular markers or TILLING®.

[0030] In some embodiments, such methods comprise making a cross between a *Glycine sp.* mutant with one or more mutations listed in Tables 29 - 37 with a second *Glycine sp.* to produce an F1 plant, or with a species in the *Fabaceae* family which can intercross with said first *Glycine sp.* The method may further comprise backcrossing the F1 plant to the second *Glycine sp.* or species in the *Fabaceae* family; and repeating the backcrossing step to generate an near isogenic line, wherein the one or more mutations are integrated into the genome of said second *Glycine sp.* or species in the *Fabaceae* family; wherein the near isogenic line derived from the second *Glycine sp.* or species in the *Fabaceae* family with the integrated mutations has altered weight, size, and/or number of one or more organs, for example, altered seed weight, seed size, seed number, and/or seed yield. Optionally, such methods can be facilitated by molecular markers or TILLING®.

[0031] The present invention provides an isolated nucleic acid sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 138, 139, 141, 142, 144, and 145, and fragments and variations derived from thereof, which encode a wheat KRP gene.

[0032] In one embodiment, the present invention provides an isolated polynucleotide encoding plant KRP protein, comprising a nucleic acid sequence that shares at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.1%, at least 99.2%, at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, or at least 99.9% identity to SEQ ID NOs: 138, 139, 141, 142, 144, and 145.

[0033] The present invention further provides an isolated amino acid sequence (e.g., a peptide, polypeptide and the like) comprising a sequence selected from the group consisting of SEQ ID NOs: 140, 143, and 146 and fragments and variations derived from thereof, which form a KRP protein.

[0034] In some embodiments, the present invention provides an isolated amino acid sequence which forms a protein that shares an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.1%, at least 99.2%, at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, or at least 99.9% identity to SEQ ID NOs: 140, 143, and 146.

[0035] In one embodiment, isolated polynucleotides of the present invention comprise a sequence selected from the group consisting of: (a) sequences recited in SEQ ID NOs: 138, 139, 141, 142, 144, and 145, or portions thereof; (b) complements of the sequences recited in SEQ ID NOs: 138, 139, 141, 142, 144, and 145, or portions thereof; (c) reverse complements of the sequences recited in SEQ ID NOs: 138, 139, 141, 142, 144, and 145 or portions thereof; (d) reverse sequences of the sequences recited in SEQ ID NOs: 138, 139, 141, 142, 144, and 145, or portions thereof; and (e) sequences having at least 50%, 75%, 90%, 95% or 98% identity, as defined herein, to a sequence of (a)-(d) or a specified region of a sequence of (a)-(d).

[0036] The present invention also provides a chimeric gene comprising the isolated nucleic acid sequence of any one of the polynucleotides described above operably linked to suitable regulatory sequences.

[0037] The present invention also provides recombinant constructs comprising the chimeric gene as described above.

[0038] The present invention further provides interfering RNA (RNAi) constructs based on nucleic acid sequences of the present invention. In some embodiments, the RNAi constructs are can be transformed into a wheat plant to down-regulate one or more KRPs. The RNAi construct can be, but is not limited to antisense oligonucleotide construct, double-strand oligonucleotide construct, siRNA construct, or inverted repeat construct. In some embodiment, the RNAi constructs comprise a plant promoter, such as a constitutive promoter, an inducible promoter, or a tissue-specific promoter. In some embodiments, the promoter is embryonic specific or seed specific.

BRIEF DESCRIPTION OF THE DRAWINGS

[0039]

Figure 1 A depicts an amplification plot of fluorescence signal versus cycle number for a wheat genomic sample homozygous for the wild type allele of wheat *KRP4B.* The upper curve represents the amplification with the wild type probe, while the bottom curve represents the amplification with the mutant probe. **Figure 1B** depicts an amplification plot of fluorescence signal versus cycle number for a wheat genomic sample homozygous for the mutant allele of wheat *KRP4B.* The upper curve represents the amplification with the mutant probe, while the bottom curve represents the amplification with the wild type probe. **Figure 1C** depicts an amplification plot of fluorescence signal versus cycle number for a wheat genomic sample heterozygous for the mutant allele of wheat *KRP4B.* **Figure 1D** depicts a sequence chromatogram showing the two nucleotides detected in a wheat genomic sample heterozygous for the mutant allele of wheat *KRP4B.*

Figure 2 depicts an allelic discrimination plot for samples in a *KRP4B* allelic discrimination assay. Samples ho-

mozygous for the wild type KRP4B allele are in blue, samples homozygous for the mutant *KRP4B* P109L allele are in red and samples heterozygous for the mutant *KRP4B* P109L allele are in green. Black squares represent no template controls and black x's are undetermined samples.

Figure 3 depicts the C-terminal amino acid sequences that include the cyclin and cyclin-dependent kinase (CDK) binding domains for KRP1A, 2D, 4A and 5A. Nonsense and Type I severe missense TILLING® mutations tested in the *in vitro* kinase assay are indicated by asterisks or mutant amino acids, respectively, below the wild-type amino acids.

Figure 4 depicts an autoradiograph of kinase assays using ZmCyclinD4/CDKA;1 kinase complex, indicated wild-type *Triticum aestivum* KRP and indicated *Triticum aestivum* KRP TILLING® mutant. Histone H1 (HH1) was used as the substrate for phosphorylation. Lanes 1 and 18: kinase complex without any wild-type or KRP TILLING® mutant. Lanes 2 and 19: only kinase complex in buffer. Lanes 3, 4, 5: kinase complex and wild-type TaKRP1A at 0.5, 0.25 and 0.1 μg, respectively. Lanes 6, 7, 8: kinase complex and mutant TaKRP1A P232L at 0.5, 0.25 and 0.1 μg, respectively. Lanes 9, 10, 11: kinase complex and mutant TaKRP1A G236S at 0.5, 0.25 and 0.1 μg, respectively. Lanes 12, 13, 14: kinase complex and mutant TaKRP1A W240* at 0.5, 0.25 and 0.1 μg, respectively. Lanes 15, 16, 17: kinase complex and wild-type TaKRP2D at 0.5, 0.25 and 0.1 μg, respectively. Lanes 20, 21, 22: kinase complex and mutant TaKRP2D P228S at 0.5, 0.25 and 0.1 μg, respectively. Lanes 23, 24, 25: kinase complex and mutant TaKRP2D D254N at 0.5, 0.25 and 0.1 μg, respectively. Lanes 26, 27, 28: kinase complex and mutant TaKRP2D R257C at 0.5, 0.25 and 0.1 μg, respectively. Lanes 29, 30: kinase complex and wild-type TaKRP4A at 0.25 and 0.1 μg, respectively. Lanes 31, 32, 33: kinase complex and mutant TaKRP4A W186* at 0.5. 0.25 and 0.1 μg, respectively.

Figure 5 depicts an autoradiograph of kinase assays using ZmCyclinD4/CDKA;1 kinase complex, indicated wild-type *Triticum aestivum* KRP and indicated *Triticum aestivum* KRP TILLING® mutant. Histone H1 (HH1) was used as the substrate for phosphorylation. Lane 1: kinase complex without any wild-type or KRP TILLING® mutant. Lanes 2 and 18: only kinase complex in buffer. Lanes 3, 4, 5: kinase complex and wild-type TaKRP2D at 0.5, 0.25 and 0.1 μg, respectively. Lanes 6, 7, 8: kinase complex and mutant TaKRP2D A238V at 0.5, 0.25 and 0.1 μg, respectively. Lanes 9, 10, 11: kinase complex and mutant TaKRP2D A239T at 0.5, 0.25 and 0.1 μg, respectively. Lanes 12, 13, 14: kinase complex and wild-type TaKRP5A at 0.5, 0.25 and 0.1 μg, respectively. Lanes 15, 16, 17: kinase complex and mutant TaKRP5A W199* at 0.5, 0.25 and 0.1 μg, respectively.

Figure 6A depicts an autoradiograph of repeat kinase assays using ZmCyclinD4/CDKA;1 kinase complex, indicated wild-type *Triticum aestivum* KRP and indicated *Triticum aestivum* KRP TILLING® mutant. Histone H1 (HH1) was used as the substrate for phosphorylation. Lane 1: kinase complex without any wild-type or KRP TILLING® mutant. Lane 2: only kinase complex in buffer. Lanes 3, 4, 5: kinase complex and wild-type TaKRP1A at 0.5, 0.25 and 0.1 μg, respectively. Lanes 6, 7, 8: kinase complex and mutant TaKRP1A W240* at 0.5, 0.25 and 0.1 μg, respectively. Lanes 9, 10: kinase complex and wild-type TaKRP4A at 0.25 and 0.1 μg, respectively. Lanes 11, 12, 13: kinase complex and mutant TaKRP4A W186* at 0.5, 0.25 and 0.1 μg, respectively. Figure 6B depicts an autoradiograph of kinase assays using ZmCyclinD4/CDKA;1 kinase complex and ZmKRP2 W250*. ZmKRP2 W250* is the mutant corn krp2 protein with an equivalent premature stop codon very close to the end of the protein. Lanes 1 and 2: kinase complex and mutant ZmKRP2 W250* at 0.3 and 3 μg, respectively.

Figure 7 depicts average grain yield (lbs/ac) of spring wheat KRP TILLING® mutants and check cultivar Express. Bozeman, MT, Fort Collins, CO. *, ** stands for significant difference between mutant and check cultivar at the 0.05 and 0.01 probability levels, respectively.

Figure 8 depicts average grain yield (lbs/ac) of wild type (WT) and homozygous (Homo) zygotic groups for each spring wheat KRP TILLING® mutant and for check cultivar 'Express'. Bozeman, MT, Fort Collins, CO. *, ** stands for significant difference between wild type (WT) and homozygous (Homo) zygotic groups at the 0.05 and 0.01 probability levels, respectively.

Figure 9 depicts average grain yield (lbs/ac) of wild type (WT) and homozygous (Homo) zygotic groups within sister F1 lines '148E04' and 149E05' from spring wheat KRP TILLING® mutant KRP4D Stop. Bozeman, MT, Fort Collins, CO.

Figure 10 depicts a phylogenetic tree of rice (Os), corn (Zm) and wheat (Ta) KRPs.

Figure 11 depicts an alignment of rice (Os), corn (Zm) and wheat (Ta) KRP proteins.

Figure 12 depicts an alignment of soy (Gm), Arabidopsis (At) and Brassica napus (Bn) KRP proteins.

SEQUENCES

[0040] Sequence listings for SEQ ID No: 1 - SEQ ID No: 152 are part of this application and are incorporated by reference herein. Sequence listings are provided at the end of this document.

DETAILED DESCRIPTION

[0041] All publications, patents and patent applications, including any drawings and appendices, and all nucleic acid sequences and polypeptide sequences identified by GenBank Accession numbers, herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

[0042] The following description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art or relevant to the presently claimed inventions, or that any publication specifically or implicitly referenced is prior art.

Definitions

[0043] As used herein, the verb "comprise" as is used in this description and in the claims and its conjugations are used in its non-limiting sense to mean that items following the word are included, but items not specifically mentioned are not excluded.

[0044] As used herein, the term "plant" refers to any living organism belonging to the kingdom Plantae (i.e., any genus/species in the Plant Kingdom). This includes familiar organisms such as but not limited to trees, herbs, bushes, grasses, vines, ferns, mosses and green algae. The term refers to both monocotyledonous plants, also called monocots, and dicotyledonous plants, also called dicots. Examples of particular plants include but are not limited to plants in the *Triticeae* tribe (e.g., plants in the *Triticum* genus), plants in the tribe of *Oryzeae* (e.g., plants in *Oryza* genus), plants in the *Andropogoneae* tribe (e.g., plants in the *Zea* genus, such has corn). Other non-limiting examples of plant include, potatoes, roses, apple trees, sunflowers, bananas, tomatoes, opo, pumpkins, squash, lettuce, cabbage, oak trees, guzmania, geraniums, hibiscus, clematis, poinsettias, sugarcane, taro, duck weed, pine trees, Kentucky blue grass, zoysia, coconut trees, brassica leafy vegetables (e.g. broccoli, broccoli raab, Brussels sprouts, cabbage, Chinese cabbage (Bok Choy and Napa), cauliflower, cavalo, collards, kale, kohlrabi, mustard greens, rape greens, and other brassica leafy vegetable crops), bulb vegetables (e.g. garlic, leek, onion (dry bulb, green, and Welch), shallot, and other bulb vegetable crops), citrus fruits (e.g. grapefruit, lemon, lime, orange, tangerine, citrus hybrids, pummelo, and other citrus fruit crops), cucurbit vegetables (e.g. cucumber, citron melon, edible gourds, gherkin, muskmelons (including hybrids and/or cultivars of cucumis melons), water-melon, cantaloupe, and other cucurbit vegetable crops), fruiting vegetables (including eggplant, ground cherry, pepino, pepper, tomato, tomatillo, and other fruiting vegetable crops), grape, leafy vegetables (e.g. romaine), root/tuber and corm vegetables (e.g. potato), and tree nuts (almond, pecan, pistachio, and walnut), berries (e.g., tomatoes, barberries, currants, elderberries, gooseberries, honeysuckles, mayapples, nannyberries, Oregon-grapes, see-buckthorns, hackberries, bearberries, lingonberries, strawberries, sea grapes, lackberries, cloudberries, loganberries, raspberries, salmonberries, thimbleberries, and wineberries), cereal crops (e.g., corn, rice, wheat, barley, sorghum, millets, oats, ryes, triticales, buckwheats, fonio, quinoa, oil palm), pome fruit (e.g., apples, pears), stone fruits (e.g., coffees, jujubes, mangos, olives, coconuts, oil palms, pistachios, almonds, apricots, cherries, damsons, nectarines, peaches and plums), vine (e.g., table grapes, wine grapes), fiber crops (e.g. hemp, cotton), ornamentals, and the like.

[0045] As used herein, the term "plant part" refers to any part of a plant including but not limited to the shoot, root, stem, seeds, stipules, leaves, petals, flowers, ovules, bracts, branches, petioles, internodes, bark, pubescence, tillers, rhizomes, fronds, blades, pollen, stamen, and the like. The two main parts of plants grown in some sort of media, such as soil, are often referred to as the "above-ground" part, also often referred to as the "shoots", and the "below-ground" part, also often referred to as the "roots".

[0046] The term "a" or "an" refers to one or more of that entity; for example, "a gene" refers to one or more genes or at least one gene. As such, the terms "a" (or "an"), "one or more" and "at least one" are used interchangeably herein. In addition, reference to "an element" by the indefinite article "a" or "an" does not exclude the possibility that more than one of the elements are present, unless the context clearly requires that there is one and only one of the elements.

[0047] As used herein, the term "nucleic acid" refers to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides, or analogs thereof. This term refers to the primary structure of the molecule, and thus includes double- and single-stranded DNA, as well as double- and single-stranded RNA. It also includes modified nucleic acids such as methylated and/or capped nucleic acids, nucleic acids containing modified bases, backbone modifications, and the like. The terms "nucleic acid" and "nucleotide sequence" are used interchangeably.

[0048] As used herein, the terms "polypeptide," "peptide," and "protein" are used interchangeably herein to refer to polymers of amino acids of any length. These terms also include proteins that are post-translationally modified through reactions that include glycosylation, acetylation and phosphorylation.

[0049] As used herein, the term "homologous" or "homologue" or "ortholog" is known in the art and refers to related sequences that share a common ancestor or family member and are determined based on the degree of sequence identity. The terms "homology", "homologous", "substantially similar" and "corresponding substantially" are used inter-

changeably herein. They refer to nucleic acid fragments wherein changes in one or more nucleotide bases do not affect the ability of the nucleic acid fragment to mediate gene expression or produce a certain phenotype. These terms also refer to modifications of the nucleic acid fragments of the instant invention such as deletion or insertion of one or more nucleotides that do not substantially alter the functional properties of the resulting nucleic acid fragment relative to the initial, unmodified fragment. It is therefore understood, as those skilled in the art will appreciate, that the invention encompasses more than the specific exemplary sequences. These terms describe the relationship between a gene found in one species, subspecies, variety, cultivar or strain and the corresponding or equivalent gene in another species, subspecies, variety, cultivar or strain. For purposes of this invention homologous sequences are compared. "Homologous sequences" or "homologues" or "orthologs" are thought, believed, or known to be functionally related. A functional relationship may be indicated in any one of a number of ways, including, but not limited to: (a) degree of sequence identity and/or (b) the same or similar biological function. Preferably, both (a) and (b) are indicated. The degree of sequence identity may vary, but in one embodiment, is at least 50% (when using standard sequence alignment programs known in the art), at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least 98.5%, or at least about 99%, or at least 99.5%, or at least 99.8%, or at least 99.9%. Homology can be determined using software programs readily available in the art, such as those discussed in Current Protocols in Molecular Biology (F.M. Ausubel et al., eds., 1987) Supplement 30, section 7.718, Table 7.71. Some alignment programs are MacVector (Oxford Molecular Ltd, Oxford, U.K.), ALIGN Plus (Scientific and Educational Software, Pennsylvania) and AlignX (Vector NTI, Invitrogen, Carlsbad, CA). Another alignment program is Sequencher (Gene Codes, Ann Arbor, Michigan), using default parameters.

[0050] As used herein, the term "nucleotide change" refers to, e.g., nucleotide substitution, deletion, and/or insertion, as is well understood in the art. For example, mutations contain alterations that produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded protein or how the proteins are made.

[0051] As used herein, the term "protein modification" refers to, e.g., amino acid substitution, amino acid modification, deletion, and/or insertion, as is well understood in the art,

[0052] As used herein, the term "derived from" refers to the origin or source, and may include naturally occurring, recombinant, unpurified, or purified molecules. A nucleic acid or an amino acid derived from an origin or source may have all kinds of nucleotide changes or protein modification as defined elsewhere herein.

As used herein, the term "at least a portion" or "fragment" of a nucleic acid or polypeptide means a portion having the minimal size characteristics of such sequences, or any larger fragment of the full length molecule, up to and including the full length molecule. For example, a portion of a nucleic acid may be 12 nucleotides, 13 nucleotides, 14 nucleotides, 15 nucleotides, 16 nucleotides, 17 nucleotides, 18 nucleotides, 19 nucleotides, 20 nucleotides, 22 nucleotides, 24 nucleotides, 26 nucleotides, 28 nucleotides, 30 nucleotides, 32 nucleotides, 34 nucleotides, 36 nucleotides, 38 nucleotides, 40 nucleotides, 45 nucleotides, 50 nucleotides, 55 nucleotides, and so on, going up to the full length nucleic acid. Similarly, a portion of a polypeptide may be 4 amino acids, 5 amino acids, 6 amino acids, 7 amino acids, and so on, going up to the full length polypeptide. The length of the portion to be used will depend on the particular application. A portion of a nucleic acid useful as hybridization probe may be as short as 12 nucleotides; in one embodiment, it is 20 nucleotides. A portion of a polypeptide useful as an epitope may be as short as 4 amino acids. A portion of a polypeptide that performs the function of the full-length polypeptide would generally be longer than 4 amino acids.

[0053] As used herein, "sequence identity" or "identity" in the context of two nucleic acid or - polypeptide sequences includes reference to the residues in the two sequences which are the same when aligned for maximum correspondence over a specified comparison window. When percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. Where sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences which differ by such conservative substitutions are said to have "sequence similarity" or "similarity." Means for making this adjustment are well-known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, e.g., according to the algorithm of Meyers and Miller, Computer Applic. Biol. Sci., 4:11-17 (1988).

[0054] As used herein, the term "substantially complementary" means that two nucleic acid sequences have at least about 65%, preferably about 70% or 75%, more preferably about 80% or 85%, even more preferably 90% or 95%, and most preferably about 98% or 99%, sequence complementarities to each other. This means that primers and probes must exhibit sufficient complementarity to their template and target nucleic acid, respectively, to hybridize under stringent conditions. Therefore, the primer and probe sequences need not reflect the exact complementary sequence of the binding region on the template and degenerate primers can be used. For example, a non-complementary nucleotide

fragment may be attached to the 5'-end of the primer, with the remainder of the primer sequence being complementary to the strand. Alternatively, non-complementary bases or longer sequences can be interspersed into the primer, provided that the primer has sufficient complementarity with the sequence of one of the strands to be amplified to hybridize therewith, and to thereby form a duplex structure which can be extended by polymerizing means. The non-complementary nucleotide sequences of the primers may include restriction enzyme sites. Appending a restriction enzyme site to the end(s) of the target sequence would be particularly helpful for cloning of the target sequence. A substantially complementary primer sequence is one that has sufficient sequence complementarity to the amplification template to result in primer binding and second-strand synthesis. The skilled person is familiar with the requirements of primers to have sufficient sequence complementarity to the amplification template.

[0055] As used herein, the terms "polynucleotide", "polynucleotide sequence", "nucleic acid sequence", "nucleic acid fragment", and "isolated nucleic acid fragment" are used interchangeably herein. These terms encompass nucleotide sequences and the like. A polynucleotide may be a polymer of RNA or DNA that is single- or double-stranded, that optionally contains synthetic, non-natural or altered nucleotide bases. A polynucleotide in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA, synthetic DNA, or mixtures thereof. Nucleotides (usually found in their 5'-monophosphate form) are referred to by a 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.

[0056] As used herein, the phrase "a biologically active variant" or "functional variant" with respect to a protein refers to an amino acid sequence that is altered by one or more amino acids with respect to a reference sequence, while still maintains substantial biological activity of the reference sequence. The variant can have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with isoleucine. Alternatively, a variant can have "nonconservative" changes, e.g., replacement of a glycine with a tryptophan. Analogous minor variations can also include amino acid deletion or insertion, or both. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without eliminating biological or immunological activity can be found using computer programs well known in the art, for example, DNASTAR software.

[0057] The term "primer" as used herein refers to an oligonucleotide which is capable of annealing to the amplification target allowing a DNA polymerase to attach, thereby serving as a point of initiation of DNA synthesis when placed under conditions in which synthesis of primer extension product is induced, i.e., in the presence of nucleotides and an agent for polymerization such as DNA polymerase and at a suitable temperature and pH. The (amplification) primer is preferably single stranded for maximum efficiency in amplification. Preferably, the primer is an oligodeoxyribonucleotide. The primer must be sufficiently long to prime the synthesis of extension products in the presence of the agent for polymerization. The exact lengths of the primers will depend on many factors, including temperature and composition (A/T vs. G/C content) of primer. A pair of bi-directional primers consists of one forward and one reverse primer as commonly used in the art of DNA amplification such as in PCR amplification.

[0058] The terms "stringency" or "stringent hybridization conditions" refer to hybridization conditions that affect the stability of hybrids, e.g., temperature, salt concentration, pH, formamide concentration and the like. These conditions are empirically optimized to maximize specific binding and minimize non-specific binding of primer or probe to its target nucleic acid sequence. The terms as used include reference to conditions under which a probe or primer will hybridize to its target sequence, to a detectably greater degree than other sequences (e.g. at least 2-fold over background). Stringent conditions are sequence dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. Generally, stringent conditions are selected to be about 5° C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength and pH) at which 50% of a complementary target sequence hybridizes to a perfectly matched probe or primer. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M Na^+ ion, typically about 0.01 to 1.0 M Na + ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30° C for short probes or primers (e.g. 10 to 50 nucleotides) and at least about 60° C for long probes or primers (e.g. greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. Exemplary low stringent conditions or "conditions of reduced stringency" include hybridization with a buffer solution of 30% formamide, 1 M NaCl, 1% SDS at 37° C and a wash in 2×SSC at 40° C. Exemplary high stringency conditions include hybridization in 50% formamide, 1M NaCl, 1% SDS at 37° C, and a wash in 0.1×SSC at 60° C. Hybridization procedures are well known in the art and are described by e.g. Ausubel et al., 1998 and Sambrook et al., 2001.

[0059] As used herein, "coding sequence" refers to a DNA sequence that codes for a specific amino acid sequence. "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.

[0060] As used herein, "regulatory sequences" may include, but are not limited to, promoters, translation leader se-

quences, introns, and polyadenylation recognition sequences.

[0061] As used herein, "promoter" refers to a DNA sequence capable of controlling the expression of a coding sequence or functional RNA. The promoter sequence consists of proximal and more distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an "enhancer" is a DNA sequence that can stimulate promoter activity, and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue specificity of a promoter. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic DNA segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions. It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, DNA fragments of some variation may have identical promoter activity.

[0062] As used herein, a "plant promoter" is a promoter capable of initiating transcription in plant cells whether or not its origin is a plant cell, e.g. it is well known that *Agrobacterium* promoters are functional in plant cells. Thus, plant promoters include promoter DNA obtained from plants, plant viruses and bacteria such as *Agrobacterium* and *Bradyrhizobium* bacteria. A plant promoter can be a constitutive promoter or a non-constitutive promoter.

[0063] As used herein, a "constitutive promoter" is a promoter which is active under most conditions and/or during most development stages. There are several advantages to using constitutive promoters in expression vectors used in plant biotechnology, such as: high level of production of proteins used to select transgenic cells or plants; high level of expression of reporter proteins or scorable markers, allowing easy detection and quantification; high level of production of a transcription factor that is part of a regulatory transcription system; production of compounds that requires ubiquitous activity in the plant; and production of compounds that are required during all stages of plant development. Non-limiting exemplary constitutive promoters include, CaMV 35S promoter, opine promoters, ubiquitin promoter, actin promoter, alcohol dehydrogenase promoter, etc.

[0064] As used herein, a "non-constitutive promoter" is a promoter which is active under certain conditions, in certain types of cells, and/or during certain development stages. For example, tissue specific, tissue preferred, cell type specific, cell type preferred, inducible promoters, and promoters under development control are non-constitutive promoters. Examples of promoters under developmental control include promoters that preferentially initiate transcription in certain tissues, such as stems, leaves, roots, or seeds.

[0065] As used herein, "inducible" or "repressible" promoter is a promoter which is under chemical or environmental factors control. Examples of environmental conditions that may effect transcription by inducible promoters include anaerobic conditions, or certain chemicals, or the presence of light.

[0066] As used herein, a "tissue specific" promoter is a promoter that initiates transcription only in certain tissues. Unlike constitutive expression of genes, tissue-specific expression is the result of several interacting levels of gene regulation. As such, in the art sometimes it is preferable to use promoters from homologous or closely related plant species to achieve efficient and reliable expression of transgenes in particular tissues. This is one of the main reasons for the large amount of tissue-specific promoters isolated from particular plants and tissues found in both scientific and patent literature. Non-limiting tissue specific promoters include, beta-amylase gene or barley hordein gene promoters (for seed gene expression), tomato pz7 and pz130 gene promoters (for ovary gene expression), tobacco RD2 gene promoter (for root gene expression), banana TRX promoter and melon actin promoter (for fruit gene expression), and embryo specific promoters, e.g., a promoter associated with an amino acid permease gene (AAP1), an oleate 12-hydroxylase:desaturase gene from Lesquerella fendleri (LFAH12), an 2S2 albumin gene (2S2), a fatty acid elongase gene (FAE1), or a leafy cotyledon gene (LEC2).

[0067] As used herein, a "tissue preferred" promoter is a promoter that initiates transcription mostly, but not necessarily entirely or solely in certain tissues.

[0068] As used herein, a "cell type specific" promoter is a promoter that primarily drives expression in certain cell types in one or more organs, for example, vascular cells in roots, leaves, stalk cells, and stem cells.

[0069] As used herein, a "cell type preferred" promoter is a promoter that primarily drives expression mostly, but not necessarily entirely or solely in certain cell types in one or more organs, for example, vascular cells in roots, leaves, stalk cells, and stem cells.

[0070] As used herein, the "3' non-coding sequences" or "3' untranslated regions" refer to DNA sequences located downstream of a coding sequence and include polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht, I. L., et al. (1989) Plant Cell 1:671-680.

[0071] As used herein, "RNA transcript" refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript. An RNA transcript is referred to as the mature RNA when it is an RNA sequence derived from post-transcriptional processing of the primary transcript. "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 an 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. "Sense" RNA refers to RNA transcript that includes the mRNA and can be translated into protein within a cell or in vitro. "Antisense RNA" refers to an RNA transcript that is complementary to all or part of a target primary transcript or mRNA, and that blocks the expression of a target gene (U.S. Pat. No. 5,107,065). The complementarity of an antisense RNA may be with any part of the specific gene transcript, i.e., at the 5' non-coding sequence, 3' non-coding sequence, introns, or the coding sequence. "Functional RNA" refers to antisense RNA, ribozyme RNA, or other RNA that may not be translated but yet has an effect on cellular processes. The terms "complement" and "reverse complement" are used interchangeably herein with respect to mRNA transcripts, and are meant to define the antisense RNA of the message.

[0072] As used herein, the term "operably linked" refers to the association of nucleic acid sequences on a single nucleic acid fragment so that the function of one is regulated by the other. For example, a promoter is operably linked with a coding sequence when it is capable of regulating the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in a sense or antisense orientation. In another example, the complementary RNA regions of the invention can be operably linked, either directly or indirectly, 5' to the target mRNA, or 3' to the target mRNA, or within the target mRNA, or a first complementary region is 5' and its complement is 3' to the target mRNA.

[0073] As used herein, the term "recombinant" refers to an artificial combination of two otherwise separated segments of sequence, e.g., by chemical synthesis or by the manipulation of isolated segments of nucleic acids by genetic engineering techniques.

[0074] As used herein, the phrases "recombinant construct", "expression construct", "chimeric construct", "construct", and "recombinant DNA construct" are used interchangeably herein. A recombinant construct comprises an artificial combination of nucleic acid fragments, e.g., regulatory and coding sequences that are not found together in nature. For example, a chimeric construct may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. Such construct may be used by itself or may be used in conjunction with a vector. If a vector is used then the choice of vector is dependent upon the method that will be used to transform host cells as is well known to those skilled in the art. For example, a plasmid vector can be used. The skilled artisan is well aware of the genetic elements that must be present on the vector in order to successfully transform, select and propagate host cells comprising any of the isolated nucleic acid fragments of the invention. The skilled artisan will also recognize that different independent transformation events will result in different levels and patterns of expression (Jones et al., (1985) EMBO J. 4:2411-2418; De Almeida et al., (1989) Mol. Gen. Genetics 218:78-86), and thus that multiple events must be screened in order to obtain lines displaying the desired expression level and pattern. Such screening may be accomplished by Southern analysis of DNA, Northern analysis of mRNA expression, immunoblotting analysis of protein expression, or phenotypic analysis, among others. Vectors can be plasmids, viruses, bacteriophages, pro-viruses, phagemids, transposons, artificial chromosomes, and the like, that replicate autonomously or can integrate into a chromosome of a host cell. A vector can also be a naked RNA polynucleotide, a naked DNA polynucleotide, a polynucleotide composed of both DNA and RNA within the same strand, a poly-lysine-conjugated DNA or RNA, a peptide-conjugated DNA or RNA, a liposome-conjugated DNA, or the like, that is not autonomously replicating.

[0075] The term "expression", as used herein, refers to the production of a functional endproduct e.g., an mRNA or a protein (precursor or mature).

[0076] As used herein, the phrase "plant selectable or screenable marker" refers to a genetic marker functional in a plant cell. A selectable marker allows cells containing and expressing that marker to grow under conditions unfavorable to growth of cells not expressing that marker. A screenable marker facilitates identification of cells which express that marker.

[0077] As used herein, the term "inbred", "inbred plant" is used in the context of the present invention. This also includes any single gene conversions of that inbred. The term single allele converted plant as used herein refers to those plants which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in addition to the single allele transferred into the inbred via the backcrossing technique.

[0078] As used herein, the term "sample" includes a sample from a plant, a plant part, a plant cell, or from a transmission vector, or a soil, water or air sample.

[0079] As used herein, the term "offspring" refers to any plant resulting as progeny from a vegetative or sexual reproduction from one or more parent plants or descendants thereof. For instance an offspring plant may be obtained by cloning or selfing of a parent plant or by crossing two parent plants and include selfings as well as the F1 or F2 or still further generations. An F1 is a first-generation offspring produced from parents at least one of which is used for the first time as donor of a trait, while offspring of second generation (F2) or subsequent generations (F3, F4, etc.) are specimens produced from selfings of F1's, F2's etc. An F1 may thus be (and usually is) a hybrid resulting from a cross between

two true breeding parents (true-breeding is homozygous for a trait), while an F2 may be (and usually is) an offspring resulting from self-pollination of said F1 hybrids.

[0080] As used herein, the term "cross", "crossing", "cross pollination" or "cross-breeding" refer to the process by which the pollen of one flower on one plant is applied (artificially or naturally) to the ovule (stigma) of a flower on another plant.

[0081] As used herein, the term "cultivar" refers to a variety, strain or race of plant that has been produced by horticultural or agronomic techniques and is not normally found in wild populations.

[0082] As used herein, the terms "dicotyledon" and "dicot" refer to a flowering plant having an embryo containing two seed halves or cotyledons. Dicotyledon plants at least include the Eudicot, Magnoliid, Amborella, Nymphaeales, Austrobaileyales, Chloranthales, and Ceratophyllum groups. Eudicots include these clades: *Ranunculales, sabiales, Proteales, Trochodendrales, Buxales,* and *Core Eudicots* (e.g., *Berberidopsidales, Dilleniales, Gunnerales*, *Caryophyllales, Santalales, Saxifragales. Vitales, Rosids* and *Asterids).* Non-limiting examples of dicotyledon plants include tobacco, tomato, pea, alfalfa, clover, bean, soybean, peanut, members of the Brassicaceae family (e.g., camelina, Canola, oilseed rape, etc.), amaranth, sunflower, sugarbeet, cotton,'oaks, maples, roses, mints, squashes, daisies, nuts; cacti, violets and buttercups.

[0083] As used herein, the term "monocotyledon" or "monocot" refer to any of a subclass (Monocotyledoneae) of flowering plants having an embryo containing only one seed leaf and usually having parallel-veined leaves, flower parts in multiples of three, and no secondary growth in stems and roots. Non-limiting examples of monocotyledon plants include lilies, orchids, corn, rice, wheat, barley, sorghum, millets, oats, ryes, triticales, buckwheats, fonio, quinoa, grasses, such as tall fescue, goat grass, and Kentucky bluegrass; grains, such as wheat, oats and barley, irises, onions, palms.

[0084] As used herein, the term "gene" refers to any segment of DNA associated with a biological function. Thus, genes include, but are not limited to, coding sequences and/or the regulatory sequences required for their expression. Genes can also include nonexpressed DNA segments that, for example, form recognition sequences for other proteins. Genes can be obtained from a variety of sources, including cloning from a source of interest or synthesizing from known or predicted sequence information, and may include sequences designed to have desired parameters.

[0085] As used herein, the term "genotype" refers to the genetic makeup of an individual cell, cell culture, tissue, organism (e.g., a plant), or group of organisms.

[0086] As used herein, the term "hemizygous" refers to a cell, tissue or organism in which a gene is present only once in a genotype, as a gene in a haploid cell or organism, a sex-linked gene in the heterogametic sex, or a gene in a segment of chromosome in a diploid cell or organism where its partner segment has been deleted.

[0087] As used herein, the terms "heterologous polynucleotide" or a "heterologous nucleic acid" or an "exogenous DNA segment" refer to a polynucleotide, nucleic acid or DNA segment that originates from a source foreign to the particular host cell, or, if from the same source, is modified from its original form. Thus, a heterologous gene in a host cell includes a gene that is endogenous to the particular host cell, but has been modified. Thus, the terms refer to a DNA segment which is foreign or heterologous to the cell, or homologous to the cell but in a position within the host cell nucleic acid in which the element is not ordinarily found. Exogenous DNA segments are expressed to yield exogenous polypeptides.

[0088] As used herein, the term "heterologous trait" refers to a phenotype imparted to a transformed host cell or transgenic organism by an exogenous DNA segment, heterologous polynucleotide or heterologous nucleic acid.

[0089] As used herein, the term "heterozygote" refers to a diploid or polyploid individual cell or plant having different alleles (forms of a given gene) present at least at one locus.

[0090] As used herein, the term "heterozygous" refers to the presence of different alleles (forms of a given gene) at a particular gene locus.

[0091] As used herein, the terms "homolog" or "homologue" refer to a nucleic acid or peptide sequence which has a common origin and functions similarly to a nucleic acid or peptide sequence from another species.

[0092] As used herein, the term "homozygote" refers to an individual cell or plant having the same alleles at one or more or all loci. When the term is used with reference to a specific locus or gene, it means at least that locus or gene has the same alleles.

[0093] As used herein, the terms "homozygous" or "HOMO" refer to the presence of identical alleles at one or more or all loci in homologous chromosomal segments. When the terms are used with reference to a specific locus or gene, it means at least that locus or gene has the same alleles.

[0094] As used herein, the term "hybrid" refers to any individual cell, tissue or plant resulting from a cross between parents that differ in one or more genes.

[0095] As used herein, the term "inbred" or "inbred line" refers to a relatively true-breeding strain.

[0096] As used herein, the term "line" is used broadly to include, but is not limited to, a group of plants vegetatively propagated from a single parent plant, via tissue culture techniques or a group of inbred plants which are genetically very similar due to descent from a common parent(s). A plant is said to "belong" to a particular line if it (a) is a primary transformant (TO) plant regenerated from material of that line; (b) has a pedigree comprised of a T0 plant of that line;

or (c) is genetically very similar due to common ancestry (e.g., via inbreeding or selfing). In this context, the term "pedigree" denotes the lineage of a plant, e.g. in terms of the sexual crosses affected such that a gene or a combination of genes, in heterozygous (hemizygous) or homozygous condition, imparts a desired trait to the plant.

[0097] As used herein, the terms "mutant" or "mutation" refer to a gene, cell, or organism with an abnormal genetic constitution that may result in a variant phenotype.

As used herein, the term "open pollination" refers to a plant population that is freely exposed to some gene flow, as opposed to a closed one in which there is an effective barrier to gene flow.

[0098] As used herein, the terms "open-pollinated population" or "open-pollinated variety" refer to plants normally capable of at least some cross-fertilization, selected to a standard, that may show variation but that also have one or more genotypic or phenotypic characteristics by which the population or the variety can be differentiated from others. A hybrid, which has no barriers to cross-pollination, is an open-pollinated population or an open-pollinated variety.

[0099] As used herein when discussing plants, the term "ovule" refers to the female gametophyte, whereas the term "pollen" means the male gametophyte.

[0100] As used herein, the term "phenotype" refers to the observable characters of an individual cell, cell culture, organism (e.g., a plant), or group of organisms which results from the interaction between that individual's genetic makeup (i.e., genotype) and the environment.

[0101] As used herein, the term "plant tissue" refers to any part of a plant. Examples of plant organs include, but are not limited to the leaf, stem, root, tuber, seed, branch, pubescence, nodule, leaf axil, flower, pollen, stamen, pistil, petal, peduncle, stalk, stigma, style, bract, fruit, trunk, carpel, sepal, anther, ovule, pedicel, needle, cone, rhizome, stolon, shoot, pericarp, endosperm, placenta, berry, stamen, and leaf sheath.

[0102] As used herein, the term "self-crossing", "self pollinated" or "self-pollination" means the pollen of one flower on one plant is applied (artificially or naturally) to the ovule (stigma) of the same or a different flower on the same plant.

[0103] As used herein, the term "transformation" refers to the transfer of nucleic acid (i. e., a nucleotide polymer) into a cell. As used herein, the term "genetic transformation" refers to the transfer and incorporation of DNA, especially recombinant DNA, into a cell.

[0104] As used herein, the term "transformant" refers to a cell, tissue or organism that has undergone transformation. The original transformant is designated as "T0" or "T_0." Selfing the T0 produces a first transformed generation designated as "T1" or "T_1."

[0105] As used herein, the term "transgene" refers to a nucleic acid that is inserted into an organism, host cell or vector in a manner that ensures its function.

[0106] As used herein, the term "transgenic" refers to cells, cell cultures, organisms (e.g., plants), and progeny which have received a foreign or modified gene by one of the various methods of transformation, wherein the foreign or modified gene is from the same or different species than the species of the organism receiving the foreign or modified gene.

[0107] As used herein, the term "transposition event" refers to the movement of a transposon from a donor site to a target site.

[0108] As used herein, the term "variety" refers to a subdivision of a species, consisting of a group of individuals within the species that are distinct in form or function from other similar arrays of individuals.

[0109] As used herein, the term "vector", "plasmid", or "construct" refers broadly to any plasmid or virus encoding an exogenous nucleic acid. The term should also be construed to include non-plasmid and non-viral compounds which facilitate transfer of nucleic acid into virions or cells, such as, for example, polylysine compounds and the like. The vector may be a viral vector that is suitable as a delivery vehicle for delivery of the nucleic acid, or mutant thereof, to a cell, or the vector may be a non-viral vector which is suitable for the same purpose. Examples of viral and non-viral vectors for delivery of DNA to cells and tissues are well known in the art and are described, for example, in Ma et al. (1997, Proc. Natl. Acad. Sci. U.S.A. 94:12744-12746).

[0110] As used herein, the phrase "seed size" refers to the volume of the seed material itself, which is the space occupied by the constituents of the seed.

[0111] As used herein, the phrase "seed number" refers to the average number of seeds produced from each fruit, each plant, or each predetermined area (e.g., 1 acre).

[0112] As used herein, the phrase "Test Weight" or "Grain Test Weight" is a determination of bulk density (mass/volume), measured for commerce under specific conditions defined in the U.S. by the USDA-FGIS. Test weight is a general indicator of grain quality and higher test weight normally means higher quality grain. Grain test weight in units of pounds per bushel specifies the weight of a "volume" bushel, which is 32 quarts (30,283 cubic centimeters) of grain. When grain is traded, samples are usually tested for quality, and test weight is one of the tests carried out. Test weights have been a part of U.S. grain grades since the United States Grain Standards Act was passed by Congress in 1916. U.S. grades for most grains specify test weight minimums for each grade level. For instance, the official minimum allowable test weight in the U.S. for No. 1 yellow corn is 56 lbs/bu and for No. 2 yellow corn is 54 lbs/bu (USDA-GIPSA, 1996). By law, a "weight" bushel of corn is exactly 56 pounds, a soybean bushel is 60 pounds, and a wheat bushel is 60 pounds, regardless of the test weight. The "weight" bushel is used for the basis of payment for grain, but price discounts are

often tied to shipments of lower grade grain possessing low test weight.

[0113] As used herein, the phrase "Grain Apparent Density" refers to grain density determined in a fashion wherein the bulk density (mass/volume) of cereal seed is sometimes measured with the aid of a gas pycnometer, which typically uses helium and measures the volume of the sample. Grain kernels contain internal void spaces and intercellular spaces and are not completely porous to helium. Since the gas cannot reach all internal spaces, the volume of material comprising the kernel can be overestimated with gas pycnometry and a density lower than the "true density" of grain material is determined (Chang, CS (1988) Cereal Chem:65:13-15).

[0114] As used herein, the phrase "Grain True Density" refers to the bulk density of grain, expressed as the quotient of mass divided by volume, whereby all void space not comprising solid materials of the seed has been eliminated before, or discounted in, determination of the volume used in the calculation (Chang, CS (1988) Cereal Chem:65:13-15).

[0115] As used herein, the term "cyclin dependent kinase inhibitor" (also referred to herein as "CDK inhibitor" or "CKI") refers to a class of proteins that negatively regulate cyclin dependent kinases (CDKs). CKIs amenable to the present invention are those having separate polypeptide regions capable of independently binding a cyclin and a CDK. Such CKIs include, for example, identified families of plant CKIs (the seven identified Arabidopsis CKIs), having homology to Kinase Inhibitor Proteins (KIPs) in animals, referred to as KIP-related proteins (KRPs) (also known as Inhibitors of "CDKs," or "ICKs").

[0116] The term "naturally occurring," in the context of CKI polypeptides and nucleic acids, means a polypeptide or nucleic acid having an amino acid or nucleotide sequence that is found in nature, i.e., an amino acid or nucleotide sequence that can be isolated from a source in nature (an organism) and which has not been intentionally modified by human intervention. As used herein, laboratory strains of plants which may have been selectively bred according to classical genetics are considered naturally-occurring plants.

[0117] As used herein, "wild-type CKI gene" or "wild-type CKI nucleic acid" refers to a sequence of nucleic acid, corresponding to a CKI genetic locus in the genome of an organism, that encodes a gene product performing the normal function of the CKI protein encoded by a naturally-occurring nucleotide sequence corresponding to the genetic locus. A genetic locus can have more than one sequence or allele in a population of individuals, and the term "wild-type" encompasses all such naturally-occurring alleles that encode a gene product performing the normal function. "Wild-type" also encompasses gene sequences that are not necessarily naturally occurring, but that still encode a gene product with normal function (e.g., genes having silent mutations or encoding proteins with conservative substitutions).

[0118] As used herein, the term "wild-type CKI polypeptide" or "wild-type CKI protein" refers to a CKI polypeptide encoded by a wild-type gene. A genetic locus can have more than one sequence or allele in a population of individuals, and the term "wild-type" encompasses all such naturally-occurring alleles that encode a gene product performing the normal function.

Breeding Methods

[0119] Classic breeding methods can be included in the present invention to introduce one or more recombinant KRPs of the present invention into other plant varieties, or other close-related species that are compatible to be crossed with the transgenic plant of the present invention.

[0120] Open-Pollinated Populations. The improvement of open-pollinated populations of such crops as rye, many maizes and sugar beets, herbage grasses, legumes such as alfalfa and clover, and tropical tree crops such as cacao, coconuts, oil palm and some rubber, depends essentially upon changing gene-frequencies towards fixation of favorable alleles while maintaining a high (but far from maximal) degree of heterozygosity. Uniformity in such populations is impossible and trueness-to-type in an open-pollinated variety is a statistical feature of the population as a whole, not a characteristic of individual plants. Thus, the heterogeneity of open-pollinated populations contrasts with the homogeneity (or virtually so) of inbred lines, clones and hybrids.

[0121] Population improvement methods fall naturally into two groups, those based on purely phenotypic selection, normally called mass selection, and those based on selection with progeny testing. Interpopulation improvement utilizes the concept of open breeding populations; allowing genes to flow from one population to another. Plants in one population (cultivar, strain, ecotype, or any germplasm source) are crossed either naturally (e.g., by wind) or by hand or by bees (commonly *Apis mellifera L.* or *Megachile rotundata F.*) with plants from other populations. Selection is applied to improve one (or sometimes both) population(s) by isolating plants with desirable traits from both sources.

[0122] There are basically two primary methods of open-pollinated population improvement. First, there is the situation in which a population is changed en masse by a chosen selection procedure. The outcome is an improved population that is indefinitely propagable by random-mating within itself in isolation. Second, the synthetic variety attains the same end result as population improvement but is not itself propagable as such; it has to be reconstructed from parental lines or clones. These plant breeding procedures for improving open-pollinated populations are well known to those skilled in the art and comprehensive reviews of breeding procedures routinely used for improving cross-pollinated plants are provided in numerous texts and articles, including: Allard, Principles of Plant Breeding, John Wiley & Sons, Inc. (1960);

Simmonds, Principles of Crop Improvement, Longman Group Limited (1979); Hallauer and Miranda, Quantitative Genetics in Maize Breeding, Iowa State University Press (1981); and, Jensen, Plant Breeding Methodology, John Wiley & Sons, Inc. (1988).

[0123] Mass Selection. In mass selection, desirable individual plants are chosen, harvested, and the seed composited without progeny testing to produce the following generation. Since selection is based on the maternal parent only, and there is no control over pollination, mass selection amounts to a form of random mating with selection. As stated herein, the purpose of mass selection is to increase the proportion of superior genotypes in the population.

[0124] Synthetics. A synthetic variety is produced by crossing inter se a number of genotypes selected for good combining ability in all possible hybrid combinations, with subsequent maintenance of the variety by open pollination. Whether parents are (more or less inbred) seed-propagated lines, as in some sugar beet and beans (Vicia) or clones, as in herbage grasses, clovers and alfalfa, makes no difference in principle. Parents are selected on general combining ability, sometimes by test crosses or topcrosses, more generally by polycrosses. Parental seed lines may be deliberately inbred (e.g. by selfing or sib crossing). However, even if the parents are not deliberately inbred, selection within lines during line maintenance will ensure that some inbreeding occurs. Clonal parents will, of course, remain unchanged and highly heterozygous.

[0125] Whether a synthetic can go straight from the parental seed production plot to the farmer or must first undergo one or two cycles of multiplication depends on seed production and the scale of demand for seed. In practice, grasses and clovers are generally multiplied once or twice and are thus considerably removed from the original synthetic.

[0126] While mass selection is sometimes used, progeny testing is generally preferred for polycrosses, because of their operational simplicity and obvious relevance to the objective, namely exploitation of general combining ability in a synthetic.

[0127] The number of parental lines or clones that enter a synthetic varies widely. In practice, numbers of parental lines range from 10 to several hundred, with 100-200 being the average. Broad based synthetics formed from 100 or more clones would be expected to be more stable during seed multiplication than narrow based synthetics.

[0128] Pedigreed varieties. A pedigreed variety is a superior genotype developed from selection of individual plants out of a segregating population followed by propagation and seed increase of self pollinated offspring and careful testing of the genotype over several generations. This is an open pollinated method that works well with naturally self pollinating species. This method can be used in combination with mass selection in variety development. Variations in pedigree and mass selection in combination are the most common methods for generating varieties in self pollinated crops.

[0129] Hybrids. A hybrid is an individual plant resulting from a cross between parents of differing genotypes. Commercial hybrids are now used extensively in many crops, including corn (maize), sorghum, sugarbeet, sunflower and broccoli. Hybrids can be formed in a number of different ways, including by crossing two parents directly (single cross hybrids), by crossing a single cross hybrid with another parent (three-way or triple cross hybrids), or by crossing two different hybrids (four-way or double cross hybrids).

[0130] Strictly speaking, most individuals in an out breeding (i.e., open-pollinated) population are hybrids, but the term is usually reserved for cases in which the parents are individuals whose genomes are sufficiently distinct for them to be recognized as different species or subspecies. Hybrids may be fertile or sterile depending on qualitative and/or quantitative differences in the genomes of the two parents. Heterosis, or hybrid vigor, is usually associated with increased heterozygosity that results in increased vigor of growth, survival, and fertility of hybrids as compared with the parental lines that were used to form the hybrid. Maximum heterosis is usually achieved by crossing two genetically different, highly inbred lines.

[0131] The production of hybrids is a well-developed industry, involving the isolated production of both the parental lines and the hybrids which result from crossing those lines. For a detailed discussion of the hybrid production process, see, e.g., Wright, Commercial Hybrid Seed Production 8:161-176, In Hybridization of Crop Plants.

Targeting Induced Local Lesions in Genomes (TILLING)

[0132] TILLING (Targeting Induced Local Lesions in Genomes) is a method in molecular biology that allows directed identification of mutations in a specific gene. TILLING® was introduced in 2000, using the model plant *Arabidopsis thaliana.* TILLING® has since been used as a reverse genetics method in other organisms such as zebrafish, corn, wheat, rice, soybean, tomato and lettuce.

[0133] The method combines a standard and efficient technique of mutagenesis with a chemical mutagen (e.g., Ethyl methanesulfonate (EMS)) with a sensitive DNA screening-technique that identifies single base mutations (also called point mutations) in a target gene. EcoTILLING is a method that uses TILLING® techniques to look for natural mutations in individuals, usually for population genetics analysis. See Comai, et al., 2003. Efficient discovery of DNA polymorphisms in natural populations by Ecotilling. The Plant Journal 37, 778-786. Gilchrist et al. 2006. Use of Ecotilling as an efficient SNP discovery tool to survey genetic variation in wild populations of Populus trichocarpa. Mol. Ecol. 15, 1367-1378. Mejlhede et al. 2006. EcoTILLING for the identification of allelic variation within the powdery mildew resistance genes

mlo and Mla of barley. Plant Breeding 125, 461-467. Nieto et al. 2007, EcoTILLING for the identification of allelic variants of melon eIF4E, a factor that controls virus susceptibility. BMC Plant Biology 7, 34-42, each of which is incorporated by reference hereby for all purposes. DEcoTILLING is a modification of TILLING® and EcoTILLING which uses an inexpensive method to identify fragments (Garvin et al., 2007, DEco-TILLING: An inexpensive method for SNP discovery that reduces ascertainment bias. Molecular Ecology Notes 7, 735-746).

[0134] The TILLING® method relies on the formation of heteroduplexes that are formed when multiple alleles (which could be from a heterozygote or a pool of multiple homozygotes and heterozygotes) are amplified in a PCR, heated, and then slowly cooled. A "bubble" forms at the mismatch of the two DNA strands (the induced mutation in TILLING® or the natural mutation or SNP in EcoTILLING), which is then cleaved by single stranded nucleases. The products are then separated by size on several different platforms.

[0135] Several TILLING® centers exists over the world that focus on agriculturally important species: UC Davis (USA), focusing on Rice; Purdue University (USA), focusing on Maize; University of British Columbia (CA), focusing on *Brassica napus;* John Innes Centre (UK), focusing on *Brassica rapa;* Fred Hutchinson Cancer Research, focusing on Arabidopsis; Southern Illinois University (USA), focusing on Soybean; John Innes Centre (UK), focusing on Lotus and Medicago ; and INRA (France), focusing on Pea and Tomato.

[0136] More detailed description on methods and compositions on TILLING® can be found in references Nos. 1 - 35b, US 5994075, US 2004/0053236 A1, WO 2005/055704, and WO 2005/048692, each of which is hereby incorporated by reference for all purposes.

Triticeae tribe

[0137] Intense use of wild Triticeae can be seen in the Levant as early as 23,000 years ago. Triticeae is a tribe within the Pooideae subfamily of grasses that includes genera with many domesticated species. Major crop genera are found in this tribe including wheat (See Wheat taxonomy), barley, and rye; crops in other genera include some for human consumption and others used for animal feed or rangeland protection. Among the world's cultivated species, this tribe has some of the most complex genetic histories. An example is bread wheat, which contains the genomes of three species, only one of them originally a wheat *Triticum* species.

[0138] Genera in the *Triticeae* tribe include, but are not limited to, Aegilops (goat grasses - jointed goatgrass, Tausch goatgrass, etc.); Agropyron (crested wheatgrasses - Desert wheatgrass, quackgrass, etc.); Amblyopyrum (Slim wheat grass - amblyopyrum, etc.); Australopyrum (Australian wheatgrasses - velvet wheatgrass,pectinated wheatgrass, etc.); Cockaynea (See Stenostachys; Cockaynea is a younger, and hence invalid, name for Stenostachys, etc.); Crithopsis (delileana grass etc.); Dasypyrum (Mosquito grass; etc.); Elymus (Elymus (wild ryes - blue wildrye,Texas ryegrass, etc.); Elytrigia; Eremium (Argentine desert ryegrass, etc.); Eremopyrum (false wheatgrasses - tapertip false wheatgrass,annual wheatgrass, etc.); Festucopsis; Haynaldia; Henrardia; Heteranthelium; Hordelymus; Hordeum (barleys - common barley, foxtail barley, etc.); Hystrix (porcupine grass- bottlebrush grass, etc.); Kengyilia; Leymus (wild rye- American dune grass,lyme grass, etc.); Lophopyrum (tall wheatgrass); Malacurus Pascopyrum(western wheatgrass etc.); Peridictyon; Psathyrostachys (Russian wildrye, etc.); Pseudoroegneria (bluebunch wheatgrasses - beardless wheatgrass, etc.); Secale (Ryes - Cereal rye, Himalayan Rye, etc.); Sitanion; Stenostachys (New Zealand wheatgrasses, etc); Taeniatherum (medusahead etc.); Thinopyrum (intermediate wheatgrass, Russian wheatgrass, thick quackgrass, etc.); Triticum (Wheats - common wheat, durum wheat, etc.).

[0139] Triticeae and its sister tribe Bromeae (possible cultivars: Bromus mango S. America) when joined form a sister clade with Poeae and Aveneae (oats). Inter-generic gene flow characterized these taxa from the early stages. For example, Poeae and Aveneae share a genetic marker with barley and 10 other members of Triticeae, whereas all 19 genera of Triticeae bear a wheat marker along with Bromeae. Genera within Triticeae contain diploid, allotetraploid and/or allohexaploid genomes, the capacity to form allopolyploid genomes varies within the tribe. In this tribe, the majority of diploid species tested are closely related to Aegilops, the more distal members (earliest branch points) include Hordeum (Barley), Eremian, Psathyrostachys.

[0140] Many genera and species of Triticeae are exemplary of allopolyploids, having more chromosomes than seen in typical diploids. Typically allopolyploids are tetraploid or hexaploid, AABB or AABBDD. The creation of polyploid species results from natural random events tolerated by polyploid capable plants. Likewise natural allopolyploid plants may have selective benefits and may allow the recombination of distantly related genetic material facilitating at a later time a reversion back to diploid. Poulard wheat is an example of a stable allotetraploid wheat.

[0141] Aegilops appears to be basal to several taxa such as Triticum, Ambylopyrum, and Crithopsis. Certain species such as Aegilops speltoides could potentially represent core variants of the taxa. The generic placement may be more a matter of nomenclature. Aegilops and Triticum genera are very closely related as the Aegilops species occupy most of the basal branch points in bread wheat evolution indicating that Triticum genus evolved from Aegilops after an estimated 4 million years ago. The divergence of the genomes is followed by allotetraploidation of a speltoid goatgrass x basal wheat species *Triticum boeoticum* with strains in the Middle Eastern region giving rise to cultivated emmer wheat.

Triticum spp.

[0142] *Triticum sp.* is a grass cultivated worldwide. In 2007 world production of wheat was 607 million tons, making it the third most-produced cereal after maize (784 million tons) and rice (651 million tons). Globally, wheat is the leading source of vegetable protein in human food, having a higher protein content than either maize (corn) or rice, the other major cereals. In terms of total production tonnages used for food, it is currently second to rice as the main human food

[0143] Wheat is planted to a limited extent as a forage crop for livestock, and its straw can be used as a construction material for roofing thatch. The husk of the grain, separated when milling white flour, is bran. Wheat germ is the embryo portion of the wheat kernel. It is a concentrated source of vitamins, minerals, and protein, and is sustained by the larger, starch storage region of the kernel-the endosperm.

[0144] Non-limiting examples of *Triticum* species include, *T. aestivum* (e.g., common wheat, or bread wheat, a.k.a. *Triticum aestivum* L. subsp. *Aestivum;* Club wheat, a.k.a. *Triticum aestivum* subspecies *compactum* (Host) MacKey; Macha wheat, a.k.a. *Triticum aestivum* subsp. *macha* (Dek. and Men.) MacKey; Vavilovi wheat, a.k.a. *Triticum aestivum* subsp. *vavilovi* (Tuman) Sears; Shot wheat, a.k.a. *Triticum aestivum* subsp. *sphacrococcum* (Perc.) MacKey), *T. aethiopicum, T araraticum, T. boeoticum* (e.g., wild Einkorn, a.k.a. *Triticum boeotictim* Boiss), *T. carthlicum, T. compactum, T. dimitrium, T. dicoccoides* (e.g., wild emmer, a.k.a. *Triticum dicoccoides* (Koern. ex Ascb. & Graebn.) Aaronsohn.), *T. dicoccum* (e.g., Emmer), *T. durum* (e.g., durum wheat), *T. ispahanicum*, *T. karamyschevii, T. macha, T. militinae*, *T. monococcum* (e.g., Einkorn, a.k.a. *Triticum monococcum* L.), *T. polonicum, T. spelta, T. sphaerococcum*, *T. timopheevii* (e.g. timopheevi wheat, a.k.a. *Triticum timopheevii* (Zbuk.) Zbuk.), *T. turanicum* (e.g., oriental wheat, a.k.a. *Triticum turanicum* jakubz), *T. turgidum* (e.g., poulard wheat, a.k.a. *Triticum turgidum L.), T. urartu, T. vavilovii,* and *T. zhukovskyi.*

[0145] Wheat genetics is more complicated than that of most other domesticated species. Some wheat species are diploid, with two sets of chromosomes, but many are stable polyploids, with four sets of chromosomes (tetraploid) or six (hexaploid). Most tetraploid wheats (e.g. emmer and durum wheat) are derived from wild emmer, *T. dicoccoides.* Wild emmer is itself the result of a hybridization between two diploid wild grasses, *T. urartu* and a wild goatgrass such as *Aegilops searsii* or *Ae. speltoides.* The unknown grass has never been identified among now surviving wild grasses, but the closest living relative is *Aegilops speltoides.* The hybridization that formed wild emmer (AABB) occurred in the wild, long before domestication, and was driven by natural selection. Hexaploid wheats evolved in farmers' fields. Common wheat *(Triticum aestivum,* 2n = 42, AABBDD) is one of the most important cereal crops in the world. Either domesticated emmer or durum wheat hybridized with yet another wild diploid grass *(Aegilops cylindrica)* to make the hexaploid wheats, spelt wheat and bread wheat. These have three sets of paired chromosomes, three times as many as in diploid wheat. Synthetic hexaploids made by crossing the wild goatgrass wheat ancestor *Aegilops tauschii* and various durum wheats are now being deployed, and these increase the genetic diversity of cultivated wheats.

[0146] Plant breeding methods for *Triticum spp.* are well known. Non-limiting methods for *Triticum spp.* breeding and agriculturally important traits (e.g., improving wheat yield, biotic stress tolerance, and abiotic stress tolerance etc.) are described in references Nos. 36 - 51, US 7652204, US 6197518, US 7034208, US 7528297, US 6407311, US20080040826, US20090300783, US20060223707, US20110027233, US20080028480, US20090320152, US20090320151, WO/2001/029237A2, WO/2008/02S097A1, and WO/2003/057848A2, each of which is incorporated by reference in its entirety for all purposes.

[0147] Genetic materials may be transferred between *Triticum spp.* and other species, for example, some plant species in the *Triticeae* tribe. Xiang et al., describe somatic hybrids between wheat and *Setaria italica* (Genome 47: 680-688 (2004)); Ge et al. describe protoplast electrofusion between common wheat and Italian ryegrass (In Vitro Cellular and Developmental Biology - Plant 42(2):179-187. 2006); Yue e al. describe asymmetic somatic hybridization between *Aeleuropus littorulis sinensis* and wheat (Plant Science, Volume 161, Issue 2, July 2001, Pages 259-266); Cai et al. describe somatic hybrids between *Festuca arundinacea Schreb.* and wheat (*Triticum aestivum* L.); Xiang et al. describe asymmetric somatic hybridization between wheat and *Avena sativa* L. (Science in China, Vol 46(3), 243-252); Zhou et al. describe asymmetric somatic hybridization between wheat and asymmetric somatic hybridization between wheat and *Avena sativa Haynaldia villosa* (Science in China, 44(3): 294-304); Xia et al. describe asymmetric somatic hybridization between wheat and *Agropyron elongatum* (Host) Nevishi (Theor Appl Genet. 2003 Jul;107(2):299-305. Epub 2003 Mar 19); Li et al. describe symmetric somatic hybridization between wheat and *Psathyrostachys juncea* (Sheng Wu Gong Cheng Xue Bao. 2004 Jul;20(4):610-4). More hybridization between *Triticum* spp. and other species are described in reference Nos. 77-86.

Oryzeae tribe

[0148] The tribe Oryzeae (Poaceae), as conventionally delimited, includes approximately 12 genera and more than 70 species distributed throughout the tropical and temperate regions of the world (Clayton and Renvoize, 1986; Vaughan, 1994). As the largest tribe in the subfamily Ehrhartoideae, Oryzeae contains more than half of both genera and species of the subfamily (Guo et al., 2005, Watson and Dallwitz, 1999; GPWG, 2001).

[0149] Genera in the *Oryzeae* tribe include, but are not limited to, Chikusichloa Koidz, Hygroryza Nees, Leersia Sw., Luziola Juss, Maltebrunia Kunth, Oryza L., Porteresia Tateoka, Potamophila R.Br., Prosphytochloa Schweick., Rhynchoryza Baill., Zizania L., and Zizaniopsis Doll & Asch.

Oryza spp.

[0150] *Oryza* is a genus of seven to twenty species of grasses in the tribe Oryzeae, native to tropical and subtropical regions of Asia, Northern Australia and Africa. They are tall wetland grasses, growing to 1-2 m tall; the genus includes both annual and perennial species.

[0151] Oryza is situated within the tribe Oryzeae, which is characterized morphologically by its single flowered spikelets whose glumes are almost completely suppressed. In Oryza, two sterile lemma simulate glumes. The tribe Oryzeae is within the subfamily Bambusoideae, a group of Poaceae tribes with certain features of internal leaf anatomy in common. The Bambusoideae are in the family Poaceae, as they all have fibrous root systems, cylindrical stems, sheathing leaves with parallel veined blades, and inflorescences with spikelets.

[0152] Non-limiting Oryza spp. include, *O. sativa* (e.g., Asian rice), *O. barthii, O. glaberrima* (e.g., Africa rice), *O. longistaminata, O. meridionalis, O. nivara, O. rufipogon* (e.g., brownbeard rice and red rice), *O. punctata, O. latifolia, O. alta, O. grandiglumis, O. eichingeri, O. officinalis, O. rhisomatis*, *O. minuta, O. australiensis*, *O. granulata, O. meyeriana*, and *O. brachyantha.*

[0153] *Oryza sativa* contains two major subspecies: the sticky, short grained japonica or sinica variety, and the non-sticky, long-grained indica variety. Japonica are usually cultivated in dry fields, in temperate East Asia, upland areas of Southeast Asia and high elevations in South Asia, while indica are mainly lowland rices, grown mostly submerged, throughout tropical Asia. Rice is known to come in a variety of colors, including: white, brown, black, purple, and red. A third subspecies, which is broad-grained and thrives under tropical conditions, was identified based on morphology and initially called javanica, but is now known as tropical japonica. Examples of this variety include the medium grain 'Tinawon' and 'Unoy' cultivars, which are grown in the high-elevation rice terraces of the Cordillera Mountains of northern Luzon, Philippines. Glaszmann (1987) used isozymcs to sort Oryza sativa into six groups: *japonica, aromatic, indica, aus, rayada,* and *ashina;* Garris et al. (2004) used SSRs to sort Oryza sativa into five groups; temperate japonica, tropical japonica and aromatic comprise the japonica varieties, while *indica* and *aus* comprise the *indica* varieties.

[0154] Plant breeding methods for *Oryza spp.* are well known. Non-limiting methods for *Oryza spp.* breeding and agriculturally important traits (e.g., improving wheat yield, biotic stress tolerance, and abiotic stress tolerance etc.) are described in references Nos. 56 - 76, US20050097639, US20040168232. US20100287664, US20080109919, US 5981842, and US20050183173, WO/2003/000904A2.

[0155] Genetic materials may be transferred between *Oryza spp.* and other species, for example, some plant species in the *Oryzeae* tribe. Yan et al. (Plant Cell Rep. 2004 Mar;22(8):569-75. Epub 2003 Nov 1.) and Yu et al. (Phytochemistry. 2008 Jul;69(10):1989-96) describe asymmetric somatic hybridization between *O. meyeriana* L. and *O. sativa* L; and Shan et al. describe asymmetric somatic hybridization between rice (*O. sativa)* and wild rice *(Zizania latifolia* Griseb.). Somatic hybrid plants of rice and barnyard grass (Terada et al., 1987), interspecies somatic hybrids between cultivated and wild species (Hayashi et al., 1988), and diploid hybrid plants form the cell fusion of haploid cells (Toriyama and Hinata 1988) have been reported. More hybridization between *Oryza* spp. and other species are described in reference Nos. 86 - 92.

Fabaceae family and Soybean

[0156] Fabaceae or Leguminosae is a large and economically important family of flowering plants, which is commonly known as the legume family, pea family, bean family or pulse family. The name 'Fabaceae' comes from the defunct genus Faba, now included into Vicia. Leguminosae is an older name still considered valid, and refers to the typical fruit of these plants, which are called legumes.

[0157] Fabaceae is the third largest family of flowering plants, behind Orchidaceae and Asteraceae, with 730 genera and over 19,400 species, according to the Royal Botanical Gardens. The largest genera are Astragalus with more than 2,000 species, Acacia with more than 900 species, and Indigofera with around 700 species. Other large genera include Crotalaria with 600 species and Mimosa with 500 species.

[0158] The species of this family are found throughout the world, growing in many different environments and climates. A number are important agricultural plants, including: *Glycine max* (soybean), *Phaseolus* (beans), *Pisum sativum* (pea), *Cicer arietinum* (chickpeas), *Medicago sativa* (alfalfa), *Arachis hypogaea* (peanut), *Ceratonia siliqua* (carob), and *Glycyrrhiza glabra* (licorice), which are among the best known members of Fabaceae. A number of species are also weedy pests in different parts of the world, including: Cytisus scoparius (broom) and Pueraria lobata (kudzu), and a number of Lupinus species.

[0159] The soybean (U.S.) or soya bean (UK) *(Glycine max)* is a species of legume native to East Asia, widely grown

for its edible bean which has numerous uses. The plant is classed as an oilseed rather than a pulse. Fat-free (defatted) soybean meal is a primary, low-cost, source of protein for animal feeds and most prepackaged meals; soy vegetable oil is another valuable product of processing the soybean crop. For example, soybean products such as textured vegetable protein (TVP) are important ingredients in many meat and dairy analogues.

Kinase Inhibitor Protein (KIP) Related Protein (KRP)

[0160] Plants have cyclin dependent kinases (CDK) that regulate the transitions between different phases of the cell cycle (Verkest et al., 2005, Switching the Cell Cycle. Kip-Related Proteins in Plant Cell Cycle Control, Plant Physiology, November 2005, Vol. 139, pp. 1099-1106, incorporated by reference in its entirety herein).

[0161] In Arabidopsis *(Arabidopsis thaliana),* at least two classes of CDKs are involved in cell cycle regulation: the A-type CDKs that are represented by only one gene in the model species Arabidopsis (designated Arath;CDKA;1) and the B-type CDK family that has four members, grouped into the B1 (Arath;CDKB1;1 and Arath;CDKB1;2) and B2 (Arath; CDKB2;1 and Arath;CDKB2;2) subclasses (Vandepoele et al., 2002, Genome-wide analysis of core cell cycle genes in Arabidopsis. Plant Cell 14: 903-916). A-type CDKs display kinase activity from late G1 phase until the end of mitosis, suggesting a role for this particular CDK at both the G1-to-S and G2-to-M transition points (Magyar et al., 1997; Porceddu et al., 2001; Sorrell et al., 2001). A central role for CDKA;1 in controlling cell number has been demonstrated using transgenic tobacco *(Nicotiana tabacum)* plants with reduced A-type CDK activity (Hemerly et al., 1995). The requirement for Arath;CKDA;1 at least for entry into mitosis has been demonstrated as well by cdka;1 null mutants that fail to progress through the second mitosis during male gametophytic development (Nowack et al., 2005). The group of B-type CDKs displays a peak of activity at the G2-to-M phase transition only (Magyar et al., 1997; Porceddu et al., 2001; Sorrell et al., 2001), suggesting that they play a role at the onset of, or progression through, mitosis. Correspondingly, cells of plants with reduced B-type CDK activity arrest in the G2 phase of the cell cycle (Porceddu et al., 2001; Boudolf et al., 2004).

[0162] CDK is regulated by cyclins. Plant cyclins are very complicated. There are at least 49 different cyclins in Arabidopsis, which were classified into seven subclasses (A, B, C, D, H, P, and T) (Vandepoele et al., 2002; Wang et al., 2004). CDK are also regulated by docking of small proteins, generally known as CDK inhibitors (CKIs). CKIs have been identified in many organisms, e.g., budding yeast (*Saccharomyces cerevisiae*), fission yeast *(Schizosaccharomyces pombe),* mammals, and plants, see, Mendenhall, 1998; Kwon T.K. et al. 1998; Vlach J. et al. 1997; Russo et al., 1996; Wang et al., 1997, 1998 and 2000; Lui et al., 2000; De Veylder et al., 2001; Jasinski et al., 2002a, 2002b; Coelho et al., 2005; Jasinski S. et al., 2002, each of which is incorporated by reference in its entirety).

[0163] Plant CKIs are also known as KIP Related Proteins (KRPs). They have cyclin binding and CDK binding domains at their C-terminal, however the mechanism regulating this protein stability and function remains unknown (Zhou et al., 2003a; Weinl et al. 2005). KRP activity can be both regulated at the transcriptional level or at the posttranslational level (Wang et al., 1998; De Veylder et al., 2001; Jasinski et al., 2002b; Ormenese et al., 2004; Coqueret, 2003; Hengst, 2004; Verkest et al., 2005; Coelho et al., 2005, each of which is incorporated by reference in its entirety). KRPs in plant normally localize in nucleus (Jasinski et al., 2002b; Zhou ct al., 2003a; Weinl et al., 2005).

[0164] KRP can function as an integrator of developmental signals, and control endocycle onset, in different cell cycle programs (e.g., proliferation, endoreduplication, and cell cycle exit). See Wang et al., 1998; Richard et al., 2001; Himanen et al., 2002; Grafi and Larkins, 1995; Joube's et al., 1999; Verkest et al., 2005; Weinl et al., 2005; Boudolf et al., 2004b.

KRP Mutations

[0165] The present invention further provides disrupted *KRP* polynucleotides and KRP amino acid sequences compared to a wild type *KRP* gene or a wild type KRP protein. In some embodiments, the present invention provides mutations in one or more *KRP* genes that can be used to increase weight, size, and/or number of one or more organs, for example, to increase seed size, seed number, seed weight, and/or seed yield in a plant.

[0166] The mutations in a mutated *KRP* gene of the present invention can be in the coding region or the non-coding region of the *KRP* genes. The mutations can either lead to, or not lead to amino acid changes in the encoded KRP polypeptides. In some embodiments, the mutations can be missense, severe missense, silent, nonsense mutations. For example, the mutation can be nucleotide substitution, insertion, deletion, or genome re-arrangement, which in turn may lead to reading frame shift, splicing change, amino acid substitution, insertion, deletion, and/or polypeptides truncation. As a result, the mutant *KRP* gene encodes a KRP polypeptide having less inhibition activity on a cyclin/CDK complex compared to a polypeptide encoded by its corresponding wild-type *KRP* gene.

[0167] As used herein, a nonsense mutation is a point mutation, e.g., a single-nucleotide polymorphism (SNP), in a sequence of DNA that results in a premature stop codon, or a nonsense codon in the transcribed mRNA, and in a truncated, incomplete, and usually nonfunctional protein product. A missense mutation (a type of nonsynonymous mutation) is a point mutation in which a single nucleotide is changed, resulting in a codon that codes for a different amino acid (mutations that change an amino acid to a stop codon are considered nonsense mutations, rather than missense

mutations). This can render the resulting protein nonfunctional. Silent mutations are DNA mutations that do not result in a change to the amino acid sequence of a protein. They may occur in a non-coding region (outside of a gene or within an intron), or they may occur within an exon in a manner that does not alter the final amino acid sequence. A severe missense mutation changes the amino acid, which lead to dramatic changes in conformation, charge status etc.

[0168] The mutations can be located at any portion of a *KRP* gene, for example, at the 5', the middle, or the 3' of a *KRP* gene, resulting mutations in any potions of the encoded KRP protein, for example, in the CDK binding domain or the cyclin binding domain, so long as the mutated gene encodes a mutant KRP polypeptide partially or completely lose the ability to inhibit one or more cyclin/CDK complexes, compared to the protein encoded by the corresponding wild type *KRP* gene. The KRP and the cyclin/CDK complexes can belong to the same plant species, different plant species in the same genus, or different plant species in different species.

[0169] The present invention provides effective systems to test if a candidate mutant KRP protein loses the inhibition ability on a cyclin/CDK complex compared to a wild type KRP protein. The effective systems comprise a kinase assay (the *"in vitro* KRP-Cylin-CDK kinase assay"), a non-limiting example of which is described herein.

[0170] Basically in this kinase assay is an *in vitro* kinase assay. In the assay, a candidate mutant KRP derived from a wild type KRP of a plant species A, the wild type KRP protein of the plant species A, a wild type cyclin protein of a plant species B, and a wild type CDK protein of the plant species B, are recombinantly expressed and purified. Then, the recombinant wild type cyclin protein and the wild type CDK protein are mixed to form a complex (alternatively, the cyclin protein and the CDK protein can be co-expressed and co-purified as a complex). In some embodiments, the recombinant proteins are expressed in insect cells. Plant species A can be the same as or different from plant species B. This kinase activity of said complex is then monitored with a standard kinase assay described below. A substrate protein that can be activated (i.e., phosphorylated) by the Cyclin-CDK complex is selected. Such substrate protein can be Histone HI (HHI) or recombinant tobacco retinoblastoma protein (Nt Rb). At least three mixtures can be made by adding recombinant proteins into a kinase buffer cocktail according to the table below:

Compositions	Mixture I	Mixture II	Mixture III
I. Kinase complex comprising the wild-type cyclin protein and the wild-type CDK protein of the plant species B	at concentration of C1	at concentration of C1	at concentration of C1
II. Wild-type KRP protein of the plant species A	0	at concentration of C2*	0
III. Candidate mutant KRP derived from the wild-type KRP of the plant species A	0	0	at concentration of C3**
IV. Substrate	at concentration of C4	at concentration of C4	at concentration of C4
Kinase Activity	100% (no inhibition)	X% (wt inhibition)	Y% (mutant inhibition)
* C2 is an amount of WT KRP that is sufficient to give between 0% and 20% kinase activity compared to mixture I. ** C3 should be no more than 50X C2			

A non-limiting example of the kinase buffer cocktail comprises KAB: 50 mM Tris pH 8.0, 10 mM $MgCl_2$, 100 μM ATP plus 0.5 μCi/ml 32 Pγ ATP and the substrate protein. Concentrations C1, C2, and C3 can be determined and optimized by one skilled in the art depending on experiment conditions.

[0171] To determine if a candidate mutant KRP loses inhibition ability on the kinase complex, C2 should be about equimolar with C1; and, C3 should be no more than 50X of C2, or no more than 40X of C2, or no more than 30X of C2, or no more than 20X of C2, or no more than 10X of C2, or no more than 5X of C2. For example, in some instances the amount of C3 is about 1X, or about 2X, or about 3X, or about 4X, or about 5X, or about 6X, or about 7X, or about 8X, or about 9X, or about 10X, or about 11X, or about 12X, or about 13X, or about 14X, or about 15X, or about 16X, or about 17X, or about 18X, or about 19X, or about 20X of the amount of C2. In some situations, however, the amount of C3 may be about 25X, or about 30X, or about 35X, or about 40X, or about 45X, or about 50X of the amount of C2. As discussed elsewhere herein, the amount of C3 which is utilized in any particular situation must be physiologically achievable in a plant cell, tissue or whole plant in order to have a dominant negative effect on the wild-type KRP.

[0172] Composition I and/or Composition III are incubated on ice for a certain amount of time (e.g., 30 minutes). Subsequently, Composition II is then added to the mixture and incubated at 4° C. for certain amount of time (e.g., 30 mins) to allow binding to the kinase complex. The kinase reaction is then initiated by adding the buffer cocktail (KAB) and to the kinase complex mixture (I, II, or III) and incubated at 27°C for a certain amount of time (e.g., 30 minutes) to allow reaction to complete. The kinase reaction in each mixture is stopped with an equal volume of 2× Laemmli buffer and boiled for 5 minutes. Next, monitor [^{32}P] phosphate incorporation to the substrate protein by autoradiography and/or Molecular Dynamics PhosphorImager following SDS-PAGE in each mixture. The signal strength of [^{32}P] phosphate incorporation in Mixture I is set as 100% percent recovery of kinase function. The strength of [^{32}P] phosphate incorporation in Mixture II is compared to that of Mixture I, calculated as X%; the strength of [^{32}P] phosphate incorporation in Mixture III is compared to that of Mixture I, calculated as Y%. For example, if the signal strength is half of what is observed for Mixture I, the calculated percent recovery of kinase activity is 50%.

[0173] The X% is compared with Y%, and the effect of the tested mutant KRP is calculated as follows: let Z% = (Y% - X%), and Zmax% is the maximum Z% within the allowable range of C2 and C3; if Zmax% is not statistically higher than 0% (i.e., Y% ≤ X%), the tested mutant KRP does not lose inhibition activity on the complex compared to the corresponding wild type KRP; if Zmax% is statistically higher that 0% (i.e., Y% > X%), but less than 30%, the tested mutant KRP has weak inhibition activity compared to the tested wild-type KRP; if Zmax% is higher that 30%, but less than 50%, the tested mutant KRP substantially loses inhibition activity compared to the tested wild-type KRP; if Zmax% is higher that 50%, the tested mutant KRP strongly loses inhibition activity compared to the tested wild-type KRP. In some embodiments, the mutant KRPs of the present invention do not substantially inhibit the kinase activity of the cyclin/CDK complex, even when present in large molar excess over the cyclin/CDK complex. Mutant KRPs with a Zmax% value higher than 0% are particularly useful for increasing weight, size, and/or number of one or more organs, for example, for increasing seed yield, seed size, seed number, and/or seed yield in a plant.

[0174] The CDK protein and the cyclin protein in the cyclin/CDK complex can be derived from any plant, for example, any dicot plants or monocot plants, especially agriculturally or industrially important plants. The CDK protein and the cyclin protein can be derived from the same plant species, or from different species. The KRP protein can be derived from the same species from which the CDK protein and the cyclin protein are derived, or from different plant species. In some embodiments, the Cyclin/CDK complexes comprise a CDK protein selected from the group consisting of *Zea mays* CDK A;1 (ZmCDKA;1), *Zea mays* CDK A;2 (ZmCDKA;2), wheat CDKs (e.g., GenBank Accession No. AAD10483, and rice CDKs (e.g., GenBank Accession No. NP_001048772 and NP_001045731), and CDKs from a plant in the *Triticeae* tribe or *Oryzeae* tribe, or the *Fabaceae* family; the cyclin protein selected from the group consisting of *Zea mays* Cyclin D1, D2, D3, D4, D5, D6, D7, wheat cyclin proteins (e.g., GenBank Accession No. AAQ08041 (cyclin D2)), rice cyclin proteins (e.g., GenBank Accession Nos. Q67V81 (cyclin-D1;1), Q8H339 (cyclin-D1;2), Q0J233 (cyclin-D2;1), Q10K98 (cyclin-D2;3), Q69QB8 (cyclin-D3;1), Q0DQA9 (cyclin-D5;1), Q53MB7 (cyclin-D7;1)), cyclin proteins from a plant in the *Triticeae* tribe or *Oryzeae* tribe, or the *Fabaceae* family, and combinations thereof, and the wild-type KRPs are selected from the group consisting of wheat KRP1 (TaKRP1), wheat KRP2 (TaKRP2), wheat KRP3 (TaKRP3), wheat KRP4 (TaKRP4), wheat KRP5 (TaKRP5), wheat KRP6 (TaKRP6), for example, SEQ ID NOs. 87-98, 140, 143, and 146, or functional variants thereof, rice KRP1, rice KRP2, rice KRP3, rice KRP4, rice KRP5, rice KRP6, rice KRP7, for example, SEQ ID NOs. 99, 108-110, or functional variants thereof, soy KRPs, for example, SEQ ID NOs. 129-137, or functional variants thereof, KRPs from a plant in the *Triticeae* tribe or *Oryzeae* tribe, the *Fabaceae* family, and combinations thereof. For example, the wild-type KRP is wheat KRP1, wheat KRP2, wheat KRP4, wheat KRP5, or wheat KRP6. The numbering of the KRPs in wheat or rice does not necessarily correspond to the numbering of the KRPs in other species (e.g., Arabidopsis or maize). For example, wheat KRP1 is not necessarily equivalent or orthologous to Arabidopsis KRP1.

[0175] In some embodiments, the *Zea mays* cyclin is selected from the 59 cyclins described in Hu et al., 2010, which is incorporated herein by reference in its entirety. In some embodiments, *Zea mays* cyclin is selected from the 21 cyclin D proteins described in Hu et al., 2010. For example, the cyclin is selected from the group consisting of *Zea mays* cyclin D1;1, D2;1, D2;2, D3;1, D3;2, D4;1, D4;2, D4;3, D4;4, D4;5, D4;6, D4;7, D4;8, D4;9, D4;10, D5;1, D5;2, D5;3, D5;4, D6;1, D7;1, and combination thereof.

[0176] In some embodiments, said mutant KRP is derived from a wheat KRP. In some embodiments, the mutant KRP is derived from wheat KRP1 (A,B, or D), wheat KRP2 (A,B, or D), wheat KRP4 (A, B, or D), wheat KRP5 (A, B, or D), or wheat KRP6 (A, B, or D). In some embodiments, said mutant KRP is derived from a rice KRP. In some embodiments, the mutant KRP is derived from rice KRP1, rice KRP2, rice KRP3, rice KRP4, or rice KRP5. In some embodiments, said mutant KRP is derived from a soybean KRP. In some other embodiments, said mutant KRP is derived from a biologically active variant, or fragment thereof of wild-type wheat, rice or soybean KRPs. The mutant KRP can be natural mutation, or a mutation induced artificially by methods well known to one skilled in the art.

[0177] Mutant KRP protein of the present invention can have one or more modifications to the wild-type KRP, or biologically active variant, or fragment thereof. Particularly suitable modifications include amino acid substitutions, insertions, deletions, or truncation. For example, amino acid substitutions can be generated as modifications in the CDK

or the cyclin-binding region that reduce or eliminate binding. Similarly, amino acid substitutions can be generated as modifications in the CDK or the cyclin-binding region of the KRP that reduce or eliminate the inhibitory activity of the KRP towards the Cyclin/CDK complex. In typical embodiments, at least one non-conservative amino acid substitution, insertion, or deletion in the CDK binding region or the cyclin binding region is made to disrupt or modify binding of the CKI polypeptide to a CDK or cyclin protein. The substitutions may be single, where only one amino acid in the molecule has been substituted, or they may be multiple, where two or more amino acids have been substituted in the same molecule. Insertional KRP mutants are those with one or more amino acids inserted immediately adjacent to an amino acid at a particular position in the wild-type KRP protein molecule, biologically active variant, or fragment thereof. The insertion can be one or more amino acids. The insertion can consist, e.g., of one or two conservative amino acids. Amino acids similar in charge and/or structure to the amino acids adjacent to the site of insertion are defined as conservative. Alternatively, mutant KRP protein includes the insertion of an amino acid with a charge and/or structure that is substantially different from the amino acids adjacent to the site of insertion. In some other embodiments, the mutant KRP is a truncated protein losing one or more domains compared to the corresponding wild type KRP protein.

Methods of Increasing Organ Weight, Organ Size, Organ Number and/or Yield

[0178] The present invention further provides methods of increasing weight, size, and/or number of one or more organs, for example, methods of increasing seed weight, seed size, seed number, and/or yield in a plant. The plant can be a dicot plant or a monocot plant. In some embodiments, the plant is a monocot plant. In some embodiments, the plant is a plant species in the *Triticeae* tribe or *Oryzeae* tribe, or the *Fabaceae* family, for example, a wheat plant or a rice plant. In some embodiments, the methods comprise disrupting one or more KRPs in the plant. The disruption can be at genomic level, transcriptional level, post-transcriptional level, translational level, and/or post translational level. In some embodiments, the methods comprise introducing one or more mutations into one or more *KRP* genes in the plant. In some embodiments, the methods comprise knocking-down expression of one or more *KRP* genes in the plant. In some embodiments, the methods comprise knocking-down KRP mRNAs stability in the plant. In some embodiments, the methods comprise down-regulating one or more KRP proteins activity in the plant.

[0179] For example, in some embodiments, the methods comprise introducing one or more KRP mutants of the present invention into the genome of the plant. In some embodiments, the methods comprise hybridizing a first plant having one or more mutated KRPs of the present invention with a second plant. In some embodiments, the hybridizing step comprises crossing the first plant with the second plant. In some embodiments, the hybridizing step comprises transferring the genetic materials in the first plant to the second plant through in vitro breeding, e.g., somatic hybridization.

[0180] Alternatively, the methods comprise mutating one or more KRPs in a plant. Methods of mutating a target gene have been known to one skilled in the art. These methods include, but are not limited to, mutagenesis (e.g., chemical mutagenesis, radiation mutagenesis, transposon mutagenesis, insertional mutagenesis, signature tagged mutagenesis, site-directed mutagenesis, and natural mutagenesis), TILLING®, homologous recombination, knock-outs/knock-ins, antisense and RNA interference. Various types of mutagenesis can be used to produce and/or isolate variant nucleic acids that encode for protein molecules and/or to further modify/mutate the proteins of the present invention. They include but are not limited to site-directed, random point mutagenesis, homologous recombination, DNA shuffling, mutagenesis using uracil containing templates, oligonucleotide-directed mutagenesis, phosphorothioate-modified DNA mutagenesis, mutagenesis using gapped duplex DNA or the like. Additional suitable methods include point mismatch repair, mutagenesis using repair-deficient host strains, restriction-selection and restriction-purification, deletion mutagenesis, mutagenesis by total gene synthesis, double-strand break repair, and the like. Mutagenesis, e.g., involving chimeric constructs, is also included in the present invention. In one embodiment, mutagenesis can be guided by known information of the naturally occurring molecule or altered or mutated naturally occurring molecule, e.g., sequence, sequence comparisons, physical properties, crystal structure or the like. For more information of mutagenesis in plants, such as agents, protocols, see reference Nos. 94 to 98, each of which is herein incorporated by reference in its entity).

[0181] In some embodiments, random mutations in *KRP* genes are created *in vitro.* For example, a library of *KRP* genes with one or more random mutations can be generated, and the produced mutant KRP genes are subjected to the in vitro KRP-Cylin-CDK kinase assay described herein to determine if the mutant KRP genes can be used for increasing weight, size, and/or number of one or more organs, for example, for increasing seed size, seed number, seed weight and/or yield. Methods for in vitro mutagenesis include, but are not limited to error-prone PCR, Rolling circle error-prone PCR, mutator strains, temporary mutator strains, insertion mutagenesis, chemical mutagenesis (e.g., EMS, nitrous acid etc.), DNA shuffling, and site directed random mutagenesis. More methods are described in Chusacultanachai et al, Fujii et al., Braman, and Trower. Commercial random mutagenesis kits are available, such as Random Mutagenesis Kits from Jena Bioscience.cat. No. PP-101, Diversify® PCR random mutagenesis kit from Clontech.

[0182] In some embodiments, mutated KRPs of the present invention are generated *in vivo* by methods such as TILLING®, site-directed mutagenesis, homologous recombination, etc. The produced mutant KRP genes are screened and subjected to the in vitro KRP-Cylin-CDK kinase assay described herein to determine if the mutant KRP genes can

be used for increasing weight, size, and/or number of one or more organs, for example, for increasing seed size, seed number, seed weight and/or yield.

[0183] In some embodiments, the methods comprise knocking down expression of one or more KRPs in the plant. Techniques which can be employed in accordance with the present invention to knock down gene expression, include, but are not limited to: (1) disrupting a gene's transcript, such as disrupting a gene's mRNA transcript; (2) disrupting the function of a polypeptide encoded by a gene, or (3) disrupting the gene itself.

[0184] For example, antisense RNA, ribozyme, dsRNAi, RNA interference (RNAi) technologies can be used in the present invention to target RNA transcripts of one or more *KRP* genes. Antisense RNA technology involves expressing in, or introducing into, a cell an RNA molecule (or RNA derivative) that is complementary to, or antisense to, sequences found in a particular mRNA in a cell. By associating with the mRNA, the antisense RNA can inhibit translation of the encoded gene product. The use of antisense technology to reduce or inhibit the expression of specific plant genes has been described, for example in European Patent Publication No. 271988, Smith et al., Nature, 334:724-726 (1988); Smith et. al., Plant Mol. Biol., 14:369-379 (1990)).

[0185] A ribozyme is an RNA that has both a catalytic domain and a sequence that is complementary to a particular mRNA. The ribozyme functions by associating with the mRNA (through the complementary domain of the ribozyme) and then cleaving (degrading) the message using the catalytic domain.

[0186] RNA interference (RNAi) is the process of sequence-specific, post-transcriptional gene silencing or transcriptional gene silencing in animals and plants, initiated by double-stranded RNA (dsRNA) that is homologous in sequence to the silenced gene. The RNAi technique is discussed, for example, in Elibashir, et al., Methods Enzymol. 26:199 (2002); McManus & Sharp, Nature Rev. Genetics 3:737 (2002); PCT application WO 01/75164; Martinez et al., Cell 110:563 (2002); Elbashir et al., supra; Lagos-Quintana et al., Curr. Biol. 12:735 (2002); Tuschl et al., Nature Biotechnol. 20:446 (2002); Tuschl, Chembiochem. 2:239 (2001); Harborth et al., J. Cell Sci. 114:4557 (2001); et al., EMBO J. 20:6877 (2001); Lagos-Quintana et al., Science 294:8538 (2001); Hutvagner et al., loc cit, 834; Elbashir et al., Nature 411:494 (2001).

[0187] The term "dsRNA" or "dsRNA molecule" or "double-strand RNA effector molecule" refers to an at least partially double-strand ribonucleic acid molecule containing a region of at least about 19 or more nucleotides that are in a double-strand conformation. The double-stranded RNA effector molecule may be a duplex double-stranded RNA formed from two separate RNA strands or it may be a single RNA strand with regions of self-complementarity capable of assuming an at least partially double-stranded hairpin conformation (i.e., a hairpin dsRNA or stem-loop dsRNA). In various embodiments, the dsRNA consists entirely of ribonucleotides or consists of a mixture of ribonucleotides and deoxynucleotides, such as RNA/DNA hybrids. The dsRNA may be a single molecule with regions of self-complementarity such that nucleotides in one segment of the molecule base pair with nucleotides in another segment of the molecule. In one aspect, the regions of self-complementarity are linked by a region of at least about 3-4 nucleotides, or about 5, 6, 7, 9 to 15 nucleotides or more, which lacks complementarity to another part of the molecule and thus remains single-stranded (i.e., the "loop region"). Such a molecule will assume a partially double-stranded stem-loop structure, optionally, with short single stranded 5' and/or 3' ends. In one aspect the regions of self-complementarity of the hairpin dsRNA or the double-stranded region of a duplex dsRNA will comprise an Effector Sequence and an Effector Complement (e.g., linked by a single-stranded loop region in a hairpin dsRNA). The Effector Sequence or Effector Strand is that strand of the double-stranded region or duplex which is incorporated in or associates with RISC. In one aspect the double-stranded RNA effector molecule will comprise an at least 19 contiguous nucleotide effector sequence, preferably 19 to 29, 19 to 27, or 19 to 21 nucleotides, which is a reverse complement to the RNA of KRPs, or an opposite strand replication intermediate, or the anti-genomic plus strand or non-mRNA plus strand sequences of KRPs. In one embodiment, said double-stranded RNA effector molecules are provided by providing to a plant, plant tissue, or plant cell an expression construct comprising one or more double-stranded RNA effector molecules. In one embodiment, the expression construct comprise a double-strand RNA derived from any one of SEQ ID NOs 60-86, 100-107, 111-128, 138-119, 141-142, and 144-145. One skilled in the art will be able to design suitable double-strand RNA effector molecule based on the nucleotide sequences of KRPs in the present invention.

[0188] In some embodiments, the dsRNA effector molecule of the invention is a "hairpin dsRNA", a "dsRNA hairpin", "short-hairpin RNA" or "shRNA", i.e., an RNA molecule of less than approximately 400 to 500 nucleotides (nt), or less than 100 to 200 nt, in which at least one stretch of at least 15 to 100 nucleotides (e.g., 17 to 50 nt, 19 to 29 nt) is based paired with a complementary sequence located on the same RNA molecule (single RNA strand), and where said sequence and complementary sequence are separated by an unpaired region of at least about 4 to 7 nucleotides (or about 9 to about 15 nt, about 15 to about 100 nt, about 100 to about 1000 nt) which forms a single-stranded loop above the stem structure created by the two regions of base complementarity. The shRNA molecules comprise at least one stem-loop structure comprising a double-stranded stem region of about 17 to about 100 bp; about 17 to about 50 bp; about 40 to about 100 bp; about 18 to about 40 bp; or from about 19 to about 29 bp; homologous and complementary to a target sequence to be inhibited; and an unpaired loop region of at least about 4 to 7 nucleotides, or about 9 to about 15 nucleotides, about 15 to about 100 nt, about 100 to about 1000 nt, which forms a single-stranded loop above the stem

structure created by the two regions of base complementarity. It will be recognized, however, that it is not strictly necessary to include a "loop region" or "loop sequence" because an RNA molecule comprising a sequence followed immediately by its reverse complement will tend to assume a stem-loop conformation even when not separated by an irrelevant "stuffer" sequence.

[0189] The plants with disrupted one or more KRPs of the present invention can be used for many purposes. In one embodiment, a plant of the present invention is used as a donor plant of genetic material which can be transferred to a recipient plant to produce a plant with desired agronomic traits which has the transferred genetic material and having increased weight, size, and/or number of one or more organs, for example, having increased seed weight, seed size, seed number and/or yield. Any suitable method known in the art can be applied to transfer genetic material from a donor plant to a recipient plant. In most cases, such genetic material is genomic material.

[0190] Descriptions of other breeding methods that are commonly used for different traits and crops can be found in one of several reference books (e.g., R. W. Allard, 1960, Principles of Plant Breeding, John Wiley and Son, pp. 115-161; N.W. Simmonds, 1979, Principles of Crop Improvement, Longman Group Limited; W. R. Fehr, 1987, Principles of Crop Development, Macmillan Publishing Co.; N. F. Jensen, 1988, Plant Breeding Methodology, John Wiley & Sons).

[0191] In some embodiments, a backcross breeding process is used. The backcross breeding process comprises the following steps: (a) crossing a first wheat plants having one or more disrupted *KRP* genes with a second plant that comprise the desired trait(s); (b) selecting the F_1 progeny plants that have the desired trait(s); (c) crossing the selected F_1 progeny plants with the first wheat plant or the second wheat plant to produce backcross progeny plants; (d) selecting for backcross progeny plants that have the desired trait(s) and one or more disrupted *KRP* genes to produce selected backcross progeny plants; and (e) repeating steps (c) and (d) one, two, three, four, five six, seven, eight, nine, or more times in succession to produce selected, second, third, fourth, fifth, sixth, seventh, eighth, ninth, or higher backcross progeny plants that comprise said disrupted *KRP* genes, and/or the desired trait(s).

[0192] The invention further provides methods for developing wheat varieties in a wheat breeding program using plant breeding techniques including recurrent selection, backcrossing, pedigree breeding, molecular markers (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 (AFLPs), and Simple Sequence Repeats (SSRs) which are also referred to as Microsatellites, etc.) enhanced selection, genetic marker enhanced selection, and transformation. Seeds, plants, and part(s) thereof produced by such breeding methods are also part of the invention.

[0193] In one embodiment, the whole genome of the plants of the present invention with disrupted KRP(s) is transferred into a recipient plant. This can be done by conventional breeding such as crossing, or somatic hybridization. In another embodiment, at least the parts having the disrupted KRP(s) of the donor plant's genome are transferred. This can be done by crossing donor plants to a recipient plant to create a F1 plant, followed with one or more backcrosses to one of the parent plants to give plants with the desired genetic background. Molecular marker assisted breeding can be utilized to monitor the transfer of the genetic material. The produced offsprings can be selected for having increased weight, size, and/or number of one or more organs, for example, having increased seed weight, seed size, seed number and/or yield.

[0194] In one embodiment, the recipient plant is an elite line having one or more certain agronomically important traits. As used herein, "agronomically important traits" include any phenotype in a plant or plant part that is useful or advantageous for human use. Examples of agronomically important traits include but are not limited to those that result in increased biomass production, increased food production, improved food quality, decrease in cracking, quicker color change when the fruit matures etc. Additional examples of agronomically important traits includes pest resistance, vigor, development time (time to harvest), enhanced nutrient content, increase in seed oil content, novel growth patterns, flavors or colors, salt, heat, drought and cold tolerance, and the like.

[0195] Other agronomically important traits include resistance to biotic and/or abiotic stresses. As used herein, the phrase "biotic stress" or "biotic pressure" refers to a situation where damage is done to plants by other living organisms, such as bacteria, viruses, fungi, parasites, insects, weeds, animals and human. As used herein, the phrase "abiotic stress" or "abiotic pressure" refers to the negative impact of non-living factors on plants in a specific environment. The non-living variable must influence the environment beyond its normal range of variation to adversely affect the population performance or individual physiology of plants in a significant way. Non-limiting examples of stressors are high winds, extreme temperatures, drought, flood, and other natural disasters, such as tornados and wildfires.

[0196] In some embodiments, the method comprises i) making a cross between a plant with one or more disrupted *KRP* genes to a second plant to produce a F1 plant, for example, a wheat, a rice, or a soybean plant with one or more disrupted *KRP* genes. Optionally, the method further comprises ii) backcrossing the F1 plant to the first or the second plant; and iii) repeating the backcrossing step to generate a near isogenic line, wherein the one or more disrupted *KRPs* are integrated into the genome of the second plant.

[0197] In some embodiments, the disrupted *KRP* gene is a wheat *KRP* selected from the group consisting of *TaKRP1A, TaKRP1B, TaKRP1D, TaKRP2A, TaKRP2B, TaKRP2D, TaKRP4A, TaKRP4B, TaKRP4D, TaKRP5A, TaKRP5B,*

TaKRP5D, TaKRP6A, TaKRP6B, or TaKRP6D, for example, SEQ ID NOs. 60-86, 138-139, 141-142, and 144-145, or functional variants thereof. In some embodiments, the first plant comprises one or more mutations selected from any one of mutations listed in Tables 2 - 12 for a particular *KRP* gene.

[0198] In some embodiments, the disrupted *KRP* gene is a rice *KRP* selected from the group consisting of *OsKRP1, OSKRP2, OsKRP4,* and *OsKRP4,* for example, SEQ ID NOs. 100-107, or functional variants thereof. In some embodiments, the first plant comprises one or more mutations selected from any one of mutations listed in Table 25 for a particular *KRP* gene.

[0199] In some embodiments, the disrupted *KRP* gene is a soybean *KRP.* In some embodiments, the *KRP* is selected from the group consisting of Gm0003x00821, Gm0013x00399, Gm0043, Gm0053x00526, Gm0087x00306, Gm0102x00087, Gm0119x00131, Gm0151x00019, Gm0067x00001, for example, SEQ ID NOs. 111-128, or functional variants thereof. In some embodiments, the first plant comprises one or more mutations selected from any one of mutations listed in Tables 29 - 37 for a particular *KRP* gene.

[0200] In some embodiments, the methods of the present invention can increase the average weight, size, and/or number of one or more organs, for example, the average seed weight, seed size, seed number and/or yield of a plant by at least 5%, at least 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 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%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 210%, 220%, 230%, 240%, 250%, 260%, 270%, 280%, 290%, 300%, 310%, 320%, 330%, 340%, 350%, 360%, 370%, 380%, 390%, 400%, or greater when compared to a control plant not having disrupted KRP(s).

[0201] The mutated KRPs in a plant genome can be viewed as quantitative trait loci (QTLs) related to weight, size, and/or number of one or more organs, for example, QTLs related to seed weight, seed size, seed number and/or yield of for yield. A QTL is a region of DNA that is associated with a particular phenotypic trait - these QTLs are often found on different chromosomes. Knowing the number of QTLs that explains variation in a particular phenotypic trait informs about the genetic architecture of the trait. It may tell that plant with preferred phenotype is controlled by many genes of small effect, or by a few genes of large effect. Therefore, QTL mapping can be applied to determine the parts of the donor plant's genome comprising the mutated KRPs, and facilitate the breeding methods.

[0202] One or more of such QTLs of mutated KRPs in a donor can be transferred to a recipient plant, confirming the phenotype of having increased weight, size, and/or number of one or more organs, for example, having increased seed weight, seed size, seed number, and/or yield. In some further embodiments, the QTLs related to mutated KRPs can be combined with one or more other QTLs that contribute to agriculturally important phenotypes, such as yield enhancement, resistance to biotic and abiotic stresses, etc. The primers in the present invention used for genotyping the mutated KRPs can be used as molecular markers indicating the presence or absence of the mutated KRPs. Instead, molecular marks closely linked to the mutated KRPs can be also used. Methods of developing molecular markers and their applications are described by Avise (Molecular markers, natural history, and evolution, Publisher: Sinauer Associates, 2004, ISBN 0878930418, 9780878930418), Srivastava et al. (Plant biotechnology and molecular markers, Publisher: Springer, 2004, ISBN1402019114, 9781402019111), and Vienne (Molecular markers in plant genetics and biotechnology, Publisher: Science Publishers, 2003), each of which is incorporated by reference in its entirety.

[0203] Without wishing to be bond by any theory, besides increased seed size, seed number, seed weight and/or yield, a plant having one or more disrupted KRPs may have one or more other phenotypes that are agriculturally or industrially important, which include, but are not limited to, increased plant vigor, organ size, increased adaptability to the environment, increased oil production, increased biomass production, and traits that allow a plant to grow better under certain environments with specific temperatures, soil conditions and levels of sunlight and precipitation compared to a wild type control plant.

Tissue Culture and Grafting

[0204] Modern plant tissue culture is performed under aseptic conditions under filtered air. Living plant materials from the environment are naturally contaminated on their surfaces (and sometimes interiors) with microorganisms, so surface sterilization of starting materials (explants) in chemical solutions (usually alcohol or bleach) is required. Explants are then usually placed on the surface of a solid culture medium, but are sometimes placed directly into a liquid medium, particularly when cell suspension cultures are desired. Solid and liquid media are generally composed of inorganic salts plus a few organic nutrients, vitamins and plant hormones. Solid media are prepared from liquid media with the addition of a gelling agent, usually purified agar.

[0205] The composition of the medium, particularly the plant hormones and the nitrogen source (nitrate versus ammonium salts or amino acids) have profound effects on the morphology of the tissues that grow from the initial explant.

For example, an excess of auxin will often result in a proliferation of roots, while an excess of cytokinin may yield shoots. A balance of both auxin and cytokinin will often produce an unorganized growth of cells, or callus, but the morphology of the outgrowth will depend on the plant species as well as the medium composition. As cultures grow, pieces are typically sliced off and transferred to new media (subcultured) to allow for growth or to alter the morphology of the culture. The skill and experience of the tissue culturist are important in judging which pieces to culture and which to discard. As shoots emerge from a culture, they may be sliced off and rooted with auxin to produce plantlets which, when mature, can be transferred to potting soil for further growth in the greenhouse as normal plants.

[0206] The tissue obtained from the plant to culture is called an explant. Based on work with certain model systems, particularly tobacco, it has often been claimed that a totipotent explant can be grown from any part of the plant. However, this concept has been vitiated in practice. In many species explants of various organs vary in their rates of growth and regeneration, while some do not grow at all. The choice of explant material also determines if the plantlets developed via tissue culture are haploid or diploid. Also the risk of microbial contamination is increased with inappropriate explants. Thus it is very important that an appropriate choice of explant be made prior to tissue culture.

[0207] The specific differences in the regeneration potential of different organs and explants have various explanations. The significant factors include differences in the stage of the cells in the cell cycle, the availability of or ability to transport endogenous growth regulators, and the metabolic capabilities of the cells. The most commonly used tissue explants are the meristematic ends of the plants like the stem tip, auxiliary bud tip and root tip. These tissues have high rates of cell division and either concentrate or produce required growth regulating substances including auxins and cytokinins. Some explants, like the root tip, are hard to isolate and are contaminated with soil microflora that become problematic during the tissue culture process. Certain soil microflora can form tight associations with the root systems, or even grow within the root. Soil particles bound to roots are difficult to remove without injury to the roots that then allows microbial attack. These associated microflora will generally overgrow the tissue culture medium before there is significant growth of plant tissue. Aerial (above soil) explants are also rich in undesirable microflora. However, they are more easily removed from the explant by gentle rinsing, and the remainder usually can be killed by surface sterilization. Most of the surface microflora do not form tight associations with the plant tissue. Such associations can usually be found by visual inspection as a mosaic, decolorization or localized necrosis on the surface of the explant.

[0208] An alternative for obtaining uncontaminated explants is to take explants from seedlings which are aseptically grown from surface-sterilized seeds. The hard surface of the seed is less permeable to penetration of harsh surface sterilizing agents, such as hypochlorite, so the acceptable conditions of sterilization used for seeds can be much more stringent than for vegetative tissues.

[0209] Tissue cultured plants are clones, if the original mother plant used to produce the first explants is susceptible to a pathogen or environmental condition, the entire crop would be susceptible to the same problem, and conversely any positive traits would remain within the line also. Plant tissue culture is used widely in plant science; it also has a number of commercial applications. Applications include:

1. Micropropagation is widely used in forestry and in floriculture. Micropropagation can also be used to conserve rare or endangered plant species.
2. A plant breeder may use tissue culture to screen cells rather than plants for advantageous characters, e.g. pathogen resistance/tolerance.
3. Large-scale growth of plant cells in liquid culture inside bioreactors as a source of secondary products, like recombinant proteins used as biopharmaceuticals.
4. To cross distantly related species by protoplast fusion and regeneration of the novel hybrid.
5. To cross-pollinate distantly related species and then tissue culture the resulting embryo which would otherwise normally die (Embryo Rescue).
6. For production of doubled monoploid (dihaploid) plants from haploid cultures to achieve homozygous lines more rapidly in breeding programs, usually by treatment with colchicine which causes doubling of the chromosome number.
7. As a tissue for transformation, followed by either short-term testing of genetic constructs or regeneration of transgenic plants.
8. Certain techniques such as meristem tip culture can be used to produce clean plant material from infected stock, such as potatoes and many species of soft fruit.
9. Micropropagation using meristem and shoot culture to produce large numbers of identical individuals.

[0210] Non-limiting exemplary tissue culture methods for wheat, rice, maize have been described by Trione et al., Dodig, et al., O'Hara et al., Zaidi et al., Wang et al., Ting et al., Hawcs ct al., and Sheridan, each of which is incorporated by reference in its entirety.

[0211] The present invention also provides a cutting, a rootstock, a scion, or an explant from the plants of the present invention.

[0212] Grafting is a method of asexual plant propagation widely used in agriculture and horticulture where the tissues

of one plant are encouraged to fuse with those of another. It is most commonly used for the propagation of trees and shrubs grown commercially. In most cases, one plant is selected for its roots, and this is called the stock or rootstock. The other plant is selected for its stems, leaves, flowers, or fruits and is called the scion. The scion contains the desired genes to be duplicated in future production by the stock/scion plant. In stem grafting, a common grafting method, a shoot of a selected, desired plant cultivar is grafted onto the stock of another type. In another common form called budding, a dormant side bud is grafted on the stem of another stock plant, and when it has fused successfully, it is encouraged to grow by cutting out the stem above the new bud.

[0213] For successful grafting to take place, the vascular cambium tissues of the stock and scion plants must be placed in contact with each other. Both tissues must be kept alive until the graft has taken, usually a period of a few weeks. Successful grafting only requires that a vascular connection takes place between the two tissues. A physical weak point often still occurs at the graft, because the structural tissue of the two distinct plants, such as wood, may not fuse.

[0214] Exemplary grafting techniques include, approach grafting, budding grafting (patch budding, chip budding, T-budding), cleft grafting, side grafting, whip grafting, stub grafting, awl grafting, veneer grafting, bark grafting, tongue grafting, et al. Detailed non-limiting grafting methods for wheat and maize are described by Lacadena, 1968, and Katsumi et al., each of which is incorporated by reference in its entirety.

Plant Transformation

[0215] The polynucleotides of the present invention can be transformed into a plant. The most common method for the introduction of new genetic material into a plant genome involves the use of living cells of the bacterial pathogen *Agrobacterium tumefaciens* to literally inject a piece of DNA, called transfer or T-DNA, into individual plant cells (usually following wounding of the tissue) where it is targeted to the plant nucleus for chromosomal integration. There are numerous patents governing Agrobacterium mediated transformation and particular DNA delivery plasmids designed specifically for use with Agrobacterium---for example, US4536475, EP0265556, EP0270822, WO8504899, WO8603516, US5591616, EP0604662, EP0672752, WO8603776, WO9209696, WO9419930, WO9967357, US4399216, WO8303259, US5731179, EP068730, WO9516031, US5693512, US6051757 and EP904362A1. Agrobacterium-mediated plant transformation involves as a first step the placement of DNA fragments cloned on plasmids into living Agrobacterium cells, which are then subsequently used for transformation into individual plant cells. Agrobacterium-mediated plant transformation is thus an indirect plant transformation method. Methods of Agrobacterium-mediated plant transformation that involve using vectors with no T-DNA are also well known to those skilled in the art and can have applicability in the present invention. See, for example, U.S. Patent No. 7,250,554, which utilizes P-DNA instead of T-DNA in the transformation vector.

[0216] Direct plant transformation methods using DNA have also been reported. The first of these to be reported historically is electroporation, which utilizes an electrical current applied to a solution containing plant cells (M. E. Fromm et al., Nature, 319, 791 (1986); H. Jones et al., Plant Mol. Biol., 13, 501 (1989) and H. Yang et al., Plant Cell Reports, 7, 421 (1988). Another direct method, called "biolistic bombardment", uses ultrafine particles, usually tungsten or gold, that are coated with DNA and then sprayed onto the surface of a plant tissue with sufficient force to cause the particles to penetrate plant cells, including the thick cell wall, membrane and nuclear envelope, but without killing at least some of them (US 5,204,253, US 5,015,580). A third direct method uses fibrous forms of metal or ceramic consisting of sharp, porous or hollow needle-like projections that literally impale the cells, and also the nuclear envelope of cells. Both silicon carbide and aluminum borate whiskers have been used for plant transformation (Mizuno et al., 2004; Petolino et al., 2000; US5302523 US Application 20040197909) and also for bacterial and animal transformation (Kaepler et al., 1992; Raloff, 1990; Wang, 1995). There are other methods reported, and undoubtedly, additional methods will be developed. However, the efficiencies of each of these indirect or direct methods in introducing foreign DNA into plant cells are invariably extremely low, making it necessary to use some method for selection of only those cells that have been transformed, and further, allowing growth and regeneration into plants of only those cells that have been transformed.

[0217] For efficient plant transformation, a selection method must be employed such that whole plants are regenerated from a single transformed cell and every cell of the transformed plant carries the DNA of interest. These methods can employ positive selection, whereby a foreign gene is supplied to a plant cell that allows it to utilize a substrate present in the medium that it otherwise could not use, such as mannose or xylose (for example, refer US 5767378; US 5994629). More typically, however, negative selection is used because it is more efficient, utilizing selective agents such as herbicides or antibiotics that either kill or inhibit the growth of nontransformed plant cells and reducing the possibility of chimeras. Resistance genes that are effective against negative selective agents are provided on the introduced foreign DNA used for the plant transformation. For example, one of the most popular selective agents used is the antibiotic kanamycin, together with the resistance gene neomycin phosphotransferase (nptII), which confers resistance to kanamycin and related antibiotics (see, for example, Messing & Vierra, Gene 19: 259-268 (1982); Bevan et al., Nature 304:184-187 (1983)). However, many different antibiotics and antibiotic resistance genes can be used for transformation purposes (refer US 5034322, US 6174724 and US 6255560). In addition, several herbicides and herbicide resistance

genes have been used for transformation purposes, including the bar gene, which confers resistance to the herbicide phosphinothricin (White et al., Nucl Acids Res 18: 1062 (1990), Spencer et al., Theor Appl Genet 79: 625-631(1990), US 4795855, US 5378824 and US 6107549). In addition, the dhfr gene, which confers resistance to the anticancer agent methotrexate, has been used for selection (Bourouis et al., EMBO J. 2(7): 1099-1104 (1983).

[0218] The expression control elements used to regulate the expression of a given protein can either be the expression control element that is normally found associated with the coding sequence (homologous expression element) or can be a heterologous expression control element. A variety of homologous and heterologous expression control elements are known in the art and can readily be used to make expression units for use in the present invention. Transcription initiation regions, for example, can include any of the various opine initiation regions, such as octopine, mannopine, nopaline and the like that are found in the Ti plasmids of *Agrobacterium tumefaciens.* Alternatively, plant viral promoters can also be used, such as the cauliflower mosaic virus 19S and 35S promoters (CaMV 19S and CaMV 35S promoters, respectively) to control gene expression in a plant (U.S. Patent Nos. 5,352,605; 5,530,196 and 5,858,742 for example). Enhancer sequences derived from the CaMV can also be utilized (U.S. Patent Nos. 5,164,316; 5,196,525; 5,322,938; 5,530,196; 5,352,605; 5,359,142; and 5,858,742 for example). Lastly, plant promoters such as prolifera promoter, fruit specific promoters, Ap3 promoter, heat shock promoters, seed specific promoters, etc. can also be used.

[0219] Either a gamete specific promoter, a constitutive promoter (such as the CaMV or Nos promoter), an organ specific promoter (e.g., stem specific promoter), or an inducible promoter is typically ligated to the protein or antisense encoding region using standard techniques known in the art. The expression unit may be further optimized by employing supplemental elements such as transcription terminators and/or enhancer elements. The expression cassette can comprise, for example, a seed specific promoter (e.g. the phaseolin promoter (U.S. Pat. No. 5,504,200). The term "seed specific promoter", means that a gene expressed under the control of the promoter is predominantly expressed in plant seeds with no or no substantial expression, typically less than 10% of the overall expression level, in other plant tissues. Seed specific promoters have been well known in the art, for example, US Patent Nos. 5,623,067, 5,717,129, 6,403,371, 6,566,584, 6,642,437, 6,777,591, 7,081,565, 7,157,629, 7,192,774, 7,405,345, 7,554,006, 7,589,252, 7,595,384, 7,619,135, 7,642,346, and US Application Publication Nos. 20030005485, 20030172403, 20040088754, 20040255350, 20050125861, 20050229273, 20060191044, 20070022502, 20070118933, 20070199098, 20080313771, and 20090100551.

[0220] Thus, for expression in plants, the expression units will typically contain, in addition to the protein sequence, a plant promoter region, a transcription initiation site and a transcription termination sequence. Unique restriction enzyme sites at the 5' and 3' ends of the expression unit are typically included to allow for easy insertion into a preexisting vector.

[0221] In the construction of heterologous promoter/structural gene or antisense combinations, the promoter is preferably positioned about the same distance from the heterologous transcription start site as it is from the transcription start site in its natural setting. As is known in the art, however, some variation in this distance can be accommodated without loss of promoter function.

[0222] In addition to a promoter sequence, the expression cassette can also contain a transcription termination region downstream of the structural gene to provide for efficient termination. The termination region may be obtained from the same gene as the promoter sequence or may be obtained from different genes. If the mRNA encoded by the structural gene is to be efficiently processed, DNA sequences which direct polyadenylation of the RNA are also commonly added to the vector construct. Polyadenylation sequences include, but are not limited to the *Agrobacterium* octopine synthase signal (Gielen et al., EMBO J 3:835-846 (1984)) or the nopaline synthase signal (Depicker et al., Mol. and Appl. Genet. 1:561-573 (1982)). The resulting expression unit is ligated into or otherwise constructed to be included in a vector that is appropriate for higher plant transformation. One or more expression units may be included in the same vector. The vector will typically contain a selectable marker gene expression unit by which transformed plant cells can be identified in culture. Usually, the marker gene will encode resistance to an antibiotic, such as G418, hygromycin, bleomycin, kanamycin, or gentamicin or to an herbicide, such as glyphosate (Round-Up) or glufosinate (BASTA) or atrazine. Replication sequences, of bacterial or viral origin, are generally also included to allow the vector to be cloned in a bacterial or phage host, preferably a broad host range prokaryotic origin of replication is included. A selectable marker for bacteria may also be included to allow selection of bacterial cells bearing the desired construct. Suitable prokaryotic selectable markers include resistance to antibiotics such as ampicillin, kanamycin or tetracycline. Other DNA sequences encoding additional functions may also be present in the vector, as is known in the art. For instance, in the case of *Agrobacterium* transformations, T-DNA sequences will also be included for subsequent transfer to plant chromosomes.

[0223] To introduce a desired gene or set of genes by conventional methods requires a sexual cross between two lines, and then repeated back-crossing between hybrid offspring and one of the parents until a plant with the desired characteristics is obtained. This process, however, is restricted to plants that can sexually hybridize, and genes in addition to the desired gene will be transferred.

[0224] Recombinant DNA techniques allow plant researchers to circumvent these limitations by enabling plant geneticists to identify and clone specific genes for desirable traits, such as resistance to an insect pest, and to introduce these genes into already useful varieties of plants. Once the foreign genes have been introduced into a plant, that plant can

then be used in conventional plant breeding schemes (e.g., pedigree breeding, single-seed-descent breeding schemes, reciprocal recurrent selection) to produce progeny which also contain the gene of interest.

[0225] Genes can be introduced in a site directed fashion using homologous recombination. Homologous recombination permits site specific modifications in endogenous genes and thus inherited or acquired mutations may be corrected, and/or novel alterations may be engineered into the genome. Homologous recombination and site-directed integration in plants are discussed in, for example, U.S. Patent Nos. 5,451,513; 5,501,967 and 5,527,695.

[0226] Methods of producing transgenic plants are well known to those of ordinary skill in the art. Transgenic plants can now be produced by a variety of different transformation methods including, but not limited to, electroporation; microinjection; microprojectile bombardment, also known as particle acceleration or biolistic bombardment; viral-mediated transformation; and Agrobacterium-mediated transformation. See, for example, U.S. Patent Nos. 5,405,765; 5,472,869; 5,538,877; 5,538,880; 5,550,318; 5,641,664; 5,736,369 and 5,736,369; International Patent Application Publication Nos. WO2002/038779 and WO/2009/117555; Lu et al., (Plant Cell Reports, 2008, 27:273-278); Watson et al., Recombinant DNA, Scientific American Books (1992); Hinchee et al., Bio/Tech. 6:915-922 (1988); McCabe et al., Bio/Tech. 6:923-926 (1988); Toriyama et al., Bio/Tech. 6:1072-1074 (1988); Fromm et al., Bio/Tech. 8:833-839 (1990); Mullins et al., Bio/Tech. 8:833-839 (1990); Hiei et al., Plant Molecular Biology 35:205-218 (1997); Ishida et al., Nature Biotechnology 14:745-750 (1996); Zhang et al., Molecular Biotechnology 8:223-231 (1997); Ku et al., Nature Biotechnology 17:76-80 (1999); and, Raineri et al., Bio/Tech. 8:33-38 (1990)), each of which is expressly incorporated herein by reference in their entirety.

[0227] *Agrobacterium tumefaciens* is a naturally occurring bacterium that is capable of inserting its DNA (genetic information) into plants, resulting in a type of injury to the plant known as crown gall. Most species of plants can now be transformed using this method, including cucurbitaceous species.

[0228] Microprojectile bombardment is also known as particle acceleration, biolistic bombardment, and the gene gun (Biolistic® Gene Gun). The gene gun is used to shoot pellets that are coated with genes (e.g., for desired traits) into plant seeds or plant tissues in order to get the plant cells to then express the new genes. The gene gun uses an actual explosive (.22 caliber blank) to propel the material. Compressed air or steam may also be used as the propellant. The Biolistic® Gene Gun was invented in 1983-1984 at Cornell University by John Sanford, Edward Wolf, and Nelson Allen. It and its registered trademark are now owned by E. I. du Pont de Nemours and Company. Most species of plants have been transformed using this method.

[0229] A transgenic plant formed using *Agrobacterium* transformation methods typically contains a single gene on one chromosome, although multiple copies are possible. Such transgenic plants can be referred to as being hemizygous for the added gene. A more accurate name for such a plant is an independent segregant, because each transformed plant represents a unique T-DNA integration event (U.S. Patent No. 6,156,953). A transgene locus is generally characterized by the presence and/or absence of the transgene. A heterozygous genotype in which one allele corresponds to the absence of the transgene is also designated hemizygous (U.S. Patent No. 6,008,437).

[0230] General transformation methods, and specific methods for transforming certain plant species (e.g., maize, rice, wheat, barley, soybean) are described in U.S. Patent Nos. 4940838, 5464763, 5149645, 5501967, 6265638, 4693976, 5635381, 5731179, 5693512, 6162965, 5693512, 5981840, 6420630, 6919494, 6329571, 6215051, 6369298, 5169770, 5376543, 5416011, 5569834, 5824877, 5959179, 5563055, and 5968830, each of which is incorporated by reference in its entirety.

[0231] This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application, as well as the Figures and the Sequence Listing, are incorporated herein by reference.

EXAMPLES

Materials and Methods

Mutagenesis

[0232] In one embodiment of the present invention, wheat seeds of tetraploid wheat (*Triticum turgidum*) cultivar 'Kronos' and the hexaploid wheat (*Triticum aestivum*) cultivar 'Express' (PVP # 9000012) were vacuum infiltrated in H_2O (approximately 1,000 seeds/100 ml H_2O for approximately 4 minutes). The seeds were then placed on a shaker (45 rpm) in a fume hood at ambient temperature. The mutagen ethyl methanesulfonate (EMS) was added to the imbibing seeds to final concentrations ranging from about 0.75% to about 1.2% (v/v). Following an 18 hour incubation period, the EMS solution was replaced 4 times with fresh H_2O. The seeds were then rinsed under running water for about 1 hour. Finally, the mutagenized seeds were planted (96/tray) in potting soil and allowed to germinate indoors. Plants that were four to six weeks old were transferred to the field to grow to fully mature M1 plants. The mature M1 plants were allowed to self-pollinate and then seeds from the M1 plant were collected and planted to produce M2 plants.

DNA Preparation

[0233] DNA from these M2 plants was extracted and prepared in order to identify which M2 plants carried a mutation at their KRP loci. The M2 plant DNA was prepared using the methods and reagents contained in the Qiagen® (Valencia, CA) DNeasy® 96 Plant Kit. Approximately 50 mg of frozen plant sample was placed in a sample tube with a tungsten bead, frozen in liquid nitrogen and ground 2 times for 1 minute each at 20 Hz using the Retsch® Mixer Mill MM 300. Next 400 μl of solution AP1 [Buffer AP1, solution DX and RNAse (100 mg/ml)] at 80° C was added to the sample. The tube was sealed and shaken for 15 seconds. Following the addition of 130 μl Buffer AP2, the tube was shaken for 15 seconds. The samples were placed in a freezer at minus 20° C for at least 1 hour. The samples were then centrifuged for 20 minutes at 5,600 X g. A 400 μl aliquot of supernatant was transferred to another sample tube. Following the addition of 600 μl of Buffer AP3/E, this sample tube was capped and shaken for 15 seconds. A filter plate was placed on a square well block and 1ml of the sample solution was applied to each well and the plate was sealed. The plate and block were centrifuged for 4 minutes at 5,600 X g. Next, 800 μl of Buffer AW was added to each well of the filter plate, sealed and spun for 15 minutes at 5,600 X g in the square well block. The filter plate was then placed on a new set of sample tubes and 80 μl of Buffer AE was applied to the filter. It was capped and incubated at room temperature for 1 minute and then spun for 2 minutes at 5,600 X g. This step was repeated with an additional 80 μl Buffer AE. The filter plate was removed and the tubes containing the pooled filtrates were capped. The individual samples were then normalized to a DNA concentration of 5 to 10 ng/μl.

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[0234] The M2 DNA was pooled into groups of two individual plants. The DNA concentration for each individual within the pool was approximately 1 to 2 ng/μl with a final concentration of 2 to 4 ng./ μl for the entire pool. Then, 5 or 10 μl of the pooled DNA samples (or 10 to 40 ng) was arrayed on microtiter plates and subjected to gene-specific PCR.

[0235] PCR amplification was performed in 15 or 20 μl reaction volumes containing 10 to 40 ng pooled DNA. A reaction included 1.24 μl of 10X ExTaq buffer (Takara®,), 0.73 μl of 25mM $MgCl_2$, 1.98 μl of 10 mM dNTPs, 0.066 μl of 100 μM primer mix, and 0.11 μl of 5U/ μl Ex-Taq (Takara®,) DNA polymerase, with 6.87 μl H_2O. PCR additives such as dimethyl sulfoxide (DMSO), betaine or Polymer-Aide PCR Enhancer (Sigma Aldrich®, St. Louis, MO) can be used to increase PCR efficiency. PCR amplification was performed using an MJ Research® thermal cycler as follows: 95° C for 2 minutes; 8 cycles of "touchdown PCR" (94° C for 20 second, followed by annealing step starting at 70-68° C for 30 seconds and decreasing 1° C per cycle, then a temperature ramp of 0.5° C per second to 72° C followed by 72° C for 1 minute); 25-45 cycles of 94° C for 20 seconds, 63-67.5° C for 30 seconds, ramp 0.5° C/sec to 72° C, 72° C for 1 minute; 72° C for 8 minutes; 98° C for 8 minutes; 80° C for 20 seconds; 60 cycles of 80° C for 7 seconds -0.3 degrees/cycle.

[0236] The PCR primers (Eurofins MWG/Operon, Huntsville, AL) were mixed as follows:

12.5% 100 μM IRD-700 labeled left primer
37.5% 100 μM left primer
25% 100 μM IRD-800 labeled right primer
25% 100 μM right primer

A label can be attached to each primer as described or to only one of the primers. Alternatively, Cy5.5 modified primers could be used. The label was coupled to the oligonucleotide using conventional phosphoramidite chemistry.

[0237] PCR products (15 or 20 μl) were digested in 96-well plates. Next, 30 μl of a solution containing 10 mM HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] (pH 7.5), 10 mM $MgSO_4$, 0.002% (w/v) Triton® X-100, 20 ng/ml of bovine serum albumin, 5ul of SURVEYOR® Nuclease (Transgenomic, Inc.) and 5ul of SURVEYOR® Enhancer (Transgenomic, Inc.) were added with mixing on ice, and the plate was incubated at 45° C for 30 minutes. Reactions were stopped by addition of 10 μl of a 2.5 M NaCl solution with 0.5 mg/ml blue dextran and 75 mM EDTA, followed by the addition of 80 μl isopropanol. The reactions were precipitated at room temperature, spun at 4,000 rpm for 30 minutes in an Eppendorf Centrifuge 5810. Pellets were resuspended in 6 μl of 33% formamide with 0.017% bromophenol blue dye, heated at 80 ° C for 7 minutes and then at 95 ° C for 5 minutes. Samples were transferred to a membrane comb using a comb-loading robot (MWG Biotech). The comb was inserted into a slab acrylamide gel (6.5%) and electrophoresed for 4h 15min at 1,500-V, 40-W, and40-mA limits at 50° C.

[0238] During electrophoresis, the gel was imaged using a LI-COR® (Lincoln, NE) scanner which was set at a channel capable of detecting the IR Dye 700 and 800 labels. The gel image showed sequence-specific pattern of background bands common to all 96 lanes. Rare events, such as mutations, create new bands that stand out above the background pattern. Plants with bands indicative of mutations of interest were evaluated by TILLING® individual members of a pool mixed with wild type DNA and then sequencing individual PCR products.

Example 1

TILLING for *Triticum KRP* mutants

[0239] Genome-specific primers were designed and used to TILL for wheat KRPs (Table 1).

Table 1. Genome-specific primers used for TILLING® of wheat KRP genes

Gene/ Genome†	Primer name	Primer Sequence (5' → 3')	SEQ ID NO
KRP1A_2-4			
	TaKRP1A_L	GGATACGATTCGAGATCTCCTTTTTGAC	6
	TaKRP1A_R	TGATAATGGTGGGAATATGTGAGCGAGTG	7
KRP1B_2-4			
	TaKRP1B_L	AAACAGCAAGGTGAGGGAATTGGGGTC	8
	TaKRP1B_R	TAATGCTTCTTTCCGGAGCATCTTTTTCC	9
KRP1D_2-4			
	TaKRP1D_L	GGATACAATTCGAGATCTCCTTTTTGCTG	10
	TaKRP1D_R	TAATGCTTCTTTCCGGAGCATCTTTTTCC	11
KRP2A2			
	TaKRP2A2L1	GCCACTCACTGCCCTAGAATTCTCCGTA	12
	TaKRP2A2R1	CAATTTGGATGGGGAGAGAGAGAGAGCTAGTGT	13
KRP2B2			
	TaKRP2B2L2	GTCCACTGCCCTAGAATTCTCCGCTACTT	14
	TaKRP2B2_altR	GCCGTGGCCTAGTGAAAGGTAAAAAGAAA	15
KRP2D2			
	KRP2D2_ENDEX1_L	TCCACTGCCCTAGAATTCTCCGCTAAT	16
	KRP2D2_ENDEX4_R	GTCATTTGCATCATGCTCTGCTCACAC	17
KRP4B2			
	KRP4B_L_2_3_NEW	TTCCTTATTTTTTATGACTATTGATATGTGTTCTT C	18
	WKP4_BR2	GTGGTCATTACAGAATGAGCTGCTAACCGTT	19
KRP4D2			
	KRP4D_L_2_3_NEW	TTACGACCACGGATGATATCGATATGTG	20
	KRP4D_R_2_3_NEW	CATTGGAGTTTTGAGGGATTAGGGTGT	21
KRP5A1			
	TaKRP5A1_L	GGCAAGTACATGCGCAAGAGCAAGG	22

(continued)

Gene/ Genome†	Primer name	Primer Sequence (5' → 3')	SEQ ID NO
	TaKRP5A1_R	GATTTTCTTCTCCATCAGGATTGAAGCGC	23
KRP5A2			
	TaKRP5A2_L	CACATTGTGTGATGTGGGGCACTTGTTA	24
	TaKRP5_ALL_EST_R	GAGCTACTGCTGACTGCGGGCTAACTCTA	25
KRP5D2			
	TaKRP5D_L_Z_2	TGTCTAGCGTGGGGCACTTGCAAATA	26
	TaKRP5_ALL_EST_R	GAGCTACTGCTGACTGCGGGCTAACTCTA	27
† number after genome letter refers to the exons or the region of the KRP gene TILL'ed. i.e. 2-4 mean exons 2-4, and the number 2 means the latter half of the gene (includes the cyclin/CDK binding domains). The number 1 means the first part of the KRP gene, excluding the cyclin/CDK binding domains.			

Example 2

Mutations of *Triticum KRP* Genes identified in TILLING®

[0240] Screening of the TILLING® population for *KRP* mutants resulted in plants with silent, splice, nonsense (premature stop codons) and/or missense (severe or non-severe) mutations in *KRP1, KRP2, KRP4 and KRP5 (A, B,* and *D* genomes) genes.

[0241] Positions and effects of mutations in *KRP1, KRP2, KRP4 and KRP5* (*A, B,* and *D* genomes) genes are displayed in Tables 2 to 12 below (* indicates the mutation results in a stop codon, = indicates silent mutation).

Table 2. Summary of *Triticum turgidum ssp. durum* (tetraploid) *KRP4B* mutants

Nucleotide Change^	Effect	Gene	Mutation Score
G389A	R4K£	KRP4B2§	Severe Missense
G390A	R4=	KRP4B2	Silent
C400T	P8S	KRP4B2	Severe Missense
C408T	S10=	KRP4B2	Silent
C457T	P27S	KRP4B2	Missense
G461A	S28N	KRP4B2	Missense
C465T	H29=	KRP4B2	Silent
G486A	V36=	KRP4B2	Silent
G496A	A40T	KRP4B2	Missense
G520A	A48T	KRP4B2	Missense
G525A	E49=	KRP4B2	Silent
C540T	F54=	KRP4B2	Silent
G550A	E58K	KRP4B2	Severe Missense
G564A	Q62=	KRP4B2	Silent
G587A	Intron	KRP4B2	
G635A	Intron	KRP4B2	
C652A	Intron	KRP4B2	

(continued)

Nucleotide Change^	Effect	Gene	Mutation Score
C802T	N75=	KRP4B2	Silent
G803A	D76N	KRP4B2	Severe Missense
C810T	P78L	KRP4B2	Severe Missense
C812T	L79F	KRP4B2	Severe Missense
C815T	P80S	KRP4B2	Missense
T870C	Non-coding	KRP4B2	
C894T	Non-coding	KRP4B2	
§The designation "2" indicates that exons 2-3 of wheat KRP4B were TILLed. £Amino acid numbering docs not start from the beginning Methionine. ^Nucleotide numbering is dependent upon the location of TILLING® primers.			

Table 3. Summary of *Triticum aestivum* (hexaploid) *KRP1A* mutants

Nucleotide Change^	Effect	Gene	Mutation Score
G512A	A140=	Krp1A§	Silent
G522A	A144T	Krp1A	Missense
C676T	P162S	Krp1A	Missense
C539T	N149=	Krp1A	Silent
G547A	R152K	Krp1A	Missense
G652A	E154K	Krp1A	Missense
G550A	Intron	Krp1A	
A554G	Intron	Krp1A	
C564T	Intron	Krp1A	
C618T	Intron	Krp1A	
G652A	E154K	Krp1A	Missense
G654A	E154=	Krp1A	Silent
G657A	T155=	Krp1A	Silent
C659T	T156M	Krp1A	Severe Missense
C661T	P157S	Krp1A	Severe Missense
C676T	P162S	Krp1A	Missense
C694T	L168=	Krp1A	Silent
G725A	G178D	Krp1A	Missense
C739T	P183S	Krp1A	Missense
C746T	T185M	Krp1A	Missense
C748T	P186S	Krp1A	Missense
C749T	P186L	Krp1A	Missense
C756T	A188=	Krp1A	Silent
C762T	A190=	Krp1A	Silent
C766T	P192S	Krp1A	Missense

(continued)

Nucleotide Change^	Effect	Gene	Mutation Score
C767T	P192L	Krp1A	Missense
C787T	P199S	Krp1A	Missense
C788T	P199L	Krp1A	Severe Missense
G808A	E206K	Krp1A	Missense
C818T	A209V	Krp1A	Severe Missense
G826A	E212K	Krp1A	Severe Missense
G828A	E212=	Krp1A	Silent
G832A	A214T	Krp1A	Missense
C834T	A214=	Krp1A	Silent
G879A	Intron	Krp1A	
G880A	Intron	Krp1A	
C925T	Intron	Krp1A	
G939A	Intron	Krp1A	
G940A	Intron	Krp1A	
G961A	A228T	Krp1A	Missense
G965A	R229H	Krp1A	Missense
C974T	P232L	Krp1A	Severe Missense
C978T	L233=	Krp1A	Silent
C983T	S235F	Krp1A	Severe Missense
G985A	G236S	Krp1A	Severe Missense
G999A	W240*	Krp1A	Nonsense
C1002T	T241=	Krp1A	Silent
C1003T	P242S	Krp1A	Missense
G1016A	S246N	Krp1A	Severe Missense
G1019A	S247N	Krp1A	Severe Missense
C1020T	S247=	Krp1A	Silent
G1027A	Non-coding	Krp1A	
G1037A	Non-coding	Krp1A	
G1043A	Non-coding	Krp1A	
G1051A	Non-coding	Krp1A	
§Exons 2-4 of wheat KRP1A were TILLed. ^Nucleotide numbering is dependent upon the location of TILLING® primers.			

Table 4. Summary of *Triticum aestivum* (hexaploid) *KRP1B* mutants

Nucleotide Change^	Effect	Gene	Mutation Score
C562T	G136=	Krp1B§	Silent
C567T	A138V	Krp1B	Missense
C589T	G145=	Krp1B	Silent

(continued)

Nucleotide Change^	Effect	Gene	Mutation Score
C595T	N147=	Krp1B	Silent
G597A	R148H	Krp1B	Missense
G603A	R150K	Krp1B	Missense
G606A	Splice Junction	Krp1B	Splice
G614A	Intron	Krp1B	
G662A	Intron	Krp1B	
C667T	Intron	Krp1B	
C674T	Intron	Krp1B	
C679T	Intron	Krp1B	
G708A	E152K	Krp1B	Severe Missense
G710A	E152=	Krp1B	Silent
C715T	T154M	Krp1B	Severe Missense
C718T	P155L	Krp1B	Severe Missense
C721T	S156F	Krp1B	Severe Missense
G724A	S157N	Krp1B	Missense
C741T	L163=	Krp1B	Silent
G752A	L166=	Krp1B	Silent
G769A	G172D	Krp1B	Missense
C776T	N174=	Krp1B	Silent
C787T	S178L	Krp1B	Missense
G788A	S178=	Krp1B	Silent
G797A	P181=	Krp1B	Silent
C798T	Q182*	Krp1B	Nonsense
C802T	T183M	Krp1B	Missense
G803A	T183=	Krp1B	Silent
C805T	P184L	Krp1B	Missense
C808T	T185I	Krp1B	Severe Missense
C811T	A186V	Krp1B	Missense
C812T	A186=	Krp1B	Silent
C827T	A191=	Krp1B	Silent
G828A	A192T	KRP1B	Missense
G830A	A192=	Krp1B	Silent
G832A	R193K	Krp1B	Missense
G839A	R195=	Krp1B	Silent
C843T	P197S	Krp1B	Severe Missense
G857A	E201=	Krp1B	Silent
C874T	A207V	Krp1B	Severe Missense
G876A	A208T	Krp1B	Missense

(continued)

Nucleotide Change^	Effect	Gene	Mutation Score
G879A	A209T	KRP1B	Severe Missense
G882A	E210K	Krp1B	Severe Missense
G884A	E210=	Krp1B	Silent
G885A	E211K	Krp1B	Missense
G891A	E213K	Krp1B	Missense
A897C	R215-	Krp1B	Silent
G918A	Intron	Krp1B	
C927T	Intron	Krp1B	
G929A	Intron	Krp1B	
C930A	Intron	Krp1B	
G934A	Intron	Krp1B	
C942T	Intron	Krp1B	
G960A	Intron	Krp1B	
C965T	Intron	KRP1B	
G966A	Intron	KRP1B	
G972A	Intron	Krp1B	
G983A	Intron	Krp1B	
C1006T	Y221=	Krp1B	Silent
C1015T	D224=	Krp1B	Silent
C1034T	L231F	Krp1B	Severe Missense
G1044A	G234D	Krp1B	Severe Missense
C1045T	G234=	Krp1B	Silent
C1046T	R235W	Krp1B	Severe Missense
G1064A	A241T	Krp1B	Missense
C1065T	A241 V	Krp1B	Missense
A1084C	Intron	Krp1B	
C1086T	Intron	KRP1B	
G1088A	Intron	Krp1B	
G1094A	Intron	Krp1B	
C1095T	Intron	Krp1B	
§Exons 2-4 of wheat KRP1B were TILLed. ^Nucleotide numbering is dependent upon the location of TILLING® primers.			

Table 5. Summary of *Triticum aestivum* (hexaploid) *KRP1D* mutants

Nucleotide Change^	Effect	Gene	Mutation Score
G638A	Splice Junction	Krp1D_2-4§	Splice
C652T	P158S	Krp1D_2-4	Severe Missense
C666T	F162=	Krp1D_2-4	Silent

(continued)

Nucleotide Change^	Effect	Gene	Mutation Score
C668T	P163L	Krp1D_2-4	Missense
C675T	D165=	Krp1D_2-4	Silent
C685T	L169=	Krp1A_2-4	Silent
C692T	S171L	Krp1D_2-4	Missense
C697T	L173=	Krp1D_2-4	Silent
G700A	A174T	Krp1D_2-4	Missense
C701T	A174V	Krp1D_2-4	Missense
G723A	S181=	Krp1D_2-4	Silent
G738A	T186=	Krp1D_2-4	Silent
C758T	P193L	Krp1D_2-4	Missense
C761T	A194V	Krp1D_2-4	Severe Missense
G765A	A 195=	Krp1D_2-4	Silent
G767A	R196K	Krp1D_2-4	Missense
C778T	P200S	Krp1D_2-4	Severe Missense
G780A	P200=	Krp1D_2-4	Silent
G798A	E206=	Krp1D_2-4	Silent
C809T	A210V	Krp1D_2-4	Severe Missense
G817A	E213K	Krp1D_2-4	Severe Missense
C831T	A217=	Krp1D_2-4	Silent
G833A	R218K	Krp1D_2-4	Missense
G845A	C222Y	Krp1D_2-4	Missense
G870A	Intron	Krp1D_2-4	
C911T	Intron	Krp1D_2-4	
C933T	Intron	Krp1D_2-4	
C938T	Intron	Krp1D_2-4	
C956T	D227=	Krp1D_2-4	Silent
C963T	R230C	Krp1D_2-4	Severe Missense
G967A	G231D	Krp1D_2-4	Missense
C974T	P233=	Krp1D_2-4	Silent
C975T	L234F	Krp1D_2-4	Severe Missense
C983T	S236=	Krp1D_2-4	Silent
C986T	G237=	Krp1D_2-4	Silent
C1001T	T242=	Krp1D_2-4	Silent
C1006T	A244V	Krp1D_2-4	Missense
G1026A	Non-coding	Krp1D_2-4	
G1060A	Non-coding	Krp1D_2-4	
C1028T	Non-coding	Krp1D_2-4	

(continued)

Nucleotide Change^	Effect	Gene	Mutation Score
G1041A	Non-coding	Krp1D_2-4	
§The designation "2-4" indicates that Exons 2-4 of wheat KRP1D were TILLed. ^Nucleotide numbering is dependent upon the location of TILLING® primers.			

Table 6. Summary of *Triticum aestivum* (hexaploid) *KRP2A* mutants

Nucleotide Change^	Effect	Gene	Mutation Score
G568A	Intron	KRP2A2§	
G584A	C153Y	KRP2A2	Severe Missense
C597T	D157=	KRP2A2	Silent
G601A	E159K	KRP2A2	Missense
C606T	S160=	KRP2A2	Silent
C608T	S161F	KRP2A2	Severe Missense
G614A	S163N	KRP2A2	Missense
G631A	G169S	KRP2A2	Missense
G632A	G169D	KRP2A2	Missense
C651 T	Intron	KRP2A2	
C663T	Intron	KRP2A2	
G669A	Intron	KRP2A2	
G742A	Intron	KRP2A2	
G764A	Intron	KRP2A2	
G765A	Intron	KRP2A2	
G773A	Splice Junction	KRP2A2	Splice
G774A	R172= (splice)	KRP2A2	Splice
C783T	T175=	KRP2A2	Silent
C787T	P177S	KRP2A2	Severe Missense
G813A	L185=	KRP2A2	Silent
C819T	D187=	KRP2A2	Silent
G825A	E189=	KRP2A2	Silent
G835A	A193T	KRP2A2	Missense
G838A	A194T	KRP2A2	Missense
C839T	A194V	KRP2A2	Missense
C843T	D195=	KRP2A2	Silent
G852A	K198=	KRP2A2	Silent
G860A	R201H	KRP2A2	Missense
G864A	R202=	KRP2A2	Silent
G873A	P205=	KRP2A2	Silent
G874A	A206T	KRP2A2	Missense
C875T	A206V	KRP2A2	Missense

(continued)

Nucleotide Change^	Effect	Gene	Mutation Score
C893T	A212V	KRP2A2	Missense
G895A	A213T	KRP2A2	Severe Missense
C903T	F215=	KRP2A2	Silent
G919A	A221T	KRP2A2	Missense
G929A	R224K	KRP2A2	Missense
C934T	P226S	KRP2A2	Severe Missense
G940A	A228T	KRP2A2	Severe Missense
C951T	I231=	KRP2A2	Silent
G952A	D232N	KRP2A2	Missense
G955A	E233K	KRP2A2	Missense
C963T	F235=	KRP2A2	Silent
C966T	A236=	KRP2A2	Silent
G978A	K240=	KRP2A2	Silent
C981T	A241=	KRP2A2	Silent
G984A	Q242=	KRP2A2	Silent
C996T	F246=	KRP2A2	Silent
C998T	A247V	KRP2A2	Severe Missense
G1005A	Splice Junction	KRP2A2	Splice
G1011A	Intron	KRP2A2	
G1026A	Intron	KRP2A2	
C1046T	Intron	KRP2A2	
C1070T	Intron	KRP2A2	
C1076T	Intron	KRP2A2	
C1084T	Intron	KRP2A2	
G1086A	Intron	KRP2A2	
A1089G	Intron	KRP2A2	
C1092T	Intron	KRP2A2	
C1104T	Intron	KRP2A2	
G1109A	Intron	KRP2A2	
C1110T	Intron	KRP2A2	
T1111A	Intron	KRP2A2	
C1118T	Intron	KRP2A2	
C1121T	Intron	KRP2A2	
G1124A	K249=	KRP2A2	Silent
G1134A	D253N	KRP2A2	Severe Missense
G1137A	V254I	KRP2A2	Missense
C1143T	R256C	KRP2A2	Severe Missense
C1145T	R256=	KRP2A2	Silent

(continued)

Nucleotide Change^	Effect	Gene	Mutation Score
G1146A	G257S	KRP2A2	Severe Missense
G1147A	G257D	KRP2A2	Severe Missense
G1149A	V258M	KRP2A2	Severe Missense
C1152T	P259S	KRP2A2	Severe Missense
C1162T	A262V	KRP2A2	Severe Missense
G1165A	G263D	KRP2A2	Severe Missense
C1167T	R264W	KRP2A2	Severe Missense
G1169A	R264=	KRP2A2	Silent
G1194A	V273I	KRP2A2	Missense
G1201A	Non-coding	KRP2A2	
C1216T	Non-coding	KRP2A2	
G1225A	Non-coding	KRP2A2	
G1227A	Non-coding	KRP2A2	
G1230A	Non-coding	KRP2A2	
G1254A	Non-coding	KRP2A2	
§The designation "2" indicates that exons 2-4 of wheat KRP2A were TILLed. ^Nucleotide numbering is dependent upon the location of TILLING® primers.			

Table 7. Summary of *Triticum aestivum* (hexaploid) *KRP2B* mutants

Nucleotide Change^	Effect	Gene	Mutation Score
C27A	Non-coding	KRP2B2§	
G57A	V7M£	KRP2B2	Missense
C67T	S10F	KRP2B2	Severe Missense
C111T	Intron	KRP2B2	
C115T	Intron	KRP2B2	
C233T	Intron	KRP2B2	
G241A	E23K	KRP2B2	Missense
G243A	E23=	KRP2B2	Silent
C248T	T25M	KRP2B2	Severe Missense
C250T	P26S	KRP2B2	Severe Missense
C263T	S30L	KRP2B2	Missense
C283T	L37=	KRP2B2	Silent
G286A	E38K	KRP2B2	Missense
C295T	Q41*	KRP2B2	Nonsense
G304A	D44N	KRP2B2	Missense
G307A	E45K	KRP2B2	Missense
G315A	K47=	KRP2B2	Silent
C322T	R50C	KRP2B2	Missense

(continued)

Nucleotide Change^	Effect	Gene	Mutation Score
G326A	R51K	KRP2B2	Missense
G329A	R52K	KRP2B2	Missense
C338T	A55V	KRP2B2	Missense
G340A	A56T	KRP2B2	Missense
G358A	A62T	KRP2B2	Severe Missense
C366T	F64=	KRP2B2	Silent
C375T	D67=	KRP2B2	Silent
G379A	E69K	KRP2B2	Missense
G403A	A77T	KRP2B2	Missense
G405A	A77=	KRP2B2	Silent
C497T	Intron	KRP2B2	
C503T	Intron	KRP2B2	
G523A	Intron	KRP2B2	
G527A	Intron	KRP2B2	
C553T	Intron	KRP2B2	
G591A	Splice Junction	KRP2B2	Splice
C623T	L109F	KRP2B2	Severe Missense
G643A	E115=	KRP2B2	Silent
G646A	W116*	KRP2B2	Nonsense
G653A	V119M	KRP2B2	Missense
C671T	Non-coding	KRP2B2	
C675T	Non-coding	KRP2B2	
C689T	Non-coding	KRP2B2	
G692A	Non-coding	KRP2B2	
T699A	Non-coding	KRP2B2	
G705A	Non-coding	KRP2B2	
G714A	Non-coding	KRP2B2	
G812A	Non-coding	KRP2B2	
G860A	Non-coding	KRP2B2	
C862_T	Non-ending	KRP2B2	

§The designation "2" indicates that exons 2-4 of wheat KRP2B were TILLed.
£Amino acid numbering does not start from the beginning Methionine.
^Nucleotide numbering is dependent upon the location of TILLING® primers.

Table 8. Summary of *Triticum aestivum* (hexaploid) *KRP2D* mutants

Nucleotide Change^	Effect	Gene	Mutation Score
G551A	Intron	KRP2D2§	
G584A	A151T	KRP2D2	Severe Missense

(continued)

Nucleotide Change^	Effect	Gene	Mutation Score
G586A	A151=	KRP2D2	Silent
G588A	C152Y	KRP2D2	Missense
C596T	R155C	KRP2D2	Severe Missense
G597A	R155H	KRP2D2	Missense
G604A	V157=	KRP2D2	Silent
G609A	S159N	KRP2D2	Missense
C610T	S159=	KRP2D2	Silent
C613T	S160=	KRP2D2	Silent
G614A	V161I	KRP2D2	Missense
C616T	V161=	KRP2D2	Silent
G618A	S162N	KRP2D2	Missense
G633A	G167D	KRP2D2	Missense
C638T	R169W	KRP2D2	Severe Missense
G639A	R169Q	KRP2D2	Severe Missense
G640A	R169=	KRP2D2	Silent
G646A	Splice Junction	KRP2D2	Splice
C652T	Intron	KRP2D2	
C653T	Intron	KRP2D2	
C655T	Intron	KRP2D2	
G663A	Intron	KRP2D2	
C668T	Intron	KRP2D2	
C682T	Intron	KRP2D2	
G761A	Intron	KRP2D2	
G762A	Intron	KRP2D2	
G769A	Intron	KRP2D2	
G778A	R171=	KRP2D2	Silent
G780A	R172K	KRP2D2	Missense
G784A	E173=	KRP2D2	Silent
C786T	T174M	KRP2D2	Severe Missense
G787A	T174=	KRP2D2	Silent
G790A	T175=	KRP2D2	Silent
C799T	S178=	KRP2D2	Silent
G805A	S180=	KRP2D2	Silent
C806T	P181S	KRP2D2	Severe Missense
G817A	L184=	KRP2D2	Silent
G819A	S185N	KRP2D2	Missense
C823T	D186=	KRP2D2	Silent
C836T	Q191*	KRP2D2	Nonsense

(continued)

Nucleotide Change^	Effect	Gene	Mutation Score
C840T	A192V	KRP2D2	Severe Missense
G844A	A193=	KRP2D2	Silent
C847T	D194=	KRP2D2	Silent
C863T	R200C	KRP2D2	Severe Missense
C865T	R200=	KRP2D2	Silent
G868A	R201=	KRP2D2	Silent
G870A	R202K	KRP2D2	Missense
G871A	R202=	KRP2D2	Silent
C876T	P204L	KRP2D2	Missense
G877T	P204=	KRP2D2	Silent
G878A	A205T	KRP2D2	Missense
C885T	T207M	KRP2D2	Missense
G886A	T207=	KRP2D2	Silent
G896A	A211T	KRP2D2	Missense
C897T	A211V	KRP2D2	Missense
G899A	A212T	KRP2D2	Missense
C902T	P213S	KRP2D2	Missense
G907A	L214=	KRP2D2	Silent
C908T	H215Y	KRP2D2	Missense
G914A	D217N	KRP2D2	Missense
C916T	D217=	KRP2D2	Silent
G920A	E219K	KRP2D2	Missense
G923A	A220T	KRP2D2	Missense
G925A	A220=	KRP2D2	Silent
G933A	R223K	KRP2D2	Missense
G935A	A224T	KRP2D2	Missense
G940A	R225=	KRP2D2	Silent
C947T	P228S	KRP2D2	Missense
G953A	A230T	KRP2D2	Missense
C964T	D233=	KRP2D2	Silent
T968A	F235I	KRP2D2	Severe Missense
C970T	F235=	KRP2D2	Silent
C976T	A237=	KRP2D2	Silent
C978T	A238V	KRP2D2	Severe Missense
G980A	A239T	KRP2D2	Severe Missense
G982A	A239=	KRP2D2	Silent
G988A	K241=	KRP2D2	Silent
C991T	A242=	KRP2D2	Silent

(continued)

Nucleotide Change^	Effect	Gene	Mutation Score
G995A	A244T	KRP2D2	Severe Missense
G1000A	E245=	KRP2D2	Silent
C1001T	R246C	KRP2D2	Severe Missense
C1006T	F247=	KRP2D2	Silent
C1008T	A248V	KRP2D2	Severe Missense
G1010A	A249T	KRP2D2	Missense
C1011T	A249V	KRP2D2	Missense
G1022A	Intron	KRP2D2	
C917insA, G1035A	Insertion, intron	KRP2D2	Add new aa, cyclin- and CDK-binding gone
G1037A	Intron	KRP2D2	
G1045A	Intron	KRP2D2	
C1048T	Intron	KRP2D2	
G1069A	Intron	KRP2D2	
C1094T	Intron	KRP2D2	
C1100T	Intron	KRP2D2	
G1106A	Intron	KRP2D2	
C1116T	Intron	KRP2D2	
C1119T	Intron	KRP2D2	
G1126A	Splice Junction	KRP2D2	Splice
G1137A	D254N	KRP2D2	Severe Missense
C1139T	D254=	KRP2D2	Silent
C1142T	V255=	KRP2D2	Silent
C1144T	A256V	KRP2D2	Missense
G1145A	A256=	KRP2D2	Silent
C1146T	R257C	KRP2D2	Severe Missense
G1154A	V259=	KRP2D2	Silent
C1156T	P260L	KRP2D2	Severe Missense
§The designation "2" indicates that exons 2-4 of wheat KRP2D were TILLed. ^Nucleotide numbering is dependent upon the location of TILLING® primers.			

Table 9. Summary of *Triticum aestivum* (hexaploid) *KRP4B* mutants

Nucleotide Change^	Effect	Gene	Mutation Score
G370A	Non-coding	KRP4B2§	
C378T	Non-coding	KRP4B2	
C401T	P8L£	KRP4B2	Severe Missense
C408T	S10=	KRP4B2	Silent
C422T	S15L	KRP4B2	Missense
G424A	G16R	KRP4B2	Missense

(continued)

Nucleotide Change^	Effect	Gene	Mutation Score
G429A	T17=	KRP4B2	Silent
C440T	P21L	KRP4B2	Severe Missense
C474T	S32=	KRP4B2	Silent
A489G	Q37=	KRP4B2	Silent
G525A	E49=	KRP4B2	Silent
C537T	F53=	KRP4B2	Silent
C540T	F54=	KRP4B2	Silent
C545T	A56V	KRP4B2	Missense
G550A	E58K	KRP4B2	Severe Missense
G780A	Splice Junction	KRP4B2	Splice
G780A	Splice Junction	KRP4B2	Splice
C810T	P78L	KRP4B2	Severe Missense
C812T	L79F	KRP4B2	Severe Missense
G832A	W85*	KRP4B2	Nonsense
G846A	*90=	KRP4B2	Silent
G846A	*90=	KRP4B2	Silent
G858A	Non-coding	KRP4B2	
G867A	Non-coding	KRP4B2	
G922A	Non-coding	KRP4B2	
§The designation "2" indicates that exons 2-3 of wheat KRP4B were TILLed. £Amino acid numbering does not start from the beginning Methionine. ^Nucleotide numbering is dependent upon the location of TILLING® primers.			

Table 10. Summary of *Triticum aestivum* (hexaploid) *KRP4D* mutants

Nucleotide Change^	Effect	Gene	Mutation Score
C332T	Non-coding	KRP4D2§	
G388A	R1=£	KRP4D2	Silent
C393T	T3I	KRP4D2	Severe Missense
G397A	R4=	KRP4D2	Silent
G400A	E5=	KRP4D2	Silent
C407T	P8S	KRP4D2	Severe Missense
C409T	P8=	KRP4D2	Silent
G418A	L11=	KRP4D2	Silent
T421C	I12=	KRP4D2	Silent
G425A	D14N	KRP4D2	Missense
C435T	T17M	KRP4D2	Missense
C447T	P21 L	KRP4D2	Severe Missense
G462A	R26K	KRP4D2	Missense

(continued)

Nucleotide Change^	Effect	Gene	Mutation Score
C469T	S28=	KRP4D2	Silent
C481T	S32=	KRP4D2	Silent
C500T	P39S	KRP4D2	Missense
G503A	A40T	KRP4D2	Missense
C518T	P45S	KRP4D2	Severe Missense
C519T	P45L	KRP4D2	Severe Missense
C525T	S47L	KRP4D2	Missense
G527A	A48T	KRP4D2	Missense
G532A	E49=	KRP4D2	Silent
C547T	F54=	KRP4D2	Silent
G556A	A57=	KRP4D2	Silent
G559A	E58=	KRP4D2	Silent
C566T	Q61*	KRP4D2	Nonsense
G571A	Q62=	KRP4D2	Silent
C572T	Q63*	KRP4D2	Nonsense
C577T	A64=	KRP4D2	Silent
G593A	Intron	KRP4D2	
G732A	Intron	KRP4D2	
C761T	Intron	KRP4D2	
C775T	Intron	KRP4D2	
G780A	Splice Junction	KRP4D2	Splice
G791A	D72N	KRP4D2	Severe Missense
C810T	P78L	KRP4D2	Severe Missense
C815T	P80S	KRP4D2	Missense
G819A	G81D	KRP4D2	Severe Missense
C820T	G81=	KRP4D2	Silent
C853T	Non-coding	KRP4D2	
G858A	Non-coding	KRP4D2	
G863A	Non-coding	KRP4D2	
C882T	Non-coding	KRP4D2	
C889A	Non-coding	KRP4D2	

§The designation "2" indicates that exons 2-3 of wheat KRP4D were TILLed.
£Amino acid numbering does not start from the beginning Methionine.
^Nucleotide numbering is dependent upon the location of TILLING® primers.

Table 11. Summary of *Triticum aestivum* (hexaploid) *KRP5A* mutants

Nucleotide Change^	Effect	Gene	Mutation Score
G84A	G25S	Krp5A1§	Severe Missense

(continued)

Nucleotide Change^	Effect	Gene	Mutation Score
C90T	R27C	Krp5A1	Severe Missense
C95T	T28=	Krp5A1	Silent
C100T	S30F	Krp5A1	Severe Missense
C101T	S30=	Krp5A1	Silent
C110T	L33=	Krp5A1	Silent
C112T	A34V	Krp5A1	Severe Missense
G114A	A35T	Krp5A1	Silent
G116A	A35=	Krp5A1	Silent
C130T	A40V	Krp5A1	Missense
G134A	P41=	Krp5A1	Silent
C137T	S42=	Krp5A1	Silent
C259T	A83V	Krp5A1	Missense
C405T	Intron	Krp5A1	
C139T	P43L	Krp5A1	Missense
C150G	R47G	Krp5A1	Missense
C150T	R47C	Krp5A1	Missense
G156A	G49S	Krp5A1	Missense
G162A	E51K	Krp5A1	Missense
G171A	D54N	Krp5A1	Missense
C173T	D54=	Krp5A1	Silent
G194A	R61=	Krp5A1	Silent
G196A	R62K	Krp5A1	Severe Missense
G197A	R62=	Krp5A1	Silent
G206A	K65=	Krp5A1	Silent
G212A	P67=	Krp5A1	Silent
C216T	P69S	Krp5A1	Severe Missense
G219A	G70R	Krp5A1	Missense
C224T	P71=	Krp5A1	Silent
G234A	E75K	Krp5A1	Missense
G236A	E75=	Krp5A1	Silent
G240A	A77T	Krp5A1	Missense
G245A	P78=	Krp5A1	Silent
C249T	P80S	Krp5A1	Severe Missense
G251A	P80=	Krp5A1	Silent
C252T	P81S	Krp5A1	Missense
C257T	A82=	Krp5A1	Silent
G272A	R87=	Krp5A1	Silent
G278A	E89=	Krp5A1	Silent

(continued)

Nucleotide Change^	Effect	Gene	Mutation Score
C279T	Q90*	Krp5A1	Nonsense
G284A	A91=	Krp5A1	Silent
G290A	S93=	Krp5A1	Silent
C292T	S94L	Krp5A1	Missense
C296T	F95=	Krp5A1	Silent
C298T	A96V	Krp5A1	Missense
C302T	A97=	Krp5A1	Silent
G307A	G99D	Krp5A1	Severe Missense
C321T	L104F	Krp5A1	Missense
G357A	D116N	Krp5A1	Severe Missense
C368T	D119=	Krp5A1	Silent
C375T	Intron	Krp5A1	
G382A	Intron	Krp5A1	
C395T	Intron	Krp5A1	
C403T	Intron	Krp5A1	
C404T	Intron	Krp5A1	
C405T	Intron	Krp5A1	
C169T	Non-coding	Krp5A2¥	
C199T	Non-coding	Krp5A2	
T201G	Non-coding	Krp5A2	
C213T	Non-coding	Krp5A2	
G227A	Non-coding	Krp5A2	
G232A	G1S£	Krp5A2	Severe Missense
G246A	T5=	Krp5A2	Silent
G257A	S9N	Krp5A2	Missense
G273A	S14=	Krp5A2	Silent
G274A	E15K	Krp5A2	Severe Missense
G276A	E15=	Krp5A2	Silent
C290T	P20L	Krp5A2	Severe Missense
C296T	S22L	Krp5A2	Severe Missense
C302T	T24I	Krp5A2	Missense
G305A	G25E	Krp5A2	Missense
G333A	R34=	Krp5A2	Silent
G345A	P38=	Krp5A2	Silent
G346A	V39I	Krp5A2	Missense
C351T	C40=	Krp5A2	Silent
G353A	R41H	Krp5A2	Severe Missense
C354T	R41=	Krp5A2	Silent

(continued)

Nucleotide Change^	Effect	Gene	Mutation Score
C366T	S45=	Krp5A2	Silent
G369A	S46=	Krp5A2	Silent
G372A	L47=	Krp5A2	Silent
C395T	A55V	Krp5A2	Missense
G402A	E57=	Krp5A2	Silent
G408A	Q59=	Krp5A2	Silent
C414T	H61=	Krp5A2	Silent
G426A	R65=	Krp5A2	Silent
G427A	D66N	Krp5A2	Severe Missense
G436A	Intron	Krp5A2	
N474A	Intron	Krp5A2	
C495T	Intron	Krp5A2	
G499A	Intron	Krp5A2	
G513A	K67=	Krp5A2	
C526T	P72S	Krp5A2	Missense
C537T	G75=	Krp5A2	Silent
C541T	P77S	Krp5A2	Severe Missense
C548T	P79L	Krp5A2	Severe Missense
G555A	R81=	Krp5A2	Silent
G568A	V86M	Krp5A2	Severe Missense
C593T	Non-coding	Krp5A2	
C599T	Non-coding	Krp5A2	
G608A	Non-coding	Krp5A2	
C614T	Non-coding	Krp5A2	
C617T	Non-coding	Krp5A2	
C626T	Non-coding	Krp5A2	
C627T	Non-coding	Krp_5A2	
C635T	Non-coding	Krp5A2	
C650T	Non-coding	Krp5A2	
G653A	Non-coding	Krp5A2	
G659A	Non-coding	Krp5A2	
G668A	Non-coding	Krp5A2	

§The designation "1" indicates that exon 1 of wheat KRP5A was TILLed.
¥The designation "2" indicates that exons 2-3 of wheat KRP5A were TILLed.
£Amino acid numbering does not start from the beginning Methionine.
^Nucleotide numbering is dependent upon the location of TILLING® primers.

Table 12. Summary of *Triticum aestivum* (hexaploid) *KRP5D* mutants

Nucleotide Change^	Effect	Gene	Mutation Score
C205T	S9=£	Krp5D2§	Silent
C206T	L10=	Krp5D2	Silent
G226A	T16=	Krp5D2	Silent
G229A	M17I	Krp5D2	Missense
G240A	G21E	Krp5D2	Severe Missense
G247A	A23=	Krp5D2	Silent
C249T	T24I	Krp5D2	Severe Missense
G258A	R27H	Krp5D2	Missense
C271T	R31=	Krp5D2	Silent
C272T	R32C	Krp5D2	Severe Missense
G280A	A34=	Krp5D2	Silent
G286A	T36=	Krp5D2	Silent
G290A	V38I	Krp5D2	Missense
C295T	C39=	Krp5D2	Silent
C296T	R40C	Krp5D2	Severe Missense
G302A	V42I	Krp5D2	Missense
G309A	S44N	Krp5D2	Severe Missense
C310T	S44=	Krp5D2	Silent
C312T	S45L	Krp5D2	Missense
G322A	M48I	Krp5D2	Missense
G323A	D49N	Krp5D2	Missense
G328A	E50=	Krp5D2	Silent
C339T	A54V	Krp5D2	Missense
C340T	A54=	Krp5D2	Silent
G344A	E56K	Krp5D2	Severe Missense
G369A	R64K	Krp5D2	Missense
G371A	E65K	Krp5D2	Severe Missense
G376A	Splice Junction	Krp5D2	Splice
C392T	Intron	Krp5D2	
G481A	Splice Junction	Krp5D2	Splice
C485T	Y67=	Krp5D2	Silent
C496T	P71L	Krp5D2	Missense
G506A	E74=	Krp5D2	Silent
C510T	P76S	Krp5D2	Severe Missense
C513T	L77F	Krp5D2	Severe Missense
C517T	P78L	Krp5D2	Severe Missense
G519A	G79R	Krp5D2	Severe Missense

(continued)

Nucleotide Change^	Effect	Gene	Mutation Score
G520A	G79E	Krp5D2	Severe Missense
G530A	E82=	Krp5D2	Silent
G532A	W83*	Krp5D2	Nonsense
C548T	C88=	Krp5D2	Silent
§The designation "2" indicates that exons 2-3 of wheat KRP5D were TILLed. £Amino acid numbering does not start from the beginning Methionine. ^Nucleotide numbering is dependent upon the location of TILLING® primers.			

Example 3

Wheat breeding program

[0242] The wheat KRP TILLING® mutants are prioritized for the breeding program in the following manner: 1) Nonsense and splice mutants; 2) Type I severe missense; and 3) Type II severe missense. Type I severe missense mutations are non-conservative amino acid substitutions in regions of the KRP protein known to be essential for binding to cyclin or cyclin-dependent kinase (CDK) and are predicted by SIFT analysis to be deleterious to protein function. Type II severe missense mutations are non-conservative amino acid substitutions outside of the cyclin and CDK binding domains but which satisfy two additional criteria. First, they are in regions of the protein determined by BLOCKS analysis (Henikoff, S. and Henikoff, J.G. (1991) Nucleic Acids Res., 19, 6565-6572) to be evolutionarily conserved and therefore possibly of functional significance. Secondly, they have a SIFT (Ng, P.C. and Henikoff, S. (2003) Nucleic Acids Res. July 1; 31(13): 3812-3814) score of less than 0.05, and are therefore predicted to be deleterious to protein function.

[0243] Tables 13 to 22 list wheat KRP TILLING® mutants that are in the breeding program and is a subset of the mutants listed in Tables 2 to 12. A given wheat (WH) group is a number that identifies a particular mutation in a particular gene.

[0244] M3 seed homozygous or heterozygous for a given KRP TILLING® mutation is grown. Backcrosses with the hexaploid spring background parent Express are performed, ideally through several rounds (to the BC3 or BC4 level), to eliminate deleterious background mutations. Background mutations could contribute to undesirable traits such as delayed maturity, premature senescence, increased susceptibility to wheat pathogens, slow germination, and/or sterility. The progeny of each backcross (F1, BC1, BC2, etc.) are also selfed to produce F2 lines. F2 lines are genotyped to identify ones that are homozygous for the wild type or for the *krp* mutant allele. Homozygote wild type and mutant siblings are seed expanded to F3 for field trials.

Wheat *krp* mutant alleles are introgressed into other spring and winter wheat to transfer the yield enhancement to commercial varieties.

[0245] Crosses between mutants are done to generate multiple stack mutants within a given KRP gene (e.g. *krp1A*/*1B, krp1B*/*krp1D, krp1A*/*1B*/*1D,* etc., all possible combinations) or across different KRP genes (e.g. *krp1A*/*2A, krp2B*/*krp4B*, *krp4D*/*krp5A, krp1B*/*krp2A*/*krp5D,* etc., all possible combinations).

[0246] The overall grain yield per unit area is determined (e.g. lbs/acre) and yield components such as seed count, seed size/weight (thousand kernel weight), seed per spike, head (spike) number, spike length, awn length, and/or tiller number, are measured. Agronomic characteristics such as stand rate, maturity rate and peduncle rate are also measured.

Table 13. ***Triticum aestivum*** **(hexaploid) KRP2A TILLING® Mutants in breeding program**

WH group	Gene	Nuc_Change from start codon	Effect-from beginning Met
1	KRP2A2	G569A	C153Y
2	KRP2A2	C593T	S161F
3	KRP2A2	G758A	Splice junction-intron2/exon3
4	KRP2A2	G759A	Splice junction-intron2/exon3
5	KRP2A2	C772T	P177S (and P77S on 5A2, hom)
6	KRP2A2	G880A	A213T

(continued)

WH group	Gene	Nuc_Change from start codon	Effect-from beginning Met
7	KRP2A2	G880A	A213T
8	KRP2A2	C919T	P226S
9	KRP2A2	G925A	A228T
10	KRP2A2	C983T	A247V
11	KRP2A2	G990A	Splice junction-exon3/intron3
12	KRP2A2	G1119A	D253N
13	KRP2A2	C1128T	R256C
14	KRP2A2	G1131A	G257S
15	KRP2A2	G1132A	G257D
16	KRP2A2	G1134A	V258M and G169S
17	KRP2A2	C1137T	P259S
18	KRP2A2	C1147T	A262V
19	KRP2A2	G1150A	G263D
20	KRP2A2	C1152T	R264W

Table 14. ***Triticum aestivum*** **(hexaploid) KRP2B TILLING® Mutants in breeding program**

WH group	Gene	Nuc_Change from start codon	Effect-from beginning Met
21	KRP2B2	G569A	V156M
22	KRP2B2	C579T	S159F
23	KRP2B2	G753A	E172K
24	KRP2B2	C760T	T174M
25	KRP2B2	C762T	P175S
26	KRP2B2	C775T	S179L
27	KRP2B2	G798A	E187K
28	KRP2B2	C807T	Q190*
29	KRP2B2	G816A	D193N
30	KRP2B2	G819A	E194K
31	KRP2B2	G838A	R200K
32	KRP2B2	G841A	R201K
33	KRP2B2	C850T	A204V
34	KRP2B2	G852A	A205V
35	KRP2B2	G870A	A211T
36	KRP2B2	G891A	E218K
37	KRP2B2	G915A	A226T
38	KRP2B2	G1103A	Splice junction-intron3/exon4
39	KRP2B2	C1135T	L258F
40	KRP2B2	G1158A	W265*

(continued)

WH group	Gene	Nuc_Change from start codon	Effect-from beginning Met
41	KRP2B2	G1165A	V268M

Table 15A. ***Triticum turgidum*** **ssp**. **durum (tetraploid) KRP4B TILLING® Mutants in breeding program**

WH group	Gene	Nuc_Change from start codon	Effect-from beginning Met
42	KRP4B2	N/A^	R105K
43	KRP4B2	N/A	P109S
46	KRP4B2	N/A	S129N
49	KRP4B2	N/A	A149T
54	KRP4B2	N/A	D177N
56	KRP4B2	N/A	P179L
58	KRP4B2	N/A	L180F
59	KRP4B2	N/A	P181S
^Full-length genomic sequence of wild type KRP4B not available; therefore nucleotide numbering is as given in Table 8.			

Table 15B. ***Triticum aestivum*** **(hexaploid) KRP4B TILLING® Mutants in breeding program**

WH group	Gene	Nuc_Change from start codon	Effect-from beginning Met
44	KRP4B2	N/A^	P109L
45	KRP4B2	N/A	S116L
47	KRP4B2	N/A	G117R
48	KRP4B2	N/A	P122L
50	KRP4B2	N/A	A157V
51	KRP4B2	N/A	E159K
52	KRP4B2	N/A	Splice junction-intron2/exon3
53	KRP4B2	N/A	P179L
55	KRP4B2	N/A	L180F
57	KRP4B2	N/A	W186*
^Full-length genomic sequence of wild type KRP4B not available; therefore nucleotide numbering is as given in Table 8.			

Table 16. ***Triticum aestivum*** **(hexaploid) KRP4D TILLING® Mutants in breeding program**

WH group	Gene	Nuc_Change from start codon	Effect-from beginning Met
60	KRP4D2	N/A^	T104I
61	KRP4D2	N/A	P109S
62	KRP4D2	N/A	D115N
63	KRP4D2	N/A	P122L
64	KRP4D2	N/A	R127K
65	KRP4D2	N/A	P140S
66	KRP4D2	N/A	A141T

(continued)

WH group	Gene	Nuc_Change from start codon	Effect-from beginning Met
67	KRP4D2	N/A	P146S
68	KRP4D2	N/A	P146L
69	KRP4D2	N/A	S148L
70	KRP4D2	N/A	A149T
71	KRP4D2	N/A	Q162*
72	KRP4D2	N/A	Q164*
73	KRP4D2	N/A	Splice junction-intron2/exon3
74	KRP4D2	N/A	Splice junction-intron2/exon3
75	KRP4D2	N/A	D173N
76	KRP4D2	N/A	P179L
77	KRP4D2	N/A	P181S
78	KRP4D2	N/A	G182D
^Full-length genomic sequence of wild type KRP4D not available; therefore nucleotide numbering is as given in Table 9.			

Table 17. *Triticum aestivum* (hexaploid) KRP5A TILLING® Mutants in breeding program

WH group	Gene	Nuc_Change from start codon	Effect-from beginning Met
79	KRP5A2	G1850A	G121S
80	KRP5A2	G1875A	S129N
81	KRP5A2	G1892A	E135K
82	KRP5A2	C1908T	P140L
83	KRP5A2	C1914T	S142L
84	KRP5A2	C1920T	T144I
85	KRP5A2	G1923A	G145E
86	KRP5A2	G1964A	V159I
87	KRP5A2	G1971A	R161H
88	KRP5A2	C2013T	A175V
89	KRP5A2	G2045A	D186N
90	KRP5A2	C2144T	P192S
91	KRP5A2	C2159T	P197S
92	KRP5A2	C2166T	P199L
93	KRP5A2	G2186A	V206M
545	KRP5A1	C268T	Q90*

Table 18. *Triticum aestivum* (hexaploid) KRP5D TILLING® Mutants in breeding program

WH group	Gene	Nuc_Change from start codon	Effect-from beginning Met
112	KRP5D2	G1767A	G137E
113	KRP5D2	C1776T	T140I

(continued)

WH group	Gene	Nuc_Change from start codon	Effect-from beginning Met
114	KRP5D2	C1799T	R148C
115	KRP5D2	C1823T	R156C
116	KRP5D2	G1829A	V158I
117	KRP5D2	G1836A	S160N
118	KRP5D2	C1839T	S161L
119	KRP5D2	G1849A	M164I
120	KRP5D2	G1850A	D165N
121	KRP5D2	G1871A	E172K
122	KRP5D2	G1898A	E181K
123	KRP5D2	G1903A	Splice junction-exon2/intron2
124	KRP5D2	G2008A	Splice junction-intron2/exon3
125	KRP5D2	C2023T	P187L
126	KRP5D2	C2037T	P192S
127	KRP5D2	C2040T	L193F
128	KRP5D2	C2044T	P194L
129	KRP5D2	G2046A	G195R
130	KRP5D2	G2047A	G195E
131	KRP5D2	G2059A	W199*

Table 19. ***Triticum aestivum*** **(hexaploid) KRP1B TILLING® Mutants in breeding program**

WH group	Gene	Nuc Change from start codon	Effect from beginning Met
147	KRP1B	C503T	A138V
148	KRP1B	G539A	R150K
149	KRP1B	G644A	E152K
150	KRP1B	C654T	P155L
151	KRP1B	C657T	S156F
152	KRP1B	G705A	G172D
153	KRP1B	C723T	S178L
154	KRP1B	C738T	T183M
155	KRP1B	C744T	T185I
156	KRP1B	G764A	A192T
157	KRP1B	G768A	R193K
158	KRP1B	C779T	P197S
159	KRP1B	G815A	A209T
160	KRP1B	G821A	E211K
161	KRP1B	G827A	E213K
162	KRP1B	G1000A	A241T

(continued)

WH group	Gene	Nuc Change from start codon	Effect from beginning Met
550	KRP1B	C733T	Q182*
551	KRP1B	G541A	Splice junction

Table 20. ***Triticum aestivum*** **(hexaploid) KRP1D TILLING® Mutants in breeding program**

WH group	Gene	Nuc Change from start codon	Effect from beginning Met
163	KRP1D	G634A	Splice Junction-intron2/exon3
164	KRP1D	C648T	P158S
165	KRP1D	C664T	P163L
166	KRP1D	C688T	S171L
167	KRP1D	G696A	A174T
168	KRP1D	C697T	A174V
169	KRP1D	C754T	P193L
170	KRP1D	C757T	A194V
171	KRP1D	G763A	R196K
172	KRP1D	C774T	P200S
173	KRP1D	C805T	A210V
174	KRP1D	G813A	E213K
175	KRP1D	G829A	R218K
176	KRP1D	G841A	C222Y
177	KRP1D	C959T	R230C
178	KRP1D	G963A	G231D
179	KRP1D	C971T	L234F
180	KRP1D	C1002T	A244V

Table 21. *Triticum aestivum* (hexaploid) KRP1A TILLING® Mutants in breeding program

WH group	Gene	Nuc Change from start codon	Effect from beginning Met
181	KRP1A	G518A	A 144T
182	KRP1A	G543A	R152K
183	KRP1A	G648A	E154K
184	KRP1A	C655T	T156M
185	KRP1A	C657T	P157S
186	KRP1A	C672T	P162S
187	KRP1A	C763T	P192L
188	KRP1A	C783T	P199S
189	KRP1A	C784T	P199L
190	KRP1A	G804A	E206K
191	KRP1A	C814T	A209V

(continued)

WH group	Gene	Nuc Change from start codon	Effect from beginning Met
192	KRP1A	G822A	E212K
193	KRP1A	G961A	R229H
194	KRP1A	C970T	P232L
195	KRP1A	C979T	S235F
196	KRP1A	G981A	G236S
197	KRP1A	G995A	W240*
198	KRP1A	C999T	P242S
199	KRP1A	G1012A	S246N
200	KRP1A	G1015A	S247N

Table 22. ***Triticum aestivum*** **(hexaploid) KRP2D TILLING® Mutants in breeding program**

WH group	Gene	Nuc Change from start codon	Effect from beginning Met
201	KRP2D	G563A	C152Y
202	KRP2D	C571T	R155C
203	KRP2D	G572A	R155H
204	KRP2D	G584A	S159N
205	KRP2D	G593A	S162N
206	KRP2D	C613T	R169W
207	KRP2D	G614A	R169Q
208	KRP2D	G753A	R171= (splice)
209	KRP2D	C761T	T174M
210	KRN2D	C815T	A192V
211	KRP2D	C838T	R200C
212	KRP2D	C860T	T207M
213	KRP2D	C877T	P213S
214	KRP2D	C883T	H215Y
215	KRP2D	C922T	P228S
216	KRP2D	G928A	A230T
217	KRP2D	C953T	A238V
218	KRP2D	G955A	A239T
219	KRP2D	G1112A	D254N
220	KRP2D	C1121T	R257C
356	KRP2D	C807T	Q190*

Example 4

Genotyping of wheat krp TILLING® mutations

[0247] Wheat *KRP* TILLING® mutations were genotyped by allelic discrimination primer/probe sets in a real-time PCR

assay. First, genome-specific primers were used to amplify a given wheat KRP gene from a wheat genomic sample. The amplification product was then genotyped in a Taqman® allelic discrimination assay (AD assay, Applied Biosystems). The following is a specific example of an assay to genotype KRP4B2 P109L.

[0248] Forward primer TTC CTT ATT TTT TAT GAC TAT TGA TAT GTG TTC TTC (SEQ ID NO: 28) and reverse primer GTG GTC ATT TCA GAA TGA GCT GCT AAC CGT T (SEQ ID NO: 29) were used to amplify KRP4B2 from wheat genomic DNA. The PCR reaction contained 2 μL genomic DNA, 2.6 μL 10X Ex Taq reaction buffer, 2.0 μL 2.5 mM dNTPs, 0.75 μL each of 5 mM forward and reverse primers, 0.1 μL Ex Taq polymerase (5 units/μL, TaKaRa) and 17.8 μL H_2O in a total reaction volume of 26 μL. PCR amplification conditions were: initial denaturation at 94°C, 2 min; 40 cycles of 94°C, 30 sec, 58°C, 30 sec, 72°C, 1min 30 sec; final extension at 72°C, 5 min.

[0249] The amplification product was then used in an AD assay with flanking forward primer (TGTGTATGTATGTTTT-GTGGCTAGCA, SEQ ID NO: 30), flanking reverse primer (CGTTCCCGAGTCCCTAATCAAG SEQ ID NO: 31), a labeled probe specific to the wild type allele (VIC - TGC AG**G** GCG TCG TC - MGB - NFQ SEQ ID NO: 32) and a labeled probe specific to the mutant allele (FAM - C TGC AG**A** GCG TCG TC - MGB - NFQ SEQ ID NO: 33). The PCR reaction contained 2 μL of KRP4B2 amplification product, 3 μL ABI genotyping real time PCR master mix and 1 μL 6X (5.4 μM each primer/1.2 μM each probe) SNP-specific AD assay primer/probe mix in a total reaction volume of 6 μL. The real-time PCR was conducted on an ABI real-time machine per ABI PCR conditions.

[0250] Figure 1A-1C demonstrates that primer/probes can be designed to distinguish between wild type and mutant KRP4B alleles in an AD assay. A mutant heterozygous for the KRP4B P109L allele was confirmed by sequencing (Figure 1D). Figure 2 further shows that populations of samples homozygous for the wild type KRP4B allele, homozygous for the mutant KRP4B P109L allele, or heterozygous for the mutant KRP4B P109L allele can be easily distinguished in an AD assay.

***In vitro* assays to test kinase inhibitory activity of mutant krp TILLING® proteins**

[0251] Selected Type I severe missense and nonsense wheat *KRP* TILLING® mutants were tested in an *in vitro* assay to determine whether the mutant KRP proteins could still inhibit the kinase activity of ZmCyclinD4/ZmCDKA;1 kinase complex (Figure 3) (assay described in Dominant Negative Mutant Kip-Related Proteins (KRP) in Zea Mays and Methods of their Use, PCT/US2011/060598).

[0252] Recombinant ZmCyclinD4 and ZmCDKA;1 were produced in and the complex isolated from *S. frugiperda* Sf9 insect cells. All wild-type TaKRP genes were synthesized by DNA2.0.

Mutagenesis of TaKRPs

[0253]

TaKRP1A P232L (pTG1947)
TaKRP1A G236S (pTG1948)
TaKRP1A W240* (pTG1949)
TaKRP2D P228S (pTG1958)
TaKRP2D A238V (pTG1959)
TaKRP2D A239T (pTG1960)
TaKRP2D D254N (pTG1961)
TaKRP2D R257C (pTG1962)
TaKRP4A W186* (pTG1950)
TaKRP5A W199* (pTG1951)

[0254] Primers to generate the mutants listed above are found in Table 28.

[0255] General methods for recombinant protein expression in bacteria, purification and detection are desribed below:

I. Insect cells and media

[0256] The baculovirus expression system is a versatile eukaryotic system for heterologous gene expression. This system provides correct protein folding, disulfide bond formation and other important post-translational modifications. All methods were taken from the *Baculovirus expression vector system:* Procedures and methods manual. (BD Biosciences, Pharmingen, San Diego, Calif. 6th Ed.). Sf9 insect cells were grown at 27° C. in TNM-FH insect cell media (BD Biosciences) for the reported studies. It should be noted that alternative media are well known to the skilled artisan and are also useful. Similarly, alternative insect cell lines such as Sf21 and High Five™ cells will also work for virus production and protein production.

II. Western blots and IPs

[0257] The recombinant protein expressed in insect cells was monitored by Western blot. Protein extracts (35 μg) were boiled in the presence of Laemmli buffer, run on 10% or 12% SDS-PAGE gels and transferred to a PVDF membrane using a submerged transfer apparatus (BioRad). Following the transfer, the membrane was blocked in TBS-T (25 mM Tris pH 7.5; 75 mM NaCl; 0.05% Tween) containing 5% non-fat dry milk powder. Primary antibody was used at 1:1000 dilution overnight in TBS-T blocking buffer. Blots were washed three times 15 minutes at room temperature. An appropriate secondary antibody conjugated to horse radish peroxidase (HRP) was used at 1:10,000 dilution in TBS-T blocking buffer. Blots were incubated in secondary antibody for 1 hour and then washed three times in TBS-T, 15 min each. Blots were then processed as described in the ECL system protocol (Amersham Biosciences). Antibodies commonly used were: anti-flag M2 monoclonal antibody (Sigma), anti-HA monoclonal or polyclonal antibody (Babco), anti-PSTAIR antibody (Sigma-Aldrich), anti-myc 9E10 monoclonal or polyclonal (A-14) (Santa Cruz Biotechnology). Secondary antibodies used were anti-mouse IgG-HRP, and anti-rabbit IgG-HRP (GE Healthcare).

III. Baculovirus vector construction

[0258] The Baculovirus system was Bac-to-bac (Invitrogen). Alternative Baculovirus genomes can also be used. All bacmids containing our genes of interest were independently transfected into 293 cells using lipid based transfection reagents such as Fugene or Lipofectamine. *S. frugiperda* Sf9 cells were seeded at 9 x 10^6 cells on 60 mm dish and transiently transfected with 1 μg bacmid using 3μl Fugene 6 transfection reagent according to the manufacturer's protocol (Roche Diagnostics). After 4 hours of transfection the Fugene/DNA solution was removed and replaced with 3 ml of TNM-FH media. Four (4) days later, the supernatant was collected and subsequently used to infect more cells for amplification of the virus. This amplification was repeated until the virus titer was at least 10^9 virus particles/ml. The virus was amplified by infecting Sf9 cells at a multiplicity of infection (moi) of < 1. The virus titer was monitored using light and fluorescence microscopy.

IV. Recombinant protein expression in bacteria and purification

[0259] All bacterial expression plasmidscarrying inserts were transformed into BL21 RosettaBlue (DE3) (Novagen). Bacterial colonies from this fresh transformation was used to inoculate 400 ml of LB containing 100 μg/ml of ampicillin and grown at 37°C. When the culture reached an OD_{600} between 0.6 and 0.8 recombinant protein expression was induced with 1 mM isopropyl -D-thiogalactopyranoside (IPTG). Cells were then grown at 30°C for three hours. Cells were collected by centrifugation in a JLA 10.500 Beckman rotor. Bacterial cell pellet was either stored at -80°C or lysed immediately. Bacteria were lysed in 10 ml Phosphate lysis buffer (100 mM Phosphate buffer pH 7.0, 150 mM NaCl, 1% Triton X100) containing protease inhibitors and lacking EDTA. The resuspended bacterial culture was lysed via a French press or repeated sonication. Lysed cells were centrifuged at 14,000 rpm in a Beckman JA20.1 rotor for 15 minutes at 4°C. Tagged KRP molecules were mainly insoluble. Insoluble tagged KRPs were solubilized in Urea buffer (8M Urea, 100 mM Phosphate buffer pH 7.0) manually with a pipette aid. Urea-insoluble proteins were eliminated by centrifugation at 14,000 rpm in a Beckman JA20.1 rotor for 15 minutes at 4°C. Tagged KRPs were purified in batch using BD Talon Co^{2+} metal affinity resin equilibrated in Urea buffer. Batch purification was incubated at 4°C 3 hrs to overnight under slow rotation. Slurry was loaded on a column and resin was washed with 36 bed volumes of Urea buffer followed by 12 bed volumes of Urea buffer containing 5 mM Imidazole pH 7.0. Bound tagged KRP protein was eluted using Urea buffer containing 300 mM Imidazole pH 7.0. Fractions were monitored for tagged KRP by SDS-PAGE and/or by Bradford protein assay (BioRad). Refolding of the denatured tagged KRP1 was carried out using step-wise dilution dialysis. Fractions containing the majority of tagged KRP protein were combined and dialyzed in a 1M Urea, 100 mM Phosphate buffer pH 7.0, and 1mM Dithiothreitol for 20 hrs at 4°C. Dialysis buffer was then changed to 0.5 M Urea, 100 mM Phosphate buffer pH 7.0, and 1mM Dithiothreitol and continued for an additional 12 hrs. Recombinant protein was collected, quantified by Bradford assay and stored at 4°C.

[0260] In *in vitro* kinase assays, a few TaKRP mutant proteins exhibited reduced inhibitory activity toward the cyclin/CDK kinase complex, while others behaved like their wild-type TaKRPs. Notably, TaKRP4A W186* (Figures 4 and 6A) and TaKRP5A W199* (Figure 5) did not inhibit the kinase activity of ZmCyclinD4/ZmCDKA;1 at 0.1, 0.25 or 0.5 μg. TaKRP1A W240* also did not inhibit the kinase activity of ZmCyclinD4/ZmCDKA;1 at 0.1, 0.25 or 0.5 μg, although its inhibitory activity was not as compromised at 0.25 and 0.5 μg (Figures 4 and 6A). TaKRP1A G236S could only strongly inhibit ZmCyclinD4/ZmCDKA;1 at 0.5 μg and weakly at 0.25 μg (Figure 4). Similarly, TaKRP2D P228S could only strongly inhibit ZmCyclinD4/ZmCDKA;1 at 0.5 μg and very weakly at 0.25 μg (Figure 4). TaKRP4A W186*, TaKRP5A W199* and TaKRP1A W240* contain premature stop codons very close to the end of the KRP proteins and thus still retain the cyclin- and CDK-binding domains. However, the elimination of the last 8 amino acids for TaKRP1A W240*, the last 6 amino acids for TaKRP5A W199* and the last 5 amino acids for TaKRP4A W186* compromises the ability of the mutant

KRPs to inhibit the kinase complex. A mutant corn KRP2 protein, ZmKRP2 W250*, with an equivalent premature stop codon near the end of the protein, also was not able to inhibit the ZmCyclinD4/ZmCDKA;1 complex at 0.3 or 3 μg (Figure 6B).

[0261] Other mutants, such as TaKRP1A P232L (Figure 4), TaKRP2D A238V (Figure 5), TaKRP2D A239T (Figure 5), TaKRP2D D254N (Figure 4) and TaKRP2D R257C (Figure 4), inhibit the ZmCyclinD4/ZmCDKA;1 complex as well as their wild-type counterparts at the indicated protein concentrations.

[0262] The inhibitory activities for various wheat *KRP* TILLING® mutants compared to their wild-type counterparts are summarized in Table 23.

Table 23. Inhibitory activity of wild-type wheat KRP or mutant wheat KRP on ZmCyclinD4/CDKA;1 kinase complex

Wheat Krp	Inhibitory activity
Krp1A wild type (WT)	++++
Krp1A (E212K)	Not tested
Krp1A (P232L)	++++
Krp1A (G236S)	++
Krp1A (W240*)	+
Krp2D WT	++++
Krp2D (P228S)	+++
Krp2D (A238V)	++++
Krp2D (A239T)	++++
Krp2D (D254N)	++++
Krp2D (R257C)	++++
Krp4A WT	++++
Krp4A (W186*)	-
Krp5A WT	++++
Krp5A (G200D)	Not tested
Krp5A (G200R)	Not tested
Krp5A (W199*)	-

Example 5

Preliminary field trial results on M generation wheat KRP TILLING® mutants

[0263] The M5 generation of selected wheat KRP TILLING® mutants were grown in Yuma, AZ in fall 2009-winter 2010 to determine yield. Sixty entries (Table 24) were in the yield trial, with each entry being replicated three times randomly throughout the trial. Each plot was 4 ft by 10 ft with 7 rows per plot and 7 inches between rows. About 75g of seed were planted per plot, or about 2500 seeds/plot or about 360 seeds per 10-ft row. Planting took place in October.

[0264] Where available, the wild type segregant of a given homozygous or heterozygous KRP TILLING® mutant was planted for comparison. The parent variety, Express, was also included in the trial. The stand rate was taken prior to harvest, the maturity rate was taken at flowering, and the peduncle rate, head number, kernel number, spike length and awn length were taken after harvest from subsamples. Maturity rate and peduncle rate were on a comparative scoring system, relative to the Express background parent. Maturity: 1 is early, 3 is similar to Express, 5 is late/vegetative. Peduncle: 1 is very thin, 3 is similar to Express, 5 is very thick (Table 24).

[0265] Harvest was conducted in two rounds in May. Plots in replicate 1 were hand sickled and plants bundled and allowed to dry in the field for a few days prior to stationary threshing. The second round utilized a plot combine to harvest the remaining plots. Prior to the second harvest, heads from five plants per plot of replicates 2 and 3 (so 10 heads total per entry) were collected for head measurements. The cut was made approximately 5-6 inches below the base of the spike.

[0266] Seed weight and seed count were determined for each 5-head subsample, from which a weight per seed could

be calculated. The plot weight was determined by hand. Seed count per plot was calculated from weight per seed and plot weight. Thousand kernel weight (TKW) is weight per seed multiplied by 1000.

[0267] A number of KRP mutants demonstrated excellent seed yield. These included WH68, WH3, WH91, WH124, WH4 and WH16 (Table 24). WH68 (KRP4D, P146L) was ranked first in yield at 3584.90 lbs/acre. Two mutants, WH3 and WH4, with changes in adjacent nucleotides that result in a splice mutation at the same intron/exon junction both showed good yield, ranking second (WH3) and sixth (WH4) out of the 60 entries. Another splice mutant in KRP5D, WH124, was ranked fifth in yield. A mutant, WH16, ranked seventh in yield interestingly has two mutations within the KRP2A gene (V258M and G169S).

Table 24. Yield and agronomic data for wheat KRP TILLING® mutants, Yuma, AZ

No	Entry	Zygosity	Group	Variant	Type	Stand rate	Maturity rate	Grain yield		Peduncle rate	Head number	Kernel number	TKW	Spike length	Awn length
								Lbs/acre					g/1000	cm	cm
1	WH11 -120M5	HOM	11	KRP2A2	SPLCE	5.00	2.00	2771.91	(18)	3.33	3.21	77.30	40.53	14.00	7.00
2	WH11 -124M5	HOM	11	KRP2A2	SPLCE	5.00	2.33	2295.06	(39)	3.33	2.92	60.70	46.92	12.50	8.50
3	WH11 -125M5	HOM	11	KRP2A2	SPLCE	5.00	2.33	2416.27	(34)	3.33	2.63	75.50	42.13	12.50	8.00
4	WH11 -126M5	HOM	11	KRP2A2	SPLCE	5.00	3.33	2137.06	(48)	3.33	2.87	73.70	37.05	12.50	6.00
5	WH11 -126M5	HOM	11	KRP2A2	SPLCE	5.00	3.00	2286.10	(40)	3.00	3.51	66.60	38.56	11.00	5.50
6	WH11 -129M5	HOM	11	KRP2A2	SPLCE	4.67	2.00	2090.37	(49)	3.00	2.95	61.60	39.44	10.50	7.00
7	WH116-1212M5	HOM	116	KRP5D2	MSSE	5.00	1.33	2633.43	(25)	2.67	3.11	63.20	46.50	13.00	7.50
8	WH124-1275M5	HOM	124	KRP5D2	SPLCE	5.00	1.67	2164.89	(46)	3.00	2.87	71.40	40.75	11.50	8.00
9	WH124 -1276M5	HOM	124	KRP5D2	SPLCE	5.00	2.00	3241.41	(5)	3.00	3.36	82.90	41.34	13.50	8.00
10	WH124-1277M5	HOM	124	KRP5D2	SPLCE	5.00	2.00	2572.98	(29)	3.33	2.82	74.20	42.79	12.00	8.50
11	WH125-1288M5	HOM	125	KRP5D2	MSSE	4.67	2.33	1862.65	(55)	2.67	2.18	65.40	43.13	12.50	6.00
12	WH125-1290M5	WT	125	KRP5D2	MSSE	4.67	2.67	1641.34	(58)	2.67	2.26	69.30	37.56	14.00	6.00
13	WH131 -1336M5	WT	131	KRP5D2	STOP	5.00	2.00	2363.50	(36)	3.33	2.77	74.90	40.26	11.50	8.00
14	WH131 -1337M5	HET	131	KRP5D2	STOP	5.00	2.00	2447.61	(32)	3.33	2.97	67.00	42.82	11.00	6.50
15	WH131 -1342M5	HOM	131	KRP5D2	STOP	5.00	2.00	1975.23	(53)	3.00	2.21	82.70	35.81	12.00	9.00
16	WH131 -1346M5	HOM	131	KRP5D2	STOP	5.00	2.00	2330.24	(38)	3.33	2.93	65.80	41.20	11.50	7.50
17	WH14 -164M5	HOM	14	KRP2A2	MSSE	4.33	2.00	2931.82	(10)	3.33	3.62	75.50	37.99	12.50	5.50
18	WH14-172M5	WT	14	KRP2A2	MSSE	5.00	2.00	2771.59	(19)	3.33	3.34	76.90	37.25	12.00	6.50
19	WH16-185M5	HOM	16	KRP2A2	MSSE	5.00	2.00	3063.27	(7)	3.33	3.37	80.70	41.37	11.50	6.50
20	WH3 -2009M5	HOM	3	KRP2A2	SPLCE	5.00	2.00	3304.74	(2)	3.67	2.78	93.30	45.17	15.00	7.50
21	WH37 -423M5	HOM	37	KRP2B2	MSSE	4.67	1.67	2753.68	(21)	3.33	3.17	73.70	39.52	11.00	6.00
22	WH37 -428M5	WT	37	KRP2B2	MSSE	4.67	2.33	2551.87	(30)	3.33	2.58	77.50	40.53	11.00	5.50
23	WH38 -432M5	HOM	38	KRP2B2	SPLCE	4.33	1.67	1534.19	(59)	3.33	1.87	55.10	44.07	11.50	7.50
24	WH38 -433M5	HOM	38	KRP2B2	SPLCE	5.00	1.67	1926.94	(54)	3.33	1.99	77.30	43.89	13.00	7.00
25	WH39 -437M5	HET	39	KRP2B2	MSSE	5.00	1.67	2962.53	(8)	3.33	2.55	82.40	46.44	14.50	7.50
26	WH4 -2060M5	HOM	4	KRP2A2	SPLCE	5.00	2.00	3117.00	(6)	3.00	3.63	74.60	43.67	14.00	6.00
27	WH4 -2066M5	HOM	4	KRP2A2	SPLCE	5.00	2.00	2623.19	(26)	3.33	2.82	76.80	45.48	13.00	6.00
28	WH4 -41M5	HET	4	KRP2A2	SPLCE	4.67	2.00	2645.26	(24)	3.33	2.77	77.40	45.39	13.50	8.00
29	WH4 -53M5	HOM	4	KRP2A2	SPLCE	4.67	2.33	2782.79	(15)	3.00	3.20	72.30	45.01	13.50	7.00
30	WH40 -440M5	WT	40	KRP2B2	STOP	5.00	2.67	2360.30	(37)	2.67	2.62	66.90	48.78	13.50	8.50
31	WH40 -443M5	HOM	40	KRP2B2	STOP	4.33	2.67	2004.66	(51)	3.00	2.20	60.60	46.35	11.50	8.50

(continued)

No	Entry	Zygosity	Group	Variant	Type	Stand rate	Maturity rate	Grain yield		Peduncle rate	Head number	Kernel number	TKW	Spike length	Awn length
								Lbs/acre					g/1000	cm	cm
32	WH44 -460M5	HOM	44	KRP4B2	MSSE	4.33	2.00	2372.77	(35)	3.00	2.38	76.90	42.89	10.50	6.50
33	WH44 -461M5	HOM	44	KRP4B2	MSSE	4.67	2.00	2911.36	(11)	3.00	2.88	80.70	45.74	13.00	9.50
34	WH44 -461M5	HOM	44	KRP4B2	MSSE	4.67	2.00	2777.99	(16)	3.33	2.85	76.30	44.97	13.00	8.00
35	WH44 -462M5	HOM	44	KRP4B2	MSSE	4.00	2.00	2246.76	(42)	3.33	2.66	64.20	40.99	10.00	8.50
36	WH48 -487M5	WT	48	KRP4B2	MSSE	5.00	2.00	2938.54	(9)	3.00	3.48	78.00	46.63	13.00	8.00
37	WH52-513M5	HOM	52	KRP4B2	SPLCE	4.67	1.67	2245.16	(43)	3.33	2.67	59.40	31.35	11.00	6.50
38	WH52 -522M5	WT	52	KRP4B2	SPLCE	5.00	2.67	1498.37	(60)	3.00	2.26	65.40	32.97	11.50	6.50
39	WH52 -524M5	HOM	52	KRP4B2	SPLCE	5.00	2.00	1688.03	(57)	3.33	2.31	66.40	37.24	12.00	7.00
40	WH63 -634M5	HOM	63	KRP4D2	MSSE	5.00	2.00	2773.19	(17)	3.00	2.78	69.70	49.53	11.50	7.50
41	WH63 -635M5	HOM	63	KRP4D2	MSSE	5.00	2.00	2514.45	(31)	3.00	2.96	59.00	48.83	11.00	6.50
42	WH68 -676M5	HOM	68	KRP4D2	MSSE	5.00	2.00	3584.90	(1)	3.33	3.63	72.80	47.40	12.50	8.50
43	WH71 -2083M5	HOM	71	KRP4D2	STOP	5.00	2.00	2691.00	(23)	3.33	2.34	86.40	45.78	11.50	6.50
44	WH71 -708M5	HET	71	KRP4D2	STOP	5.00	2.00	2820.21	(13)	3.00	3.24	73.20	42.86	12.00	8.50
45	WH71 -712M5	HET	71	KRP4D2	STOP	5.00	1.67	2580.66	(27)	3.00	3.10	73.70	42.43	12.00	8.00
46	WH71 -713M5	HOM	71	KRP4D2	STOP	4.67	2.33	2808.69	(14)	3.00	2.90	66.80	45.58	13.00	7.00
47	WH71 -714M5	HOM	71	KRP4D2	STOP	5.00	2.33	2902.08	(12)	3.33	3.10	73.10	46.60	13.00	7.50
48	WH72 -724M5	HOM	72	KRP4D2	STOP	5.00	2.00	2763.92	(20)	3.33	2.86	71.50	47.42	11.50	6.50
49	WH72 -725M5	WT	72	KRP4D2	STOP	4.33	2.00	2173.52	(44)	3.33	2.31	76.00	49.16	12.50	7.50
50	WH72 -729M5	HOM	72	KRP4D2	STOP	5.00	2.00	2447.61	(33)	3.00	2.25	76.60	48.64	12.00	7.00
51	WH72 -734M5	HOM	72	KRP4D2	STOP	5.00	2.00	2730.02	(22)	3.33	2.90	73.10	47.05	10.50	6.00
52	WH73 -739M5	HOM	73	KRP4D2	SPLCE	5.00	2.00	2167.13	(45)	3.00	2.41	76.10	40.87	12.50	3.50
53	WH73 -740M5	HOM	73	KRP4D2	SPLCE	5.00	2.00	2151.13	(47)	3.33	2.56	73.30	42.25	12.00	6.00
54	WH73 -747M5	HOM	73	KRP4D2	SPLCE	5.00	2.00	2068.30	(50)	3.33	2.28	79.10	40.77	15.00	4.50
55	WH91 -1000M5	HOM	91	KRP5A2	MSSE	4.67	2.00	2257.00	(41)	2.67	3.53	72.10	34.98	13.00	9.00
56	WH91 -1003M5	WT	91	KRP5A2	MSSE	5.00	2.00	2576.18	(28)	3.00	2.60	89.50	39.92	13.50	7.00
57	WH91 -1004M5	WT	91	KRP5A2	MSSE	5.00	2.00	1817.24	(56)	2.67	3.02	72.80	31.98	14.00	7.00
58	WH91 -1005M5	HET	91	KRP5A2	MSSE	5.00	1.33	3256.13	(4)	3.00	2.83	83.90	46.63	12.50	9.00
59	WH91 -1008M5	WT	91	KRP5A2	MSSE	4.67	2.33	2000.18	(52)	3.00	2.44	74.10	39.26	13.50	7.50
60	Express	RP	1000	Express	CTL	5.00	2.00	3269.88	(3)	3.33	2.98	81.30	46.49	12.00	8.50
p < F						**	**	**		NA	*	**	**	*	**

(continued)

No	Entry	Zygosity	Group	Variant	Type	Stand rate	Maturity rate	Grain yield	Peduncle rate	Head number	Kernel number	TKW	Spike length	Awn length
								Lbs/acre				g/1000	cm	cm
CV (%)						7	17	18	13	17	10	5	10	13
LSD (0.05)						0.57	0.57	723.95	0.65	0.95	14.96	4.16	2.50	1.87
Mean						4.86	2.07	2493.27	3.15	2.81	73.24	42.58	12.36	7.17

Example 6

Rice krp TILLING® mutant

[0268] A rice TILLING® library was screened for mutations in rice *KRPs 1, 2, 4 and 5* (SEQ ID NOs: 100-107). A mutant containing a premature stop codon in OsKRP4 was identified (Table 25). M3 seeds from two distinct M2 sibling plants heterozygous for the R167* mutation were planted in the greenhouse and genotyped to identify plants homozygous for the R167* allele or homozygous for the wild type allele. Homozygous R167* and wild type plants were selfed to obtain homozygous mutant and wild type M4 seed. Additionally, homozygous R167* plants were backcrossed to the recurrent Cypress parent to obtain F1 seed.

[0269] Seed number and seed weight were determined for the homozygous R167* and homozygous wild type M4 seed (Table 26). There is an indication that RI527 R167* homozygotes have at least a general increase in thousand kernel weight (TKW) compared to their wild type siblings (Table 27), and the RI526 R167* homozygotes may be better than their control for seed number. These same measurements will be taken again on seed from successive backcrossed generations.

[0270] F1 and M4 seeds were planted in the greenhouse in the next cycle. F1 plants were genotyped to confirm the heterozygosity of R167*. Heterozygous R167* plants are backcrossed a second time to the recurrent parent to obtain BC1F1 seed. Simultaneously, heterozygous R167* F1 plants are selfed to obtain segregating F2 seed. Homozygous R167* F2 plants are identified by genotyping and seed expanded to F3 for field trials.

[0271] BC1F1 seed are grown in the greenhouse and the plants genotyped to identify ones heterozygous for the R167* allele or wild type allele. Heterozygous R167* plants are backcrossed to obtain BC2F1 and selfed to obtain BC1F2. Homozygous R167* BC1F2 plants are identified by genotyping and seed expanded to BC1F3 for field trials. Backcrossing may be continued to the BC3 or BC4 level. Throughout the backcrossing and selfing, wild type siblings are carried forward to serve as controls. Backcrosses are also done to Nipponbare to move the mutation into short grain rice.

Table 25. *Oryza sativa* KRP4 TILLING® Mutant in breeding program

RI group	Gene	Nuc Change	Effect-from beginning Met
526^	KRP4_2-3§	C593T	R167*
527	KRP4_2-3	C593T	R167*

^The two RI groups represent two distinct M2 sibling plants heterozygous for the R167* mutation.
§The designation "2-3" indicates that exons 2-3 of OsKRP4 were TILL'ed.

Table 26. Seed number and seed weight for OsKRP4 R167* M4 seed

Plant ID	# of seeds	Total seed weight (g)	weight/seed (g)	TKW (g)
526 HOMO #12	178	3.76	0.021	21.1
526 HOMO #13	164	3.39	0.021	20.7
526 HOMO #15	33	0.69	0.021	20.9
526 HOMO #21	258	4.91	0.019	19.0
526 HOMO #22	220	5.14	0.023	23.4
526 HOMO #27	261	5.90	0.023	22.6
526 HOMO #33	209	4.43	0.021	21.2
526 HOMO #35	141	2.81	0.020	19.9
526 HOMO #37	155	3.33	0.021	21.5
526 HOMO #4	169	3.77	0.022	22.3
526 HOMO #41	245	5.33	0.022	21.8
526 HOMO #8	151	2.94	0.019	19.5
526 WT #14	72	1.39	0.019	19.3
526 WT #19	161	3.43	0.021	21.3

(continued)

Plant ID	# of seeds	Total seed weight (g)	weight/seed (g)	TKW (g)
526 WT #23	229	5.10	0.022	22.3
526 WT #24	165	3.74	0.023	22.7
526 WT #25	273	5.98	0.022	21.9
526 WT #3	181	3.70	0.020	20.4
526 WT #30	165	3.69	0.022	22.4
526 WT #32	121	2.51	0.021	20.7
526 WT #36	1	0.02	0.020	20.0
526 WT #6	137	2.80	0.020	20.4
526 WT #7	260	5.48	0.021	21.1
526 WT #9	203	4.40	0.022	21.7
527 HOMO #1	197	4.50	0.023	22.8
527 HOMO #14	220	4.93	0.022	22.4
527 HOMO #2	215	5.13	0.024	23.9
527 HOMO #20	4	0.11	0.028	27.5
527 HOMO #24	179	4.40	0.025	24.6
527 HOMO #35	142	3.06	0.022	21.5
527 HOMO #38	37	0.61	0.016	16.5
527 HOMO #6	142	2.96	0.021	20.8
527 WT #10	201	4.67	0.023	23.2
527 WT #11	6	0.10	0.017	16.7
527 WT #13	38	0.70	0.018	18.4
527 WT #19	12	0.20	0.017	16.7
527 WT #23	261	6.19	0.024	23.7
527 WT #30	248	5.52	0.022	22.3
527 WT #32	189	4.20	0.022	22.2
527 WT #34	187	4.32	0.023	23.1
527 WT #36	68	1.54	0.023	22.6
527 WT #7	189	3.85	0.020	20.4
Nipponbare	10	0.24	0.024	24.0
Cypress	10	0.23	0.023	23.0

Table 27. Mean seed number and seed weight for OsKRP4 genotypes

Plant ID	Mean seed number	Mean TKW (g)
526 homo	182.00	21.15
526 wild type	164.00	21.18
527 homo	142.00	22.51
527 wild type	139.90	20.93

Table 28. Primer sequences used to generate mutant Krps

Wheat Krp	forward primer 5'->3'	reverse primer 5'->3'
Krp1A (E212K)	Cgaagagttctttgcggcggctaaagaggcggaagcacgccg (SEQ ID NO: 34)	Cggcgtgcttccgcctctttagccgccgcaaagaactcttcg (SEQ ID NO: 35)
Krp1A (P232L)	Cgacgttgcacgcggcgtgcttctggattccggtcgctatgag (SEQ ID NO: 36)	Ctcatagcgaccggaatccagaagcacgccgcgtgcaacgtcg (SEQ ID NO: 37)
Krp1A (G236S)	Cggcgtgcctctggattccagtcgctatgagtggaccccggc (SEQ ID NO: 38)	Gccgggggtccactcgtagcgactggaatccagaggcacgccg (SEQ ID NO: 39)
ICrp1A (W240*)	Ggattccggtcgctatgagtgaaccccggcagtttccagcag (SEQ ID NO: 40)	Ctgctggaaactgccgggggttcactcatagcgaccggaatcc (SEQ ID NO: 41)
Krp2D (P228S)	Ccgtgcccgtgcgcgtatgtcaccggcagcggaaatcgacg (SEQ ID NO: 42)	Cgtcgatttccgctgccggtgacatacgcgcacgggcacgg (SEQ ID NO: 43)
Krp2D (A238V)	Cgacgagttttcgcggttgcggagaaagcccaggcagag (SEQ ID NO: 44)	Ctctgcctgggctttctccgcaaccgcgaaaaactcgtcg (SEQ ID NO: 45)
Krp2D (A239T)	Cgacgagttttcgcggctacggagaaagcccaggcagagcg (SEQ ID NO: 46)	Cgctctgcctgggctttctccgtagccgcgaaaaactcgtcg (SEQ ID NO: 47)
Krp2D (D254N)	Cgccgcgaagtataacttaatgtggcccgtggcgttccgctg (SEQ ID NO: 48)	Cagcggaacgccacgggccacattaaagttatacttcgcggcg (SEQ ID NO: 49)
Krp2D (R257C)	Ctttgatgtggcctgtggcgttccgctgaatgctggtcgc (SEQ ID NO: 50)	Gcgaccagcattcagcggaacgccacaggccacatcaaag (SEQ ID NO: 51)
Krp4A (W186*)	Gccaggtcgttatgaataggtcaagctggactaactcgag (SEQ ID NO: 52)	Ctcgagttagtccagcttgacctattcataacgacctggc (SEQ ID NO: 53)
Krp5A (G200E)	Ctcgtggctgcccgctgccggatcgttacgagtggaccgtc (SEQ ID NO: 54)	Gacggtccactcgtaacgatccggcagcgggcagccacgag (SEQ ID NO: 55)
Krp5A (G200R)	Ctcgtggctgcccgctgccgaggcgttacgagtggaccgtc (SEQ ID NO: 56)	Gacggtccactcgtaacgcctcggcagcgggcagccacgag (SEQ ID NO: 57)
Krp5A (W199*)	Gctgccgggtcgttacgagtgaaccgtcctggactgctaactc (SEQ ID NO: 58)	Gagttagcagtccaggacggttcactcgtaacgacccggcagc (SEQ ID NO: 59)

Example 7

Mutations of *Glycine max KRP* Genes identified in TILLING®

[0272] Candidate *Glycine max* (soybean) KRP genes were searched on the Phytozome and nine KRP genes (SEQ ID NOs: 111-128) were identified. A soy TILLING® library was screened for mutations in these soy KRPs.

[0273] Representative mutations in *Glycine max KRPs* are displayed in Tables 29 to 37 below (* indicates the mutation results in a stop codon). This is only a representative list and should not be construed to be limiting in any way.

Table 29. *Glycine max* Gm 0003x00821 representative TILLING® Mutants

Gene	Nuc Change^	Effect from beginning Met	Mutation Score
KRP Gm0003x00821	C533T	A17V	Missense
KRP Gm0003x00821	C542T	A20V	Missense
KRP Gm0003x00821	G610A	A43T	Missense
KRP Gm0003x00821	G628A	G49R	Missense
KRP Gm0003x00821	C650T	S56F	Missense
KRP Gm0003x00821	G655A	A58T	Missense
KRP Gm0003x00821	A674T	N64I	Missense
KRP Gm0003x00821	G721A	E80K	Severe Missense
KRP Gm0003x00821	G873A	D92N	Missense
KRP Gm0003x00821	A1274G	Splice Junction	Splice
KRP Gm0003x00821	G1275A	Splice Junction	Splice
KRP Gm0003x00821	A1277T	R102*	Nonsense
KRP Gm0003x00821	C1320T	S116F	Severe Missense
KRP Gm0003x00821	G1328A	E119K	Missense
KRP Gm0003x00821	T1332A	V120E	Missense
KRP Gm0003x00821	A1365T	K131I	Missense
KRP Gm0003x00821	C1392T	T140M	Severe Missense
KRP Gm0003x00821	G1400A	E143K	Severe Missense
KRP Gm0003x00821	G1421A	A150T	Missense
KRP Gm0003x00821	A1428G	E152G	Severe Missense
^Nucleotide numbering is dependent upon the location of TILLING® primers.			

Table 30. *Glycine max* Gm0013 representative TILLING® Mutants

Gene	Nuc Change^	Effect from beginning Met	Mutation score
KRP Gm0013	C574T	S28F	Severe Missense
KRP Gm0013	C622T	S44F	Missense
KRP Gm0013	T648C	S53P	Severe Missense
KRP Cm0013	C649T	S53F	Severe Missense
KRP Gm0013	A685T	Q65L	Missense
KRP Gm0013	C727T	S79F	Severe Missense
KRP Gm0013	T732G	C81G	Severe Missense

(continued)

Gene	Nuc Change^	Effect from beginning Met	Mutation score
KRP Gm0013	T732A	C81S	Severe Missense
KRP Gm0013	C736T	S82F	Severe Missense
KRP Gm0013	A738T	S83C	Severe Missense
KRP Gm0013	G783A	D98N	Severe Missense
KRP Gm0013	G792A	Splice Junction	Splice
KRP Gm0013	G1315C	E121Q	Missense
KRP Gm0013	G1320A	M122I	Missense
KRP Gm0013	A1334T	E127V	Missense
KRP Gm0013	G1360A	E136K	Missense
KRP Gm0013	C1448T	A165V	Missense
KRP Gm0013	C1462T	Q170*	Nonsense
KRP Gm0013	G1671A	G189R	Severe Missense
KRP Gm0013	A1690T	Q195L	Missense
^Nucleotide numbering is dependent upon the location of TILLING® primers.			

Table 31. *Glycine max* Gm0043 representative TILLING® Mutants

Gene	Nuc Change^	Effect from beginning Met	Mutation score
KRP Gm0043_1-2§	A582T	Q42L	Missense
KRP Gm0043_1-2	T585A	F43Y	Severe Missense
KRP Gm0043_1-2	T586A	F43L	Severe Missense
KRP Gm0043_1-2	G599A	V48I	Missense
KRP Gm0043_1-2	G638A	V61I	Missense
KRP Gm0043_1-2	G645A	G63D	Missense
KRP Gm0043_1-2	G647T	D64Y	Missense
KRP Gm0043_1-2	A720T	Q88L	Missense
KRP Gm0043_1-2	T904A	Y102N	Missense
KRP Gm0043_3-4€	C1194T	S113F	Severe Missense
KRP Gm0043_3-4	C1215T	A120V	Missense
KRP Gm0043_3-4	C1227T	A124V	Missense
KRP Gm0043_3-4	C1235T	R127W	Missense
KRP Gm0043_3-4	G1259A	A135T	Missense
KRP Gm0043_3-4	G1289A	E145K	Severe Missense
KRP Gm0043_3-4	G1302A	R149Q	Missense
KRP Gm0043_3-4	C1513T	P165L	Severe Missense
KRP Gm0043_3-4	G1521A	G168S	Severe Missense

(continued)

Gene	Nuc Change^	Effect from beginning Met	Mutation score
KRP Gm0043_3-4	C1524T	R169C	Severe Missense
^Nucleotide numbering is dependent upon the location of TILLING® primers. §The designation "1-2" indicates that exons 1-2 of soy KRP Gm0043 were TILLed. €The designation "3-4" indicates that exons 3-4 of soy KRP Gm0043 were TILLed.			

Table 32. *Glycine max* Gm0053 representative TILLING® Mutants

Gene	Nuc Change^	Effect from beginning Met	Mutation score
KRP Gm0053	G629A	R10Q	Missense
KRP Gm0053	C728T	S43F	Severe Missense
KRP Gm0053	C758T	A53V	Missense
KRP Gm0053	C814A	Q72K	Missense
KRP Gm0053	C866T	S89F	Severe Missense
KRP Gm0053	A868T	S90C	Severe Missense
KRP Gm0053	G869A	S90N	Severe Missense
KRP Gm0053	C1125T	T117M	Severe Missense
KRP Gm0053	G1408A	R127K	Missense
KRP Gm0053	A1409T	R127S	Severe Missense
KRP Gm0053	G1527A	E167K	Severe Missense
^Nucleotide numbering is dependent upon the location of TILLING® primers.			

Table 33. *Glycine max* Gm0087 representative TILLING® Mutants

Gene	Nuc Change^	Effect from beginning Met	Mutation score
KRP Gm0087_2-3§	G3098A	R137K	Severe Missense
KRP Gm0087_2-3	G3178A	A164T	Missense
KRP Gm0087_2-3	G3191A	R168Q	Missense
KRP Gm0087_2-3	G3194A	R169K	Severe Missense
KRP Gm0087_2-3	C3227T	T180I	Severe Missense
KRP Gm0087_2-3	G3289A	E201K	Severe Missense
KRP Gm0087_2-3	C3424T	L213F	Severe Missense
KRP Gm0087_2-3	G3430A	G215R	Severe Missense
KRP Gm0087_2-3	G3445A	E220K	Missense
^Nucleotide numbering is dependent upon the location of TILLING® primers. §The designation "2-3" indicates that exons 2-3 of soy KRP Gm0087 were TILLed.			

Table 34. *Glycine max* Gm0102 representative TILLING® Mutants

Gene	Nuc Change^	Effect from beginning Met	Mutation score
KRP Gm0102_3-4§	C722T	S120F	Missense
KRP Gm0102_3-4	G724A	G121R	Severe Missense

(continued)

Gene	Nuc Change^	Effect from beginning Met	Mutation score
KRP Gm0102_3-4	G733A	E124K	Missense
KRP Gm0102_3-4	A734T	E124V	Missense
KRP Gm0102_3-4	C743T	A127V	Missense
KRP Gm0102_3-4	C770T	A136V	Missense
KRP Gm0102_3-4	T776A	L138Q	Missense
KRP Gm0102_3-4	C790T	P143S	Severe Missense
KRP Gm0102_3-4	C794T	P144L	Missense
KRP Gm0102_3-4	G799A	A146T	Missense
KRP Gm0102_3-4	C800T	A146V	Missense
KRP Gm0102_3-4	A809T	E149V	Severe Missense
KRP Gm0102_3-4	G811A	E150K	Missense
KRP Gm0102_3-4	G848A	R162Q	Severe Missense
KRP Gm0102_3-4	G1154A	D174N	Severe Missense
KRP Gm0102_3-4	C1160T	P176S	Severe Missense
KRP Gm0102_3-4	G1166A	E178K	Severe Missense
KRP Gm0102_3-4	A1179T	Q182L	Severe Missense
^Nucleotide numbering is dependent upon the location of TILLING® primers. §The designation "3-4" indicates that exons 3-4 of soy KRP Gm0102 were TILLed.			

Table 35. *Glycine max* Gm0119 representative TILLING® Mutants

Gene	Nuc Change^	Effect from beginning Met	Mutation score
KRP Gm0119_2-3§	G2664A	Splice Junction	Splice
KRP Gm0119_2-3	C2717T	H133Y	Missense
KRP Gm0119_2-3	G2857A	Splice Junction	Splice
KRP Gm0119_2-3	G3040A	R193Q	Severe Missense
KRP Gm0119_2-3	A3010T	D183V	Severe Missense
^Nucleotide numbering is dependent upon the location of TILLING® primers. §The designation "2-3" indicates that exons 2-3 of soy KRP Gm0119 were TILLed.			

Table 36. *Glycine max* Gm0151 representative TILLING® Mutants

Gene	Nuc Change^	Effect from beginning Met	Mutation score
KRP Gm0151_2-3§	A2306T	R122W	Severe Missense
KRP Gm0151_2-3	C2367T	T142I	Severe Missense
KRP Gm0151_2-3	G2399A	E153K	Missense
KRP Gm0151_2-3	G2412A	R157K	Missense
KRP Gm0151_2-3	G2485A	M181I	Missense

(continued)

Gene	Nuc Change^	Effect from beginning Met	Mutation score
KRP Gm0151_2-3	A2647T	E191D	Missense
^Nucleotide numbering is dependent upon the location of TILLING® primers. §The designation "2-3" indicates that exons 2-3 of soy KRP Gm0151 were TILLed.			

Table 37. *Glycine max* Gm0067 representative TILLING® Mutants

Gene	Nuc Change^	Effect from beginning Met	Mutation score
KRP Gm0067_1-2§	G631A	V4I	Severe Missense
KRP Gm0067_1-2	G652A	A11T	Severe Missense
KRP Gm0067_1-2	C671T	S17F	Severe Missense
KRP Gm0067_1-2	T679A	S20T	Missense
KRP Gm0067_1-2	C682T	P21S	Missense
KRP Gm0067_1-2	G689A	R23K	Severe Missense
KRP Gm0067_1-2	C739T	P40S	Missense
KRP Gm0067_1-2	G748A	E43K	Missense
KRP Gm0067_1-2	C773T	P51L	Severe Missense
KRP Gm0067_1-2	C776T	A52V	Severe Missense
KRP Gm0067_1-2	T778A	S53T	Severe Missense
KRP Gm0067_1-2	C788T	S56F	Severe Missense
KRP Gm0067_1-2	G818A	R66Q	Missense
KRP Gm0067_1-2	A826T	K69*	Nonsense
KRP Gm0067_1-2	T832A	S71T	Missense
KRP Gm0067_1-2	C833T	S71L	Missense
KRP Gm0067_1-2	G841A	E74K	Severe Missense
KRP Gm0067_1-2	T845A	Splice Junction	Splice
^Nucleotide numbering is dependent upon the location of TILLING® primers. §The designation "1-2" indicates that exons 1-2 of soy KRP Gm0067 were TILLed.			

Example 8

Preliminary field evaluation results on F2:3 generation wheat KRP TILLING® mutants

[0274] The objective was to assess the feasibility of obtaining reliable data from field evaluations of KRP TILLING® lines in spring wheat and to determine the performance of homozygous (F2:3) mutant lines.

Materials & Methods

[0275] Five field experiments were conducted during the growing season in Fort Collins, CO and Bozeman, MT (Table 38) in which a number of mutant lines were evaluated (Table 39).

Table 38. Sites of evaluation of spring wheat mutant materials

Location	Water regime	Planting date
Bozeman, MT	Partially irrigated	May 5

(continued)

Location	Water regime	Planting date
Fort Collins, CO	Dryland	April 11

Table 39. Mutant materials evaluated in and Bozeman, MT and Fort Collins, CO

Experiment	Number of Entries	WH group	Gene	Genome	Type	Zygosity	F1 families
1	39 + Check	4	KRP2	A	Splice	Homo WT	39D03 41D05 42D06
2	54 + Check	11	KRP2	A	Splice	Homo WT	9051_A10
3	37 + Check	71	KRP4	D	Stop	Homo WT	148E94 148E95
4	12 + Check	4/38	KRP2	A/B	Splice/Splice	Homo WT	9052_E03
5	17 + Check	44/71	KRP4	B/D	Miss/Stop	Homo WT	9052_G02

Major results

[0276] Mutant materials yielded significantly lower than the check cultivar (Figure 7). This is not surprising, since the mutant lines had been crossed only once to the Express parent and may still have deleterious lesions from the EMS mutagenesis compared to the unmutagenized Express cultivar. Notably, the yield difference between mutant line and check was not significantly different for the KRP4D Stop and the KRP2A Splice WH4 under high yielding conditions (Montana). The impact of mutation appeared to be less for single mutants than for double mutants (Figure 7). Mutant KRP2AB Splice/Splice had the highest reduction in yield (46% and 39% reductions in MT & CO), and mutant KRP4D Stop had the lowest reduction in yield (4% & 11% reductions in MT & CO).

[0277] Between zygotic groups, the wild type (WT) lines tended to yield higher than their mutant counterparts (Homo), although under high yielding conditions (Montana), the opposite appeared to be the case for the double mutants KRP4BD missense/stop and KRP2AB splice/splice (Figure 8). Among Homo groups, mutants KRP4D Stop and KRP2A Splice WH11 showed the highest relative yield performance. Homozygous mutant KRP2A Splice WH11 performed consistently closer to the average yield of its WT counterpart under both CO and MT growing conditions.

[0278] For KRP TILLING® mutants in which F1 sister lines were included, line '149E05' within the KRP4D Stop mutant showed a differential response: homozygous lines yielded above or at least the same as their wild type counterparts (Figure 9).

[0279] The relative grain yield response between the wild type and homozygous zygotic groups can be expressed by the WT/Homo ratio for grain yield and various yield components. Putative mechanisms associated with the relative grain yield response could be discerned (Table 40). In Montana the largest positive effect on yield (WT/Homo ratio = 0.92) was observed in the 'KRP4BD Missense/Stop' double mutant and was driven mainly by spike number, thousand kernel weight (TKW) and plant height, whereas the greatest negative effect on yield (WT/Homo ratio = 1.09) was observed in the 'KRP2A Splice WH4' mutant, line 42D06, driven mainly by disruptions in kernel number. In Colorado the largest positive effect on yield (WT/Homo ratio = 0.93) was observed in the 'KRP4D Stop' mutant, line 149E05, and was driven mainly by TKW and plant height, whereas the largest negative effect on yield (WT/Homo ratio = 1.18) was observed in the variant 'KRP2A Splice WH4' mutant, line 42D06, driven mainly by disruptions in spike number and kernel number.

Table 40. Relative performance ratio between wild type and homozygous zygotic groups for the mutants evaluated. Bozeman, MT, Fort Collins, CO.

Gene	F1 line	Spike number Spikes/plant	Kernel number Kernels/spike	TKW g/1000	Plant height inches	Grain yield Lb/ac
			Montana			
KRP2A Splice	39D03	1.00	1.03	1.01	1.02	1.02
KRP2A Splice	41D05	0.99	1.08	1.01	1.01	1.08
KRP2A Splice	42D06	1.00	1.11	0.98	0.99	1.09
KRP2A Splice	9051_A10	1.05	1.02	1.00	1.01	1.03

(continued)

Gene	F1 line	Spike number Spikes/plant	Kernel number Kernels/spike	TKW g/1000	Plant height inches	Grain yield Lb/ac
			Montana			
KRP4D Stop	148E04	1.12	1.05	1.01	0.99	1.04
KRP4D Stop	149E05	**0.97**	**0.97**	1.01	**0.98**	**1.00**
KRP2AB Splice/Splice	9052_E03	1.01	1.03	**0.99**	**0.96**	**0.93**
KRP4BD Miss/Stop	9052_G02	**0.97**	0.99	**0.96**	**0.91**	**0.92**
			Colorado			
KRP2A Splice	39D03	1.09	1.03	0.97	1.00	1.09
KRP2A Splice	41D05	1.04	1.03	1.01	0.99	1.07
KRP2A Splice	42D06	1.06	1.10	1.01	1.01	1.18
KRP2A Splice	9051_A10	1.04	1.01	0.99	1.02	1.04
KRP4D Stop	148E04	1.06	1.03	0.97	1.00	1.05
KRP4D Stop	149E05	0.99	0.99	**0.96**	**0.98**	**0.93**
KRP2AB Splice/Splice	9052_E03	1.02	0.98	1.04	0.98	1.03
KRP4BD Miss/Stop	9052_G02	--	--	--	--	--

Example 9

Identification and Retrieval of other wheat KRPs

[0280] Rice serves as a diploid model cereal species for wheat. In rice two additional KRP genes are present and expressed: KRP3 and KRP6. KRP3 is expressed at a very specific time point in seed development, while KRP6 seems to be expressed throughout seed development in rice (Mizutani et al 2010). In order to complete the portfolio of wheat KRP TILLING® mutants, other wheat KRPs are identified for future TILLING®.

[0281] The rice KRP6 DNA sequence was used to identify a wheat KRP6 EST from a BLAST search at NCBI. Then longer wheat KRP6 sequences were pulled out from a wheat genomic sequences database (WheatBP) at Univeristy of Bristol using the wheat KRP6 EST from NCBI. The wheat database had 5X coverage of the Chinese Spring variety. The wheat TILLING® library is based on the variety Express, but not many polymorphisms are expected between the two varieties.

[0282] Wheat KRP6 sequences ranged from ~300 to 800bp. ContigExpress from Vector NTI (Invitrogen) was used to assemble the various reads into contigs. Reads were assembled into three contigs, which represented TaKRP6A, TaKRP6B and TaKRP6D. Further characterization is done to determine which contig corresponds to which genome. Exon/intron boundaries for the wheat KRP6 genes were deduced based on the rice KRP6 sequence. The deduced coding sequences were translated in Vector to generate protein sequences. Table 41 gives a comparison of KRP6 gene, cDNA and protein lengths for wheat, rice, Brachypodium, corn and sorghum.

Table 41. Comparison of wheat, rice, Brachypodium, corn and sorghum KRP6 sequences

	Total length of sequence available (bp)	5'	3'	Length Start-Stop (3 exons, 2 introns)	cDNA	Protein
Ta KRP6-A (wheat)	1678 (contig of 17 reads)	94	613	971	264	87 aa
Ta KRP6-B (wheat)	1834 (contig of 18 reads)	164	729	941	264	87 aa
Ta KRP6-D (wheat)	2141 (contig of 12 reads)	369	867	905	270	89 aa

(continued)

	Total length of sequence available (bp)	5'	3'	Length Start-Stop (3 exons, 2 introns)	cDNA	Protein
Os KRP6 (rice)	1565			1269	261	87 aa
Brachypodium KRP6	1377			891	258	86 aa
Zm KRP6 (corn)	1458			679	249	83 aa
Sb KRP6 (sorghum)	1253			995	258	86 aa

[0283] Using the sequences identified, three primer pairs were designed (Table 42). No wheat KRP6 amplification products were obtained from wheat genomic DNA using proofreading Phusion polymerase at annealing temperatures ranging from 55°C to 65°C. Using SureBand PCR Optimization kit (Bioline) at 58°C annealing temperature and one of the twelve buffers from the kit yielded a faint band with TaKRP6 49F and TaKRP6 258R. Repeating the amplification with the same buffer and primer pair, and testing a range of annealing temperatures from 54°C to 66.5°C, yielded strong, unique bands at 60°C and higher. The band was cut from the gel and the DNA purified and cloned using TA cloning and Zero Blunt cloning (Invitrogen). Colony screening showed several positive transformants. Minipreps were done on cultures from 30 colonies and all were sequenced from both ends of the insert using M13F and M13R primers.

[0284] Sequences obtained were first assembled into contigs (M13F and M13R sequences from each of the 30 clones) and then those contigs were assembled into larger contigs. After assembly and alignments, it was determined that all three versions of wheat KRP6 had been cloned: 14 clones for KRP6A, 1 clone for KRP6B and 14 clones for KRP6D.

Table 42. Primers to amplify wheat KRP6

Primer	Sequence (5' to 3')	SEQ ID NO
TaKRP6 START	atg gcc gcc acc gcc gcg gc	147
TaKRP6 nearSTOP	tcg gac cca ctc gta ccg ccc	148
TaKRP6 upstr	cct aat cct atc gtt atc tcc tcc ca	149
TaKRP6 downstr	cta cga gac aat gta cac aga taa cg	150
TaKRP6 49F	agc tgc agc aag ggc gag a	151
TaKRP6 258R	cct cac tcg gac cca ctc gta	152

[0285] The rice KRP3 cDNA sequence was used in a BLAST search at NCBI. Several wheat ESTs were identified. The wheat KRP6 sequences identified above were used to search for wheat KRP contigs in the cereal database. Using this approach, a contig was identified in the wheat database that looked similar but was not identical to KRP6 or other known wheat KRPs.

Example 10

TILLING® of other wheat KRPs and characterization of mutants

[0286] Design and validation of genome-specific TILLING® primers and TILLING® of other wheat KRPs, such as wheat KRP6, is done as described in Materials and Methods and Example 1 above. The KRP TILLING® mutants obtained are backcrossed, introgressed into other wheat varieties and combined with other wheat KRP mutants as described in Example 3 above. Determination of yield, yield components and agronomic characteristics is as described in Examples 3, 5 and 8 above.

[0287] Unless defined otherwise, all technical and scientific terms herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials, similar or equivalent to those described herein, can be used in the practice or testing of the present invention, the preferred methods and materials are described herein. All publications, patents, and patent publications cited are incorporated by reference herein in their entirety for all purposes.

[0288] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

[0289] While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope of the appended claims.

REFERENCES

[0290]

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[0291] Embodiment 1. A plant cell, plant part, plant tissue culture or whole plant comprising at least one *Kinase Inhibitor Protein (KIP) Related Protein (KRP)* gene, wherein the function of one or more copies of the *KRP* gene is disrupted, and wherein the disruption is due to one or more nucleotide changes of a wild type KRP gene as set forth in Tables 2-12, 25 and 29-37.

[0292] Embodiment 2. The plant cell, plant part, plant tissue culture or whole plant of embodiment 1, wherein the plant is a monocotyledonous plant and the one or more nucleotide changes of the wild type KRP gene are as set forth in Tables 2-12 and 25.

[0293] Embodiment 3. The plant cell, plant part, plant tissue culture or whole plant of embodiment 2, wherein the monocotyledonous plant is a species in the *Triticeae* tribe and the one or more nucleotide changes of the wild type KRP gene are as set forth in Tables 2-12.

[0294] Embodiment 4. The plant cell, plant part, plant tissue culture or whole plant of embodiment 3, wherein the plant

in the *Triticeae* tribe is a plant in the *Triticum* genus.

[0295] Embodiment 5. The plant cell, plant part, plant tissue culture or whole plant of embodiment 4, wherein the plant in the *Triticum* genus is wheat.

[0296] Embodiment 6. The plant cell, plant part, plant tissue culture or whole plant of embodiment 5, wherein the wheat plant is tetraploid or hexaploid.

[0297] Embodiment 7. The plant cell, plant part, plant tissue culture or whole plant of embodiment 2, wherein the monocotyledonous plant is a species in the *Oryzeae* tribe and the one or more nucleotide changes of the wild type KRP gene are as set forth in Table 25.

[0298] Embodiment 8. The plant cell, plant part, plant tissue culture or whole plant of embodiment 7, wherein the plant in the *Oryzeae* tribe is a plant in the *Oryza* genus.

[0299] Embodiment 9. The plant cell, plant part, plant tissue culture or whole plant of embodiment 8, wherein the plant in the *Oryza* genus is rice.

[0300] Embodiment 10. The plant cell, plant part, plant tissue culture or whole plant of embodiment 1, wherein the plant is a dicotyledonous plant and the one or more nucleotide changes of the wild type KRP gene are as set forth in Tables 29-37.

[0301] Embodiment 11. The plant cell, plant part, plant tissue culture or whole plant of embodiment 10, wherein the dicotyledonous plant is a species in the *Fabaceae* family.

[0302] Embodiment 12. The plant cell, plant part, plant tissue culture or whole plant of embodiment 11, wherein the plant in the *Fabaceae* family is soybean.

[0303] Embodiment 13. The plant cell, plant part, plant tissue culture or whole plant of embodiment 12, wherein the plant is *Glycine max.*

[0304] Embodiment 14. A wheat plant cell, plant part, plant tissue culture or whole plant comprising at least one *Kinase Inhibitor Protein (KIP) Related Protein (KRP)* gene, wherein the function of one or more copies of the *KRP* gene is disrupted, and wherein the disruption is due to one or more nucleotide changes of a wild type KRP gene selected from the group consisting of *TaKRP1, TaKRP2, TaKRP4, TaKRP5* and *TaKRP6.*

[0305] Embodiment 15. The wheat plant cell, plant part, plant tissue culture or whole plant of embodiment 14, wherein wheat is tetraploid wheat plant and the disrupted KRP gene is *TaKRP1A, TaKRP1B, TaKRP2A, TaKRP2B, TaKRP4A, TaKRP4B, TaKRP5A, TaKRP5B, TaKRP6A,* and/or *TaKRP6B,* and wherein the *KRP* in the hexaploid wheat is *TaKRP1A, TaKRP1B, TaKRP1D, TaKRP2A, TaKRP2B, TaKRP2D, TaKRP4A, TaKRP4B, TaKRP4D, TaKRP5A, TaKRP5B, TaKRP5D, TaKRP6A, TaKRP6B,* and/or *TaKRP6D.*

[0306] Embodiment 16. A rice plant cell, plant part, plant tissue culture or whole plant comprising at least one *Kinase Inhibitor Protein (KIP) Related Protein (KRP)* gene, wherein the function of one or more copies of the *KRP* gene is disrupted, and wherein the disruption is due to one or more nucleotide changes of a wild type KRP gene selected from the group consisting of *OsKRP1, OsKRP2, OsKRP4* and *OsKRP5.*

[0307] Embodiment 17. A soy plant cell, plant part, plant tissue culture or whole plant comprising at least one *Kinase Inhibitor Protein (KIP) Related Protein (KRP)* gene, wherein the function of one or more copies of the *KRP* gene is disrupted, and wherein the disruption is due to one or more nucleotide changes of a wild type KRP gene selected from the group consisting of Gm0003x00821, Gm0013x00399, Gm0043, Gm0053x00526, Gm0087x00306, Gm0102x00087, Gm0119x00131, Gm0151x00019, and Gm0067x00001.

[0308] Embodiment 18. A method of increasing organ weight, organ size, organ number and/or yield of a plant in the *Triticeae* tribe, the *Oryzeae* tribe, or the *Fabaceae* family comprising disrupting one or more *KRPs* in the plant.

[0309] Embodiment 19. The method of embodiment 18, wherein the organ is seed.

[0310] Embodiment 20. The method of embodiment 19, wherein the plant in the *Triticeae* tribe is a plant in the *Triticum* genus, wherein the plant in the *Oryzeae* tribe is a plant in the *Oryza* genus, and wherein the plant in the *Fabaceae* family is in the *Glycine* genus.

[0311] Embodiment 21. The method of embodiment 20, wherein the plant in the *Triticum* genus is a wheat plant, wherein the plant in the *Oryza* genus is a rice plant, and wherein the plant in the *Glycine* genus is a soybean plant. Embodiment 22. The method of embodiment 18, wherein the *KRP* in the wheat is *TaKRP1, TaKRP2, TaKRP4, TaKRP5,* or *TaKRP6,* the *KRP* in the rice is *OsKRP1, OsKRP2, OsKRP4, or OsKRP5,* and the *KRP* in the soybean is Gm0003x00821, Gm0013x00399, Gm0043, Gm0053x00526, Gm0087x00306, Gm0102x00087, Gm0119x00131, Gm0151 x00019, or Gm0067x00001.

[0312] Embodiment 23. The method of embodiment 22, wherein the *KRP* in a tetraploid wheat plant is *TaKRP1A, TaKRP1B, TaKRP2A, TaKRP2B, TaKRP4A, TaKRP4B, TaKRP5A, TaKRP5B,* or *TaKRP6,* and wherein the *KRP* in a hexaploid wheat is *TaKRP1A, TaKRP1B, TaKRP1D, TaKRP2A, TaKRP2B, TaKRP2D, TaKRP4A, TaKRP4B, TaKRP4D, TaKRP5A, TaKRP5B, TaKRP5D, TaKRP6A, TaKRP6B,* and *TaKRP6C.*

[0313] Embodiment 24. The method of embodiment 18, wherein the *KRP* gene function is disrupted by nucleotide substitution, deletion, insertion, homologous recombination, T-DNA, transposon, antisense oligonucleotide, double stranded oligonucleotide, siRNA, shRNA, inverted oligonucleotide repeat, or combination thereof.

[0314] Embodiment 25. The method of embodiment 21, wherein the wheat plant comprises one or more mutations selected from any one of mutations listed in Tables 2 - 12 for a particular *KRP* gene; wherein the rice plant comprises one or more mutations selected from any one of mutations listed in Table 25 for a particular *KRP* gene; and wherein the soybean plant comprises one or more mutations selected from any one of mutations listed in Tables 29 - 37 for a particular *KRP* gene.

[0315] Embodiment 26. A method of producing a plant with increased organ size, organ weight, organ number and/or yield compared to a wild type plant, comprising

i) making a cross between a first plant to a second plant to produce a F1 plant, wherein the first plant is in the *Triticeae* tribe, the *Oryzeae* tribe, or the *Fabaceae* family comprising one or more disrupted *KRP* genes.

[0316] Embodiment 27. The method of embodiment 26, wherein the method further comprises

ii) backcrossing the F1 plant to the first or the second plant; and
iii) repeating the backcrossing step to generate a near isogenic line, wherein the one or more disrupted *KRPs* in the first plant are integrated into the genome of the near isogenic line.

[0317] Embodiment 28. The method of embodiment 26 or 27, wherein the plant in the *Triticeae* tribe is a plant in the *Triticum* genus, wherein the plant in the *Oryzeae* tribe is a plant in the *Oryza* genus, and wherein the plant in the *Fabaceae* family is in the *Glycine* genus.

[0318] Embodiment 29. The method of embodiment 28, wherein the plant in the *Triticum* genus is a wheat plant, wherein the plant in the *Oryza* genus is a rice plant, and wherein the plant in the *Glycine* genus is a soybean plant.

[0319] Embodiment 30. The method of embodiment 29, wherein the *KRP* in the wheat is *TaKRP1, TaKRP2, TaKRP4, TaKRP5,* or *TaKRP6,* the *KRP* in the rice is *OsKRP1, OsKRP2, OsKRP4, or OsKRP5,* and the *KRP* in the soybean is Gm0003x00821, Gm0013x00399, Gm0043, Gm0053x00526, Gm0087x00306, Gm0102x00087, Gm0119x00131, Gm0151 x00019, or Gm0067x00001.

[0320] Embodiment 31. The method of embodiment 30, wherein the *KRP* in a tetraploid wheat plant is *TaKRP1A, TaKRP1B, TaKRP2A, TaKRP2B, TaKRP4A, TaKRP4B, TaKRP5A, TaKRP5B, TaKRP6A,* and/or *TaKRP6B,* and wherein the *KRP* in a hexaploid wheat is *TaKRP1A, TaKRP1B, TaKRP1D, TaKRP2A, TaKRP2B, TaKRP2D, TaKRP4A, TaKRP4B, TaKRP4D, TaKRP5A, TaKRP5B, TaKRP5D, TaKRP6A, TaKRP6B,* and/or *TaKRP6D.*

[0321] Embodiment 32. The method of embodiment 26 or 27, wherein the *KRP* gene function is disrupted by nucleotide substitution, deletion, insertion, homologous recombination, T-DNA, transposon, antisense oligonucleotide, double stranded oligonucleotide, siRNA, shRNA, inverted oligonucleotide repeat, or combination thereof.

[0322] Embodiment 33. The method of embodiment 32, wherein the plant is a wheat plant comprising one or more mutations selected from any one of mutations listed in Tables 2 - 12 for a particular *KRP* gene; wherein the plant is a rice plant comprising one or more mutations selected from any one of mutations listed in Table 25 for a particular *KRP* gene; and wherein the plant is a soybean plant comprising one or more mutations selected from any one of mutations listed in Tables 29 - 37 for a particular *KRP* gene.

[0323] Embodiment 34. An isolated polynucleotide comprising a sequence selected from the group consisting of: (a) sequences recited in SEQ ID NOs: 138, 139, 141, 142, 144, and 145, and portions thereof; (b) complements of the sequences recited in SEQ ID NOs: 138, 139, 141, 142, 144, and 145, and portions thereof; (c) reverse complements of the sequences recited in SEQ ID NOs: 138, 139, 141, 142, 144, and 145, and portions thereof; (d) reverse sequences of the sequences recited in SEQ ID NOs: 138, 139, 141, 142, 144, and 145, and portions thereof; and (e) sequences having at least 90% identity to a sequence of (a)-(d) or a specified region of a sequence of (a)-(d).

[0324] Embodiment 35. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of (a) sequences recited in SEQ ID NOs: 140, 143, and 146, and portions thereof; (b) sequences having at least 90% identity to a sequence of (a).

[0325] Embodiment 36. A recombinant vector, recombinant nucleic acid, plant cell, plant part, plant tissue culture or whole plant comprising at least one isolated polynucleotide of embodiment 34.

[0326] Embodiment 37. The plant cell, plant part, plant tissue culture or whole plant of embodiment 36, wherein the plant is a plant of the *Triticeae* tribe.

[0327] Embodiment 38. The plant cell, plant part, plant tissue culture of whole plant of embodiment 37, wherein the plant is a plant of the *Triticum* genus. Embodiment 39. The plant cell, plant part, plant tissue culture or whole plant of embodiment 38, wherein the plant in the *Triticum* genus is a wheat plant.

[0328] Embodiment 40. The plant cell, plant part, plant tissue culture or whole plant of embodiment 39, wherein the wheat plant is tetraploid or hexaploid.

SEQUENCE LISTING

<110> Targeted Growth, Inc.

<120> IDENTIFICATION AND THE USE OF KRP MUTANTS IN PLANTS

<130> JWJ01948EP1

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<150> PCT/US2012/033047
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35 40 45

Ser Ser Cys Ser Gly Ser Asn Glu Tyr Lys Lys Lys Glu Leu Ile His
50 55 60

Leu Glu Glu Glu Asp Lys Asp Gly Asp Thr Glu Thr Ser Thr Tyr Arg
65 70 75 80

Arg Gly Thr Lys Arg Lys Leu Phe Glu Asn Leu Arg Glu Glu Glu Lys
85 90 95

Glu Glu Leu Ser Lys Ser Met Glu Asn Tyr Ser Ser Glu Phe Glu Ser
100 105 110

Ala Val Lys Glu Ser Leu Asp Cys Cys Cys Ser Gly Arg Lys Thr Met
115 120 125

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130 135 140

Glu Met Pro Thr Glu Ser Glu Ile Glu Asp Phe Phe Val Glu Ala Glu
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accaaccaac caaccaccga tcatcaacaa ctttttcttt cactcactcg ctcacatatt 1440

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ttccccggcg acctgagcga cctggagtcg gatctggcgg ggcagaacag cggccggtcg 540

tcgctgccgc aaacgccgac cgcccaggcc cagcccgccg cgaggtcgag ggtcccgccg 600

gcggccgaga tcgaggagtt cttcgcggcc gccgaggagg ccgaggccag gcggttcgct 660

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<210> 63
<211> 738
<212> DNA
<213> Triticum sp.

<400> 63
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ggcgacctga gcgacctgga gtcggatctg gcgggccaga acagcggccg gtcgtcgctg 540

ccgcaaacgc cgaccgccca ggtccagccg gccgcgaggt cgaggatccc gccggcggcc 600

gagatcgagg agttcttcgc ggccgccgag gaggccgagg ccaggcgctt cgcttgcaag 660

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gcggtgagca gcaactag 738

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<212> DNA
<213> Triticum sp.

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aaagaagcat tatagcctaa ccaaccaacc taccaccgat catc 1304

<210> 65
<211> 747
<212> DNA
<213> Triticum sp.

<400> 65
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gcttgcaagt acaacttcga cgtggcccgc ggcgtgcccc tcgactccgg ccggtacgag 720

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<212> DNA
<213> Triticum sp.

<220>
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<223> n is a, c, g, or t

<400> 66
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cctgctggga gtgaaaagag acgctgatcc aacccgcaaa ggaaaacagt aaagagaaag 1320

aggagtgaaa aaagaacaga ataatcccat gcacagcagc ctagagctag a 1371

<210> 67
<211> 822
<212> DNA
<213> Triticum sp.

<400> 67
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gcggcggtcg tggccggggt ctcgcgctgc tccagcaccg cgtcgacggc ggtggacgtg 420

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aacgccggcc ggttcgagtg gaccccggtg gccaccgtct ga 822

<210> 68
<211> 1364
<212> DNA
<213> Triticum sp.

<400> 68
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aaaaagaaca gaataatccc atgcacagca ggcctagagc taga 1364

<210> 69
<211> 816
<212> DNA
<213> Triticum sp.

<400> 69
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ggccggttcg agtggacccc ggtggccacc gtctga 816

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<211> 1388
<212> DNA
<213> Triticum sp.

<400> 70
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<211> 825
<212> DNA
<213> Triticum sp.

<400> 71
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<211> 652
<212> DNA
<213> Triticum sp.

<400> 72
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tttcttatta agctttgctg tttattgggg atttttttctg tcccaactct tc 652

<210> 73
<211> 905
<212> DNA
<213> Triticum sp.

<400> 73
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caacagcaag ccttcatcga caagtacgac attgtttggt tctctcagtc agttaacctt 480

gtctaattaa aaaaaatctt tcaatatctt tgcagtgaag aatgccaact cagcgtgcaa 540

tgtggttttg acacgtgata tgttcatgcc tttgctcttg ataaaaagtg tgattataac 600

actaacaaca tggtttcatg gcttaataat cttcaggtac aactttgatc ctgtgaacga 660

ctgtcctctc ccaggccgat acgagtgggt gaagctagac tgataattct ccaggaagga 720

gagcaccatg tatctctctg ctccctccac cttagcgtcg tggtagaggc gcgcaccgtc 780

gtgttagctt tgtttccgtt gtaaaaagaa ttagggttag cctgtagtag cctcaatggt 840

tgtgtaacat acagaagtaa tgctgagtta caccctatcc ctcaaactcc ccaaatgtcg 900

gtagc 905

<210> 74
<211> 573
<212> DNA
<213> Triticum sp.

<400> 74
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ggggcgccgg gggccaagga ccagggggag tacctggagc tcaggagccg gaagctcgag 180

aagctgcccc cgccgccgcc gccggcgagg aggagggcgg ccgcggcgga gcgtgtcgag 240

gccgaggccg aggccgacaa ggtgtccttc ggggagaacg tgctcgagcc ggaggccatg 300

gggaggggta ccagggagac gacgccctgc agcttgatta gggactcggg aatgataagc 360

actcctggat ccacaacaag accgagccac tcgaattccc atcgcagggt gcaagctcca 420

gcgcgccata ttattccaag ttcagcagag atgaatgagt tcttctctgc tgcagagcaa 480

ccgcaacagc aagccttcat cgacaagtac aactttgatc ctgtgaacga ctgtcctctc 540

ccaggccgat acgagtgggt gaagctagac tga 573

<210> 75
<211> 651
<212> DNA
<213> Triticum sp.

<400> 75
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gcatgggcaa gtacatgcgc aagcccaagg tctccggcga ggtggccgtc atggaggtcg 120

ccgccgcgcc gctaggggtc cgcacccgcg cacgagcgct cgcgatgcag aggcagccgc 180

agggggcggc ggtggccaag gaccaggggg agtacctgga gctcaggagt cggaagctcg 240

agaagctgcc cccgccgccg ccgccggcga ggaggagggc ggccgcggcg gagcgtgtcg 300

aggccgaggc cgaggccgac gaggtgtcct tcggtgagaa cgtgctcgag tcggaggcca 360

tggggaggtg agccttctcc tgcgccggcg attttcttcg gttattgggg ttttatttct 420

cggcgggggg attattaccg tgctagggtt tagggttttg tgtcgtaccg agaagctttg 480

gattgcttct tctatttcgc gcttcggctc gtttcatttc tccttgtcaa tttggcttgt 540

tctatccgtg ctgcgtgcgg ggctcgaatt tggtgtggat gctattttcc ccaatatctt 600

tgttactatt aaactttgct gtttattggg gatttttccg tctaactctt c 651

<210> 76
<211> 1071
<212> DNA
<213> Triticum sp.

<400> 76
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tggccccaat ctgtttgaac taaccttagg caagtttggt aagaaatgtg tggcaaattg 180

tggcattgct aggcctagtt agaaccaaac aaccccggat actcataagg gggggattcc 240

ttatttttta tgactattga tatgtgttct tctttttcca taccctgtta agttacaggt 300

gatttttttcc cttttgctat gcttcctctc tatggttgtt tctaaaaatt gagtgtgtat 360

gtatgttttg tggctagcag gggtaccagg gagacgacgc cctgcagctt gattagggac 420

tcgggaacga taagcactcc tggatccaca acaagaccga gccactcgaa ttcccatcgc 480

agggtgcaag ctccagcgcg ccatattatt ccatgttcag cagagatgaa tgagttcttc 540

tctgctgcgg agcaaccgca acagcaagcc ttcatcgaca agtacggcat tgtttggttc 600

tctcagtcag ttaaccttgt ctaatttaaa aaaagggaaa tctttcaata tcttcgcagt 660

gaagaatgcc aactcagcgt gcaatgtggt tttgacacgt gatatgttta cgcctttgct 720

cttgataaaa agtgtgatta taacactaac aacatggttt catggcttaa taatcttcag 780

gtacaacttt gatcctgtga acgactgtcc tctcccaggc cgatacgagt gggtgaagct 840

agactgataa ttctccagga aggagagcat catgtacttc tccgctccct ccaccttagc 900

gtcgtggtaa aggcgcgccc cgtcgtgtta gctttgtttc cgttgtaaaa agaattaggt 960

tagcctgtag tagcctcaat ggtcgtgtaa catacagaag taatgctgag ttacacccta 1020

atccctcaaa ctccaatgta acggttagca gctcattctg aaatgaccac a 1071

<210> 77
<211> 573
<212> DNA
<213> Triticum sp.

<400> 77
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gccgcgccgc taggggtccg cacccgcgca cgagcgctcg cgatgcagag gcagccgcag 120

ggggcggcgg tggccaagga ccaggggggag tacctggagc tcaggagtcg gaagctcgag 180

aagctgcccc cgccgccgcc gccggcgagg aggagggcgg ccgcggcgga gcgtgtcgag 240

gccgaggccg aggccgacga ggtgtccttc ggtgagaacg tgctcgagtc ggaggccatg 300

gggaggggta ccagggagac gacgccctgc agcttgatta gggactcggg aacgataagc 360

actcctggat ccacaacaag accgagccac tcgaattccc atcgcagggt gcaagctcca 420

gcgcgccata ttattccatg ttcagcagag atgaatgagt tcttctctgc tgcggagcaa 480

ccgcaacagc aagccttcat cgacaagtac aactttgatc ctgtgaacga ctgtcctctc 540

ccaggccgat acgagtgggt gaagctagac tga 573

<210> 78
<211> 651
<212> DNA
<213> Triticum sp.

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gcatgggcaa gtacatgcgc aagcccaagg tctccggcga ggtggccgtc atggaggtcg 120

ccgccgcgcc gctgggtgtc cgcacccgcg cgcgagcgct cgcgatgcag aggcagccgc 180

aggggcgcc gggggccaag gaccaggggg agtacctgga gctcaggagc cggaagctcg 240

agaagctgcc cctgccgccg ccgccggcga ggaggagggc ggccgcggcg gagcgtgtcg 300

aggccgaggc cgaggccgac gaggtgtcct tcggggagaa cgtgctcgag tcggaggcca 360

tggggaggtg agccgccttc tcctgcgccg gcgattttct tcggttctgg ggttttattt 420

ctcggcgggg ggattattac cgtgctaggg tttagggttt tgtgtcgtac cgagaagctt 480

tggattgctt gttccatttc acgcttcggc tcgtttcttt tttccttgtc agtttggctt 540

gttctgtccg tgctgcgtgc ggggctcgaa tttggtgtgg atgctatttt ccccaatatc 600

tttgttaagc ttggctgttt tattggggat ttttttcctg gctaactctt c 651

<210> 79
<211> 1073
<212> DNA
<213> Triticum sp.

<400> 79
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tgctagcctt tatatggcat atgagataga ttgaagggct gtttggttag gcaacttgtg 120

gctccaattt gtttgaacta accttaggca agtttggtga gaaatgtgtg gcaaattgtg 180

gcattgctag gcctagttag aaccaaacaa ccccggatac tcataagggg gggattcctt 240

atttcttacg accacggatg atatcgatat gtgttcttct ttttgcatac cctgttaagt 300

tacaggtgat ttttcccctt ttgctatact tcctctctat ggttgtttct aaaaattgag 360

tgtgtatgta tgttttgtgg ctagcagggg taccagggag acgacgccct gcagcttgat 420

tagggactcg ggaacgataa gcactcctgg atccacaaca agaccaagcc actcgaattc 480

ccatcgcagg gtgcaagctc cagcgcgcca tattattcca tgttcagcag agatgaatga 540

gttcttctct gctgcggagc aaccgcaaca gcaagccttc atcgacaagt acggcattgt 600

ttggttctct cagtcagtta accttgtcta attaaaaaaa tctttcaata tcttcgcagt 660

gaagaatgcc aactcagagt gcaatgtggt tttgacacgt gatatgttca cgcctttgct 720

cttgataaaa agtgtgatta taacactaac aacatggttt catggcttaa taatcttcag 780

gtacaacttt gatcctgtga acgactgtcc tctcccaggc cgatacgagt gggtgaagct 840

agactgataa ttctccagga aggagagcac catgtacctc tccgctccct ccaccttagc 900

gtcgtggtag aggcgcgcac cgccgtgtta gctttgtttc cgttgtaaaa agaattaggg 960

ttagcctgta gtagcctcaa tggtcttgta acatacagaa gtaatgctga gttacaccct 1020

aatccctcaa aactccaatg taacggttag cagctcattc tgtaatgacc aca 1073

<210> 80

<211> 573
<212> DNA
<213> Triticum sp.

<400> 80
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gccgcgccgc tgggtgtccg cacccgcgcg cgagcgctcg cgatgcagag gcagccgcag 120

ggggcgccgg gggccaagga ccaggggggag tacctggagc tcaggagccg gaagctcgag 180

aagctgcccc tgccgccgcc gccggcgagg aggagggcgg ccgcggcgga gcgtgtcgag 240

gccgaggccg aggccgacga ggtgtccttc ggggagaacg tgctcgagtc ggaggccatg 300

gggaggggta ccagggagac gacgccctgc agcttgatta gggactcggg aacgataagc 360

actcctggat ccacaacaag accaagccac tcgaattccc atcgcagggt gcaagctcca 420

gcgcgccata ttattccatg ttcagcagag atgaatgagt tcttctctgc tgcggagcaa 480

ccgcaacagc aagccttcat cgacaagtac aactttgatc ctgtgaacga ctgtcctctc 540

ccaggccgat acgagtgggt gaagctagac tga 573

<210> 81
<211> 2259
<212> DNA
<213> Triticum sp.

<220>
<221> misc_feature
<222> (2103)..(2103)
<223> n is a, c, g, or t

<400> 81
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agcagcgcgc tccgtcccct tcgccgcagc gcaagggcca cgaggacggc gactacctcg 180

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cgccgcagcc gccggccgcc ggtgggagga ggatggagca ggcgccgtcg tcgttcgccg 300

ccgagggctt cgaggccgac ctcgaggtct ccttcggcga caacgtcctg gactgggacg 360

ccaccgacag gtaacaacag agcaccagac ttttctctcc tccccttcct cttccgccaa 420

tccccccccc ccgcctcagt cagcaatccc ctctcgcgcc ccgccccggc cgatacgaat 480

acgactgagg tttagggttt atccgccgcc gtgtcgccgt cctgctccat tagcgccgcc 540

gcgtgctctc aaatctcaac tctcaggcgc cggcaacctc aagaaccccc tccctatcag 600

ttttctcaga cgagcgccgc cgctggtccg gcgattttct tctccatcag gattgaagcg 660

cccaaatagc cacaccttcc gctgattgtg cccggatgcc tgcaagaatc aaggcctccg 720

ctggccttga tttcctcaag ccttagccgt tggctggctg gagcttgaaa gaatcgaaga 780

aacgcctgtc cgctgtgttg acccggggaa aaaggccccc ctatttcccc ccctccagaa 840

aagccgccat tttccccctc caaacaaaga tgcatccagg cgcactcaat caacccccaa 900

tcaaagtggg cgctgcactt gattagtgga gcctcctcct cctcctcctc cagtggccgt 960

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cctcacacca ccaccaggag ctcctccatt gatctctgt 2259

<210> 82
<211> 630
<212> DNA
<213> Triticum sp.

<400> 82
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gaggccgacc tcgaggtctc cttcggcgac aacgtcctgg actgggacgc caccgacagg 360

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gggtcggcga ccggaggagc ccgcaaccac tcccgccgca gggcgcagac gccggtctgc 480

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caccagacct tcagggacaa gtacaacttc tgtcctgcga ggggctgccc gctccccggg 600

cggtacgagt ggacggtgct agactgctag 630

<210> 83
<211> 2282
<212> DNA
<213> Triticum sp.

<400> 83
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gcggctcgag aagcagccgc cgccgggggc cagggagaag gaggacgcgc cgcagccggc 360

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gttggcgtcc gctcgaaaga atccaagaaa gaaacgcctg cccgccgtgt tgacccggcg 900

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cctcctccag aaatcagtgg agcctcctcc ccctccagtg gccgcggccg tggctccgcc 1080

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gt 2282

<210> 84
<211> 660
<212> DNA
<213> Triticum sp.

<400> 84
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<210> 85
<211> 2430
<212> DNA
<213> Triticum sp.

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<221> misc_feature
<222> (2415)..(2415)
<223> n is a, c, g, or t

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cggcgcgctg ctcggcgtcc gcacccgctc ccgcaccctc gccgcgcagc agcagcgcgc 120

tccgtcccct tcgccgcagc gcaagggcca cgaggacggc gactacctcg agctcaggag 180

caggaggctc gagaagcagc cgccgccggg gcccaaggac aaggaggacg cgccgcagcc 240

gccggccgcc ggtgggaggg ggatggagtc gttcgcggcc gaggggttcg aggccgacct 300

cgaggtctcc ttcggcgaca acgtgctgga ctgggacgcc accgacaggt aagaacagag 360

caccagcgcc ttctttcctc ccccttcct ctcccctcaa tccttcccct ccggttcagt 420

cggcaatccc ctccgccccg gccgatacca atacgattga ggtttagggt tcatatccgc 480

cgctgtttcg ttctgctcca ttagcgccgc cgctgcgcgg cctcgaatct caacacgaat 540

cccctccct ctcaaacgag cgccgccgct ggcccgctgg ttttctccac aggattgagc 600

caaaccttgt gctgatttcg cccggatgct tgcgggaata atcccttgca gtttcctgat 660

tttcctcaag ctggagccgt tggccgtagc tttgaaagaa tccaagaaac gcctgcccgc 720

cgtgttgacc cggcgaaaaa gggcccccat tttccccct ccaaaaaagc cgccattttt 780

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gccgtgggtg aaaatggctt gctgctcatc attggccctt gtaccagagc gccaccgccg 1140

ccattgaatg cttgctggcc tcttgttagc ttcctgaccg gacgttgaat ggacaccgga 1200

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gaagacctca gtaaatatat ctttctccgg cttatttaat tctacttacg ttcgtatgat 1380

tgatctcgtg gtcccgttgt ccggcgtatg catgttgaac gcgcccattt agcttgcttg 1440

gccgtgttag gccaagttgt tgtttgtttg tcagcatcca gtcattcagt gtgcttgtgc 1500

tgcaccaatt atcaggtaca cctgacattg tctagcgtgg ggcacttgca aataatgaaa 1560

tggacaaaat catgctagaa catgagctgt cgtgttcaac ttcctgtagc ttggtctcat 1620

ctgagctcac caacccagct tgcatctgca gtaatttgca agacctcgtg tgcatttcag 1680

cttctgaacc tcatgttgct tgcaggggcg ccagggagac gacgccgtgc agcctgatct 1740

acagctcgga gacgatgagc acccccgggt cggcgaccgg ggcccgcaac cattcccgcc 1800

gcagggcgca gacgccggtc tgccgctacg tcccgagctc gctcgagatg gacgagttct 1860

tcgccgccgc ggagcagcag caacaccaga ccttcaggga gaagtaagaa ctctgcctcc 1920

tcctaccacc atcatttaaa catgctcact gaagatcaag cttcttgttc atacaattgt 1980

tctaacactc gctgcttcat tctaatcagg tacaacttct gtcccgcgag cgagcgcccg 2040

ctccccggac ggtacgagtg gacggtgctg gactgctagg gcttcttcat acctcacatc 2100

accaccacca ccaggagctc ctccattgat ctctgtaaca ccagaatgac caccaccatc 2160

agcagcagca gcagcatgtc atatgccgtg ggcgcgatgc aaatgcagta gcgttaggtt 2220

tctgattcac ctgttgtaaa aacttagagt tagcccgcag tcagcagtag ctcagccagc 2280

cagccatctc tcagcctgat ccccaacctc actgtaaccg tcgttagtta acaacatctc 2340

atttccgtag gctctagctt gattagcagc tcggttatct tctgtatccc ggtcctccat 2400

caatgaatga atcanagcta gatttatttt 2430

<210> 86
<211> 615
<212> DNA
<213> Triticum sp.

<400> 86
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ggcgcgctgc tcggcgtccg cacccgctcc cgcaccctcg ccgcgcagca gcagcgcgct 120

ccgtcccctt cgccgcagcg caagggccac gaggacggcg actacctcga gctcaggagc 180

aggaggctcg agaagcagcc gccgccgggg cccaaggaca aggaggacgc gccgcagccg 240

ccggccgccg gtgggagggg gatggagtcg ttcgcggccg aggggttcga ggccgacctc 300

gaggtctcct tcggcgacaa cgtgctggac tgggacgcca ccgacagggg cgccagggag 360

acgacgccgt gcagcctgat ctacagctcg gagacgatga gcacccccgg gtcggcgacc 420

ggggcccgca accattcccg ccgcagggcg cagacgccgg tctgccgcta cgtcccgagc 480

tcgctcgaga tggacgagtt cttcgccgcc gcggagcagc agcaacacca gaccttcagg 540

gagaagtaca acttctgtcc cgcgagcgag cgcccgctcc ccggacggta cgagtggacg 600

gtgctggact gctag 615

<210> 87
<211> 247
<212> PRT
<213> Triticum sp.

<400> 87

Met Gly Lys Tyr Met Arg Lys Cys Arg Ala Glu Asp Gly Ala Val Gly
1 5 10 15

Gly Val Glu Val Thr Gln Ala Val Gly Val Arg Thr Arg Ser Arg Ala
20 25 30

Ala Ala Ala Asn Val Val Val Ser Lys Arg Arg Arg Pro Leu Pro Pro
35 40 45

Gly Ser Pro Ser Ala Ser Ser Ser Leu Ala Arg Ala Gln Gly Gly Ser
50 55 60

Cys Tyr Leu Lys Leu Arg Ser Arg Met Leu Phe Met Ala Pro Pro Ala
65 70 75 80

Pro Ala Ser Gly Ala Ala Ala Gly His Gly Pro Ala Pro Pro Leu Pro
85 90 95

Ala Gly Leu Ser Arg Cys Ser Ser Thr Ala Ser Ser Val Asp Ala Ser
100 105 110

Ala Ala Ala Gln Asp Arg Ser Leu Leu Ser Cys Gly Ser Asp Ala Ala
115 120 125

Ala Asn Asn Lys Ala Gly Ala Pro Glu Gly Ser Ala Ser Asn Asn Ala
130 135 140

Glu Ser Gly Gly Asn Arg Glu Arg Arg Glu Thr Thr Pro Ser Ser His
145 150 155 160

Phe Pro Gly Asp Leu Ser Asp Leu Glu Ser Asp Leu Ala Gly Gln Asn
165 170 175

Ser Gly Arg Ser Ser Leu Pro Gln Thr Pro Thr Ala Gln Ala Gln Pro
180 185 190

Ala Ala Arg Ser Arg Val Pro Pro Ala Ala Glu Ile Glu Glu Phe Phe
195 200 205

Ala Ala Ala Glu Glu Ala Glu Ala Arg Arg Phe Ala Cys Lys Tyr Asn
210 215 220

Phe Asp Val Ala Arg Gly Val Pro Leu Gly Ser Gly Arg Tyr Glu Trp
225 230 235 240

Thr Pro Ala Val Ser Ser Ser
245

<210> 88
<211> 245
<212> PRT
<213> Triticum sp.

<400> 88

Met Gly Lys Tyr Met Arg Lys Cys Arg Ala Glu Asp Gly Val Gly Gly
1 5 10 15

Val Glu Val Thr Gln Ala Val Gly Val Arg Thr Arg Ser Arg Ala Ala
20 25 30

Ala Ala Asn Val Val Val Ser Lys Arg Arg Arg Pro Leu Pro Pro Ser
35 40 45

Ser Pro Leu Gly Gly Ala Ala Ala Arg Ala Gln Ser Gly Ser Cys Tyr
50 55 60

Leu Lys Leu Arg Ser Arg Met Leu Phe Met Ala Pro Pro Ala Pro Ala
65 70 75 80

Ser Ala Ala Gly Pro Gly His Arg Pro Ala Pro Pro Leu Pro Ala Gly
85 90 95

Leu Ser Arg Cys Ser Ser Thr Ala Ser Ser Val Asp Ala Ser Ala Ala
100 105 110

Gly Gln Asp Arg Ser Leu Pro Ser Cys Gly Ser Asp Ala Ala Ala Asn
115 120 125

Ser Lys Ala Gly Ala Pro Glu Gly Ser Ala Ser Asn Asn Ala Glu Ser
130 135 140

Gly Gly Asn Arg Glu Arg Arg Glu Thr Thr Pro Ser Ser His Phe Pro
145 150 155 160

Gly Asp Leu Ser Asp Leu Glu Ser Asp Leu Ala Gly Gln Asn Ser Gly
165 170 175

Arg Ser Ser Leu Pro Gln Thr Pro Thr Ala Gln Val Gln Pro Ala Ala
180 185 190

Arg Ser Arg Ile Pro Pro Ala Ala Glu Ile Glu Glu Phe Phe Ala Ala
195 200 205

Ala Glu Glu Ala Glu Ala Arg Arg Phe Ala Cys Lys Tyr Asn Phe Asp
210 215 220

Val Ala Arg Gly Val Pro Leu Asp Ser Gly Arg Tyr Glu Trp Thr Pro
225 230 235 240

Ala Val Ser Ser Asn
245

<210> 89
<211> 248
<212> PRT
<213> Triticum sp.

<400> 89

Met Gly Lys Tyr Met Arg Lys Cys Arg Ala Glu Asp Val Ala Val Gly
1 5 10 15

Gly Val Glu Val Thr Gln Ala Val Gly Val Arg Thr Arg Ser Arg Ala
20 25 30

Ala Ala Ala Asn Val Val Val Ser Lys Arg Arg Arg Pro Leu Pro Pro
35 40 45

Ala Ser Pro Ser Ala Ser Ser Ala Leu Ala Arg Ala Gln Gly Gly Ser
50 55 60

Cys Tyr Leu Lys Leu Arg Ser Arg Met Leu Phe Met Ala Pro Pro Ala
65 70 75 80

Pro Ala Ser Ala Ser Ala Ala Ala Ala Gly His Gly Ala Pro Pro Pro
85 90 95

Leu Pro Ala Gly Leu Ser Arg Cys Ser Ser Thr Ala Ser Ser Val Asp
100 105 110

Ala Ser Ala Ala Ala Gln Asp Arg Ser Leu Pro Ser Cys Gly Ser Asp
115 120 125

Ala Ala Ala Asn Lys Ala Gly Ala Pro Glu Gly Ser Ala Ser Asn Asn
130 135 140

Ala Glu Ser Gly Gly Asn Arg Glu Arg Arg Glu Thr Thr Pro Ser Ser
145 150 155 160

His Phe Pro Gly Asp Leu Ser Asp Leu Glu Ser Asp Leu Ala Gly Lys
165 170 175

Asn Ser Gly Arg Ser Ser Leu Pro Gln Thr Leu Ala Ala Gln Ala Gln
180 185 190

Pro Ala Ala Arg Ser Arg Val Pro Pro Ala Ala Glu Ile Glu Glu Phe
195 200 205

Phe Ala Ala Ala Glu Glu Ala Glu Ala Arg Arg Phe Ala Cys Lys Tyr
210 215 220

Asn Phe Asp Val Ala Arg Gly Val Pro Leu Asp Ser Gly Arg Tyr Glu
225 230 235 240

Trp Thr Pro Ala Val Ser Ser Ser
245

<210> 90
<211> 273
<212> PRT
<213> Triticum sp.

<400> 90

Met Gly Lys Tyr Met Arg Lys Cys Arg Ala Ala Pro Arg Arg Gly Arg
1 5 10 15

Arg Gln Gly Gly Ala Ala Val Val Glu His Arg Ala Pro Val Ala Leu
20 25 30

Gly Val Arg Thr Arg Ser Arg Ala Ala Ala Leu Asn Ala Lys Met Arg
35 40 45

Lys Gln Gln Gln Ala Thr Thr Ser Thr Ala Ala Arg Ala Val Glu Asp
50 55 60

Ala Leu Leu Gly Arg Asp Gly Gly Asp Ala Ala Ala Gly Cys Tyr Leu
65 70 75 80

His Leu Arg Ser Arg Arg Leu Phe Met Pro Ala Ser Ala Ala Val Asp
85 90 95

Gln Leu Arg Gly Leu Gly Ala Asp Glu Glu Ala Ser Thr Ala Gly Leu
100 105 110

Pro Asp Ser Arg Pro Ser Val Glu Ala Ala Val Val Ala Gly Val Ser
115 120 125

Arg Cys Ser Ser Thr Ala Ser Thr Ala Val Asp Val Ala Ala Arg Glu
130 135 140

Arg Ser Gly Asp Glu Ala Glu Ala Cys Glu Ser Gly Asp Val Glu Ser
145 150 155 160

Ser Val Ser Asp Ser Glu Cys Gly Gly Arg Asp Arg Arg Glu Thr Thr
165 170 175

Pro Ser Ser His Ser Pro Ala Asp Leu Ser Asp Leu Glu Ser Ser Gln
180 185 190

Ser Ala Asp Glu Gln Lys His Lys Arg Arg Arg Tyr Pro Ala Thr Thr
195 200 205

Thr Thr Thr Ala Ala Pro Phe Arg Leu Asp Leu Glu Ala Arg Ala Arg
210 215 220

Met Pro Pro Ala Ala Glu Ile Asp Glu Phe Phe Ala Ala Ala Glu Lys
225 230 235 240

Ala Gln Ala Glu Arg Phe Ala Ala Lys Tyr Asn Phe Asp Val Ala Arg
245 250 255

Gly Val Pro Leu Asn Ala Gly Arg Phe Glu Trp Thr Pro Val Ala Thr
260 265 270

Val

<210> 91
<211> 271
<212> PRT
<213> Triticum sp.

<400> 91

Met Gly Lys Tyr Met Arg Lys Cys Arg Gly Ala Ala Ala Gly Gly Gly
1 5 10 15

Arg Ala Ala Pro Ala Val Val Glu His Arg Ala Pro Val Ala Leu Gly
20 25 30

Val Arg Thr Arg Ser Arg Ala Ala Ala Phe Asp Ala Lys Arg Arg Lys
35 40 45

```
Gln Gln Ala Thr Thr Ser Thr Ala Ala Arg Ala Val Asp Asp Ala Leu
    50                  55                  60

Leu Gly Arg Asp Gly Gly Asp Ala Ala Gly Gly Cys Tyr Leu His Leu
65                  70                  75                  80

Arg Ser Arg Arg Leu Phe Met Pro Ala Ser Ala Val Val Asp Arg Leu
                85                  90                  95

Arg Gly Gln Gly Ala Asp Glu Glu Ala Ser Thr Ala Arg Leu Ala Asp
            100                 105                 110

Ser Gly Pro Ser Val Glu Ala Gly Val Val Ala Gly Val Ser Arg Cys
        115                 120                 125

Ser Ser Thr Ala Ser Thr Ala Ala Asp Val Ala Ala Arg Glu Arg Ser
    130                 135                 140

Gly Asp Glu Ala Glu Ala Cys Glu Ser Arg Asp Val Glu Ser Ser Val
145                 150                 155                 160

Ser Asp Ser Glu Cys Gly Gly Arg Asp Arg Arg Glu Ala Thr Pro Ser
                165                 170                 175

Ser Arg Ser Pro Val Asp Leu Ser Asp Leu Glu Ser Ser Gln Ala Ala
            180                 185                 190

Asp Glu Gln Lys His Lys Arg Arg Arg Cys Pro Ala Ala Thr Thr Ala
        195                 200                 205

Ala Ala Ala Pro Phe His Leu Asp Ser Glu Ala Arg Ala Arg Met Pro
    210                 215                 220

Pro Ala Ala Glu Ile Asp Glu Phe Phe Ala Ala Ala Glu Lys Ala Gln
225                 230                 235                 240

Ala Glu His Phe Ala Ala Lys Tyr Asn Phe Asp Val Ala Arg Gly Val
                245                 250                 255

Pro Leu Asn Ala Gly Arg Phe Glu Trp Thr Pro Val Ala Thr Val
            260                 265                 270
```

<210> 92
<211> 274
<212> PRT
<213> Triticum sp.

<400> 92

```
Met Gly Lys Tyr Met Arg Lys Cys Arg Ala Ala Ala Ala Gly Gly Gly
1               5                  10                  15

Arg Ala Ala Pro Ala Val Val Glu His Arg Ala Pro Val Ala Leu Gly
            20                  25                  30

Val Arg Thr Arg Ser Arg Ala Ala Ala Leu Asp Ala Lys Met Arg Lys
        35                  40                  45

Gln Gln Gln Ala Thr Thr Ser Thr Ala Ala Arg Ala Val Glu Asp Ala
    50                  55                  60

Leu Leu Gly Arg Asp Gly Gly Asp Ala Ala Ala Gly Cys Tyr Leu His
65                  70                  75                  80

Leu Arg Ser Arg Arg Leu Phe Met Pro Ala Ala Ala Val Val Asp Gln
                85                  90                  95

Leu Arg Gly Gln Gly Val Cys Glu Glu Ala Ser Thr Ala Gly Leu Pro
            100                 105                 110

Asp Ser Gly Pro Ser Val Glu Ala Ala Val Gly Ala Gly Val Ser Arg
        115                 120                 125

Cys Ser Ser Thr Ala Ser Thr Ala Val Asp Val Ala Ala Arg Glu Arg
    130                 135                 140

Ser Gly Asp Glu Ala Glu Ala Cys Glu Ser Arg Asp Val Glu Ser Ser
145                 150                 155                 160

Val Ser Asp Ser Glu Cys Gly Gly Arg Asp Arg Arg Glu Thr Thr Pro
                165                 170                 175

Ser Ser Arg Ser Pro Val Asp Leu Ser Asp Leu Glu Ser Ser Gln Ala
            180                 185                 190

Ala Asp Glu Gln Lys His Lys Arg Arg Arg Cys Pro Ala Thr Thr Thr
        195                 200                 205

Thr Thr Ala Ala Pro Leu His Tyr Asp Leu Glu Ala Arg Ala Arg Ala
    210                 215                 220

Arg Met Pro Pro Ala Ala Glu Ile Asp Glu Phe Phe Ala Ala Ala Glu
225                 230                 235                 240

Lys Ala Gln Ala Glu Arg Phe Ala Ala Lys Tyr Asn Phe Asp Val Ala
                245                 250                 255
```

Arg Gly Val Pro Leu Asn Ala Gly Arg Phe Glu Trp Thr Pro Val Ala
260 265 270

Thr Val

<210> 93
<211> 190
<212> PRT
<213> Triticum sp.

<400> 93

Met Gly Lys Tyr Met Arg Lys Pro Lys Val Ser Gly Glu Val Ala Val
1 5 10 15

Met Glu Val Ala Ala Ala Pro Leu Gly Val Arg Thr Arg Ala Arg Ala
20 25 30

Leu Ala Met Gln Arg Gln Pro Gln Gly Ala Pro Gly Ala Lys Asp Gln
35 40 45

Gly Glu Tyr Leu Glu Leu Arg Ser Arg Lys Leu Glu Lys Leu Pro Pro
50 55 60

Pro Pro Pro Pro Ala Arg Arg Arg Ala Ala Ala Ala Glu Arg Val Glu
65 70 75 80

Ala Glu Ala Glu Ala Asp Lys Val Ser Phe Gly Glu Asn Val Leu Glu
85 90 95

Pro Glu Ala Met Gly Arg Gly Thr Arg Glu Thr Thr Pro Cys Ser Leu
100 105 110

Ile Arg Asp Ser Gly Met Ile Ser Thr Pro Gly Ser Thr Thr Arg Pro
115 120 125

Ser His Ser Asn Ser His Arg Arg Val Gln Ala Pro Ala Arg His Ile
130 135 140

Ile Pro Ser Ser Ala Glu Met Asn Glu Phe Phe Ser Ala Ala Glu Gln
145 150 155 160

Pro Gln Gln Gln Ala Phe Ile Asp Lys Tyr Asn Phe Asp Pro Val Asn
165 170 175

Asp Cys Pro Leu Pro Gly Arg Tyr Glu Trp Val Lys Leu Asp
180 185 190

<210> 94
<211> 190
<212> PRT
<213> Triticum sp.

<400> 94

```
Met Gly Lys Tyr Met Arg Lys Pro Lys Val Ser Gly Glu Val Ala Val
1               5                  10                  15

Met Glu Val Ala Ala Ala Pro Leu Gly Val Arg Thr Arg Ala Arg Ala
            20                  25                  30

Leu Ala Met Gln Arg Gln Pro Gln Gly Ala Ala Val Ala Lys Asp Gln
        35                  40                  45

Gly Glu Tyr Leu Glu Leu Arg Ser Arg Lys Leu Glu Lys Leu Pro Pro
    50                  55                  60

Pro Pro Pro Ala Ala Arg Arg Arg Ala Ala Ala Ala Glu Arg Val Glu
65                  70                  75                  80

Ala Glu Ala Glu Ala Asp Glu Val Ser Phe Gly Glu Asn Val Leu Glu
                85                  90                  95

Ser Glu Ala Met Gly Arg Gly Thr Arg Glu Thr Thr Pro Cys Ser Leu
            100                 105                 110

Ile Arg Asp Ser Gly Thr Ile Ser Thr Pro Gly Ser Thr Thr Arg Pro
        115                 120                 125

Ser His Ser Asn Ser His Arg Arg Val Gln Ala Pro Ala Arg His Ile
    130                 135                 140

Ile Pro Cys Ser Ala Glu Met Asn Glu Phe Phe Ser Ala Ala Glu Gln
145                 150                 155                 160

Pro Gln Gln Gln Ala Phe Ile Asp Lys Tyr Asn Phe Asp Pro Val Asn
                165                 170                 175

Asp Cys Pro Leu Pro Gly Arg Tyr Glu Trp Val Lys Leu Asp
            180                 185                 190
```

<210> 95
<211> 190
<212> PRT
<213> Triticum sp.

<400> 95

```
Met Gly Lys Tyr Met Arg Lys Pro Lys Val Ser Gly Glu Val Ala Val
1               5                   10                  15

Met Glu Val Ala Ala Ala Pro Leu Gly Val Arg Thr Arg Ala Arg Ala
            20                  25                  30

Leu Ala Met Gln Arg Gln Pro Gln Gly Ala Pro Gly Ala Lys Asp Gln
        35                  40                  45

Gly Glu Tyr Leu Glu Leu Arg Ser Arg Lys Leu Glu Lys Leu Pro Leu
    50                  55                  60

Pro Pro Pro Pro Ala Arg Arg Arg Ala Ala Ala Ala Glu Arg Val Glu
65                  70                  75                  80

Ala Glu Ala Glu Ala Asp Glu Val Ser Phe Gly Glu Asn Val Leu Glu
                85                  90                  95

Ser Glu Ala Met Gly Arg Gly Thr Arg Glu Thr Thr Pro Cys Ser Leu
            100                 105                 110

Ile Arg Asp Ser Gly Thr Ile Ser Thr Pro Gly Ser Thr Thr Arg Pro
        115                 120                 125

Ser His Ser Asn Ser His Arg Arg Val Gln Ala Pro Ala Arg His Ile
    130                 135                 140

Ile Pro Cys Ser Ala Glu Met Asn Glu Phe Phe Ser Ala Ala Glu Gln
145                 150                 155                 160

Pro Gln Gln Gln Ala Phe Ile Asp Lys Tyr Asn Phe Asp Pro Val Asn
                165                 170                 175

Asp Cys Pro Leu Pro Gly Arg Tyr Glu Trp Val Lys Leu Asp
            180                 185                 190
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<210> 96
<211> 209
<212> PRT
<213> Triticum sp.

<400> 96

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Met Gly Lys Tyr Met Arg Lys Ser Lys Ala Ser Gly Glu Val Ala Val
1               5                   10                  15

Met Glu Val Ala Gly Ala Leu Leu Gly Val Arg Thr Arg Ser Arg Thr
            20                  25                  30
```

Leu Ala Ala Gln Gln Gln Arg Ala Pro Ser Pro Ser Pro Gln Arg Lys
35 40 45

Gly His Glu Asp Gly Asp Tyr Leu Glu Leu Arg Ser Arg Arg Leu Glu
50 55 60

Lys Gln Pro Pro Pro Gly Pro Lys Asp Lys Glu Asp Ala Pro Gln Pro
65 70 75 80

Pro Ala Ala Gly Gly Arg Arg Met Glu Gln Ala Pro Ser Ser Phe Ala
85 90 95

Ala Glu Gly Phe Glu Ala Asp Leu Glu Val Ser Phe Gly Asp Asn Val
100 105 110

Leu Asp Trp Asp Ala Thr Asp Arg Gly Ala Arg Glu Thr Thr Pro Cys
115 120 125

Ser Leu Ile Tyr Ser Ser Glu Thr Met Ser Thr Pro Gly Ser Ala Thr
130 135 140

Gly Gly Ala Arg Asn His Ser Arg Arg Arg Ala Gln Thr Pro Val Cys
145 150 155 160

Arg Tyr Val Pro Ser Ser Leu Glu Met Asp Glu Phe Phe Ala Ala Ala
165 170 175

Glu Gln Gln Gln His Gln Thr Phe Arg Asp Lys Tyr Asn Phe Cys Pro
180 185 190

Ala Arg Gly Cys Pro Leu Pro Gly Arg Tyr Glu Trp Thr Val Leu Asp
195 200 205

Cys

<210> 97
<211> 140
<212> PRT
<213> Triticum sp.

<400> 97

Met Gly Lys Tyr Met Arg Lys Ser Lys Ala Ser Gly Glu Val Ala Val
1 5 10 15

Met Glu Val Ala Gly Ala Leu Leu Gly Val Arg Thr Arg Ser Arg Thr
20 25 30

Leu Ala Ala Gln Gln Gln Arg Ala Pro Ser Pro Ser Pro Ser Pro Gln
35 40 45

Arg Lys Gly Gln Glu Asp Gly Asp Pro Gly Ala Gly Asp Tyr Leu Glu
50 55 60

Leu Arg Ser Arg Arg Leu Glu Lys Gln Pro Pro Pro Gly Ala Arg Glu
65 70 75 80

Lys Glu Asp Ala Pro Gln Arg Pro Arg Gly Gly Pro Pro Pro Leu Ala
85 90 95

Gly Gly Gly Trp Ser Arg Arg Arg Arg Ser Pro Pro Arg Gly Ser Arg
100 105 110

Pro Thr Ser Arg Ser Pro Ser Ala Thr Thr Cys Trp Thr Gly Thr Pro
115 120 125

Pro Thr Gly Ala Pro Gly Arg Arg Arg Arg Ala Ala
130 135 140

<210> 98
<211> 204
<212> PRT
<213> Triticum sp.

<400> 98

Met Gly Lys Tyr Met Arg Lys Ser Lys Ala Ser Gly Glu Val Ala Val
1 5 10 15

Met Glu Val Ala Gly Ala Leu Leu Gly Val Arg Thr Arg Ser Arg Thr
20 25 30

Leu Ala Ala Gln Gln Gln Arg Ala Pro Ser Pro Ser Pro Gln Arg Lys
35 40 45

Gly His Glu Asp Gly Asp Tyr Leu Glu Leu Arg Ser Arg Arg Leu Glu
50 55 60

Lys Gln Pro Pro Pro Gly Pro Lys Asp Lys Glu Asp Ala Pro Gln Pro
65 70 75 80

Pro Ala Ala Gly Gly Arg Gly Met Glu Ser Phe Ala Ala Glu Gly Phe
85 90 95

Glu Ala Asp Leu Glu Val Ser Phe Gly Asp Asn Val Leu Asp Trp Asp
100 105 110

Ala Thr Asp Arg Gly Ala Arg Glu Thr Thr Pro Cys Ser Leu Ile Tyr
115 120 125

Ser Ser Glu Thr Met Ser Thr Pro Gly Ser Ala Thr Gly Ala Arg Asn
130 135 140

His Ser Arg Arg Arg Ala Gln Thr Pro Val Cys Arg Tyr Val Pro Ser
145 150 155 160

Ser Leu Glu Met Asp Glu Phe Phe Ala Ala Ala Glu Gln Gln Gln His
165 170 175

Gln Thr Phe Arg Glu Lys Tyr Asn Phe Cys Pro Ala Ser Glu Arg Pro
180 185 190

Leu Pro Gly Arg Tyr Glu Trp Thr Val Leu Asp Cys
195 200

<210> 99
<211> 262
<212> PRT
<213> Oryza sativa

<400> 99

Met Gly Lys Tyr Met Arg Lys Phe Arg Gly Ala Thr Gly Glu Glu Leu
1 5 10 15

Ala Ala Met Glu Val Thr Gln Val Val Gly Val Arg Thr Arg Ser Arg
20 25 30

Ser Ala Ala Ala Ala Gly Ala Thr Thr Thr Lys Val Lys Ala Ala Ser
35 40 45

Ala Ala Ser Thr Arg Arg Arg Lys Ala Leu Leu Pro Thr Ala Val Val
50 55 60

Gly Thr Thr Arg Arg Asp Gly Gly Ser Cys Tyr Leu Gln Leu Arg Ser
65 70 75 80

Arg Met Leu Phe Met Ala Pro Pro Arg Pro Ala Pro Ala Ala Arg Ala
85 90 95

Pro Val Val Ala Glu Ala Ala Gly Ser Gly Asn Gly Ala Ala Ala His
100 105 110

Ala Ala Ala Gly Leu Ser Arg Cys Ser Ser Thr Ala Ser Ser Val Asp
115 120 125

Ala Ala Ala Gln Asp Arg Ser Leu Ala Cys Arg Ser Asp Val Ala Glu
130 135 140

Ala Gly Ser Glu His Val Pro Glu Gly Ser Ala Ser Asp Ser Ala Ser
145 150 155 160

Gly Arg Asp Arg Glu Arg Arg Glu Thr Thr Pro Ser Ser Phe Leu Pro
165 170 175

Gly Glu Val Ser Asp Leu Glu Ser Asp Leu Ala Gly Gly Gln Lys Arg
180 185 190

Ser Arg Pro Leu Pro Ser Ala Ala Thr Ala Ser Ala Gln Gln Ala Thr
195 200 205

Arg Pro Lys Ile Pro Pro Ala Ala Glu Ile Glu Ala Phe Phe Ala Ala
210 215 220

Ala Glu Glu Ala Glu Ala Lys Arg Phe Ala Ala Lys Tyr Asn Phe Asp
225 230 235 240

Val Val Arg Gly Val Pro Leu Asp Ala Gly Arg Phe Glu Trp Thr Pro
245 250 255

Val Val Ser Ser Arg Ser
260

<210> 100
<211> 1265
<212> DNA
<213> Oryza sativa

<400> 100
agggaaacgg ccaaagaagg cgacggcggc ggcgcaggag gggcgcgcac gcggcgcaga 60

tgggcaagta catgaggaag ttcaggggggg ccacggggga ggagttggcc gccatggagg 120

tcacgcaggt ggttggcgtc cggacgaggt cgaggtcggc agcggcggcg ggcgcgacga 180

cgacgaaggt gaaggcggcg tcggcggcgt ccaccaggag gaggaaggcg ctgctgccga 240

cggcggtcgt ggggactact cgccgtgacg gcgggagctg ctacctccag ctgaggagcc 300

gcatgctgtt catggccccg ccgaggccgg cgccggccgc gagggctccg gttgtagcgg 360

aggcggcggg ttccgggaac ggagcggcgg cgcatgcggc ggctggcctc tcgcgttgct 420

ccagcacggc gtcgtccgtg gacgcggcgg ctcaggacag gagcctcgcg tgccgctccg 480

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gccgtgaccg cgagaggaga gaaacaactc catcaagctt tctccccgga gaggtgagcg 600

atctggagtc ggatctggct ggaggacaga agcgcagccg tccactacct tctgcggcaa 660

cagcctcagc acagcaagcc acgcggccga agattccgcc ggccgccgag atcgaggcgt 720

tcttcgcggc ggccgaggag gctgaggcca agcgcttcgc cgccaagtac aacttcgacg 780

tcgttcgcgg cgtgcccctc gacgccggtc ggttcgagtg gactccggtg gtcagcagcc 840

gaagctgaag cgagcgtgca gattaagcgg aagctagaaa ggaaggtaca ggggggcgcc 900

gtgtagaaag ggaaggcgag ctagagagag gagaagaaga agaagaaaag atgctcatcc 960

aaagggaata aactggaaaa gtgggagact acaaaaaaag aagcattata gcctaacaac 1020

caccgattcg actctttttt ctttcacatt ttctttgcat ttttactctt actgtgtact 1080

agaaagtagt agcagtagta aactagtaat tcgtcccagt atttatcaga ggtttatctc 1140

gataggaata gatatattat ccccttactg taattgcctc catcttgtat ttggatggaa 1200

attaaattta ctgtacagca gcagcagctg ttctgcaagt ttaagttaac catcaccgtt 1260

ttatt 1265

<210> 101
<211> 789
<212> DNA
<213> Oryza sativa

<400> 101
atgggcaagt acatgaggaa gttcaggggg gccacggggg aggagttggc cgccatggag 60

gtcacgcagg tggttggcgt ccggacgagg tcgaggtcgg cagcggcggc gggcgcgacg 120

acgacgaagg tgaaggcggc gtcggcggcg tccaccagga ggaggaaggc gctgctgccg 180

acggcggtcg tggggactac tcgccgtgac ggcgggagct gctacctcca gctgaggagc 240

cgcatgctgt tcatggcccc gccgaggccg gcgccggccg cgagggctcc ggttgtagcg 300

gaggcggcgg gttccgggaa cggagcggcg gcgcatgcgg cggctggcct ctcgcgttgc 360

tccagcacgg cgtcgtccgt ggacgcggcg gctcaggaca ggagcctcgc gtgccgctcc 420

gacgtcgcgg aggcaggcag cgagcatgtc ccggagggct ccgcgagcga ctcggcgagc 480

ggccgtgacc gcgagaggag agaaacaact ccatcaagct ttctccccgg agaggtgagc 540

gatctggagt cggatctggc tggaggacag aagcgcagcc gtccactacc ttctgcggca 600

acagcctcag cacagcaagc cacgcggccg aagattccgc cggccgccga gatcgaggcg 660

ttcttcgcgg cggccgagga ggctgaggcc aagcgcttcg ccgccaagta caacttcgac 720

gtcgttcgcg gcgtgcccct cgacgccggt cggttcgagt ggactccggt ggtcagcagc 780

cgaagctga 789

<210> 102
<211> 750
<212> DNA
<213> Oryza sativa

<400> 102
atggggaaga agaagaagcg cgacggcgcg gcggcgagga ggcaggcgcg ggtggtggtc 60

ggcggcgtcc gtacgcgggc cgccgtcacg gcgaggaggg tggtggcgag cgcggaggag 120

ggttgtggtt tggtgggccg tggcggtggc ggtggcagtg gcggagacga tggcgagggc 180

ggatgctatc tgcgtctgcg gagcaggagg ctgcccttcg tggcggccgc ggtggtgtcg 240

tcgcggaggg aggaggcgct cggtgattcg gtggcggagg cggcttcgtc gtcgtcgtcg 300

cgggcggtgg aattgttggg ctgttctggt gaggaggagg ctatggccga gaaggtttgc 360

acgcaggcag gcgaggatca cgacgaggag agctccgtcg gcgactccgg ctgcggccgc 420

gagaggagcg cgacgacgcc gtcgagccgc cggccgccgg gagacgcgga ctcgagcgac 480

gcggagtcaa accaggaggc caagcagcaa atgtgccgcc ggagttcgac gacctcagca 540

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<212> DNA
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35 40 45

Gly Gly Gly Gly Ser Gly Gly Asp Asp Gly Glu Gly Gly Cys Tyr Leu
50 55 60

Arg Leu Arg Ser Arg Arg Leu Pro Phe Val Ala Ala Ala Val Val Ser
65 70 75 80

Ser Arg Arg Glu Glu Ala Leu Gly Asp Ser Val Ala Glu Ala Ala Ser
85 90 95

Ser Ser Ser Ser Arg Ala Val Glu Leu Leu Gly Cys Ser Gly Glu Glu
100 105 110

Glu Ala Met Ala Glu Lys Val Cys Thr Gln Ala Gly Glu Asp His Asp
115 120 125

Glu Glu Ser Ser Val Gly Asp Ser Gly Cys Gly Arg Glu Arg Ser Ala
130 135 140

Thr Thr Pro Ser Ser Arg Arg Pro Pro Gly Asp Ala Asp Ser Ser Asp
145 150 155 160

Ala Glu Ser Asn Gln Glu Ala Lys Gln Gln Met Cys Arg Arg Ser Ser
165 170 175

Thr Thr Ser Ala Ala Ala Phe His Ala Gly Ala Thr Thr Arg Ser Phe
180 185 190

Arg Met Met Ala Pro Pro Ala Ala Ala Ala Glu Ile Glu Glu Phe Leu
195 200 205

Ala Ala Ala Glu Arg Ser Glu Ala Glu Arg Phe Ala Ala Lys Tyr Asn
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Phe Asp Val Val Arg Gly Val Pro Leu Asp Ala Gly Gly Ala Gly Arg
225 230 235 240

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35 40 45

Leu Glu Leu Arg Ser Arg Arg Leu Glu Lys Leu Pro Pro Pro Pro Pro
50 55 60

Pro Pro Pro Arg Arg Arg Ala Thr Ala Ala Ala Ala Thr Ala Asp Ala
65 70 75 80

Thr Ala Ala Glu Ser Ala Glu Ala Glu Val Ser Phe Gly Gly Glu Asn
85 90 95

Val Leu Glu Leu Glu Ala Met Glu Arg Asn Thr Arg Glu Thr Thr Pro
100 105 110

Cys Ser Leu Ile Arg Asp Pro Asp Thr Ile Ser Thr Pro Gly Ser Thr
115 120 125

Thr Arg Arg Ser His Ser Ser Ser His Cys Lys Val Gln Thr Pro Val
130 135 140

Arg His Asn Ile Ile Pro Ala Ser Ala Glu Leu Glu Ala Phe Phe Ala
145 150 155 160

Ala Glu Glu Gln Arg Gln Arg Gln Ala Phe Ile Asp Lys Tyr Asn Phe
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Asp Pro Val Asn Asp Cys Pro Leu Pro Gly Arg Phe Glu Trp Val Lys
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<400> 110

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Leu Ala Leu Gln Arg Thr Thr Ser Ser Gln Lys Pro Pro Glu Lys Gly
35 40 45

Glu Gly Asp Pro Gly Ala Gly Ala Gly Ala Gly Ala Glu Tyr Leu Glu
50 55 60

Leu Arg Ser Arg Arg Leu Glu Lys Pro Pro Pro His Thr Pro Pro Ala
65 70 75 80

Lys Glu Lys Glu Thr Ala Arg Arg Ala Ser Ala Ala Ala Ala Ala Ala
85 90 95

Val Arg Met Pro Ala Ala Pro Gln Ala Ala Glu Glu Phe Glu Ala Glu
100 105 110

Val Glu Val Ser Phe Gly Asp Asn Val Leu Asp Leu Asp Gly Asp Ala
115 120 125

Met Glu Arg Ser Thr Arg Glu Thr Thr Pro Cys Ser Leu Ile Arg Ser
130 135 140

Ser Glu Met Ile Ser Thr Pro Gly Ser Thr Thr Lys Thr Asn Thr Ser
145 150 155 160

Ile Ser Ser Arg Arg Arg Met Glu Thr Ser Val Cys Arg Tyr Val Pro
165 170 175

Ser Ser Leu Glu Met Glu Glu Phe Phe Ala Ala Ala Glu Gln Gln Gln
180 185 190

His Gln Ala Phe Arg Glu Arg Tyr Asn Phe Cys Pro Val Asn Asp Cys
195 200 205

Pro Leu Pro Gly Arg Tyr Glu Trp Thr Arg Leu Asp Cys
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<211> 618
<212> DNA
<213> Glycine sp.

<400> 114
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accagggaaa gcacccctttg tagttttata agggactcaa atgccattca tacccctggt 420

tcaaccacaa ggccaaggac tcgccaaata atccatgagc acatccaaag aaacattcca 480

acggcttatg agatggagga gttctttgct tatgccgaga agcagcaaca aacaatattt 540

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<211> 1728
<212> DNA
<213> Glycine sp.

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<210> 116
<211> 489

<212> DNA
<213> Glycine sp.

<400> 116
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gattcagcgg aggagcagat cacccaaacc aaatctttgc cgccgcagaa aatgccgacg 420

gagttggagc tcgatgaatt cttcgccgct gctgagaaag atattcggaa acgcttctca 480

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<210> 117
<211> 1466
<212> DNA
<213> Glycine sp.

<400> 117
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<211> 366
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<213> Glycine sp.

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ggagtcggag ctcgaagatt tcttcgctgc tgcggagaaa gacattcaga aacgcttcac 360

agacaa 366

<210> 119
<211> 1648
<212> DNA
<213> Glycine sp.

<400> 119
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<210> 120
<211> 597
<212> DNA
<213> Glycine sp.

<400> 120
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<210> 121
<211> 1724
<212> DNA
<213> Glycine sp.

<400> 121
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<211> 618
<212> DNA
<213> Glycine sp.

<400> 122
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<210> 123
<211> 2714
<212> DNA
<213> Glycine sp.

<400> 123
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<211> 675
<212> DNA
<213> Glycine sp.

<400> 124
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<210> 125
<211> 1775
<212> DNA
<213> Glycine sp.

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aatcgttcag gtttttgttc tacttttttt ttttttttgt tctacttgtt tctcatgtac 840

gcacgatcaa attttcaaac gaaacgttgt cgcaactgaa acgctacatt tatttattta 900

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aggcggagat cgaagagttt ttcgcgatgg ctgaaaagta cgagcaaaaa cggttcacag 1080

agaagtaagt agtagtatat atagttgatt gctacaaata aaagttttaa tattgcaaat 1140

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agtttgtagt gtatgtttag ccattggagt acactggtga ggaagctaac ctgacacgag 1560

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agtggtgatg ggttggttta tgtatagcta actgttttct ctttcttttt atgtgggata 1680

caacaaggtg gcttttctgc aaactctgca ctcagaatag aactagtaga accttctttg 1740

tgaggtgatg aagaaaaaga aagaaaagga aaaag 1775

<210> 126
<211> 564
<212> DNA
<213> Glycine sp.

<400> 126
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ttccagttat gctcttccga tatgcagttt ccccacacta tcgtctcgcc ggaagtttca 180

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acggtagaag actcaaccag cctcaatttc aaatcgttca gtttgttgag tgagttttcc 360

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aagacgccgc cgaaggcgga gatcgaagag tttttcgcga tggctgaaaa gtacgagcaa 480

aaacggttca cagagaagta caactttgat attgttagag atttgccgtt ggagggtcgc 540

taccagtggg ttcgtttaca ttga 564

<210> 127
<211> 2149
<212> DNA
<213> Glycine sp.

<400> 127
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aacttatttg taaaaaaaag aaaagaaggt atttttattt actgatttca aattattctg 180

taattttggt cataaaaatc taaaactgaa cataagttta ttattattta gaagagagaa 240

gaagaggcta aaagggagag tagcagtagc aatgatcgta acgatgtcgt ttagcgggta 300

ctgtagctgg agaaagacaa gagggaccat ccacgaatcg attcactgcg ctcagtaaat 360

attcctctgc gatcactctc tttttaagct ccctccacct ctctccctaa gcctagccca 420

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aaaaaccacc gcttccttcc agttacgctc ttccgatacg cagtttcccg acactatcgt 600

ctcgccggaa gcttccgtca gttctaccgg cacggttgtt tccggcgatt tttgctccga 660

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atacaaagaa cacaagaatt tccgctatga atattggtga tctaaaatat gttaatgtaa 1860

ttgagagttt gagaccgtct ccttcagtgt ttccagaaca ataaggggt tcagctaatt 1920

catgcggctg ttttttttta aaaaataatt attcattatc agaagaattc atggatagaa 1980

gaatattaat tgttatttgg ttactaattt tctatgaaaa gtgtcttttc ttcgaccttc 2040

ccctcttgcc tgcatgtttc cttttcagct ttccgtagct tcattcaatt acctttttt 2100

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<210> 128
<211> 531
<212> DNA
<213> Glycine sp.

<400> 128
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acgcagtttc ccgacactat cgtctcgccg gaagcttccg tcagttctac cggcacggtt 180

gtttccggcg atttttgctc cgatcgctct tgctgcagct ccagccactt taaggacctc 240

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cgctacttca agccgttcag tttgttgagt gagttttctg gagactcgga ggaatcggcg 360

aagtcttccg cggcagtgcg gaaattgaag acgccaccac aagcagagat cgaagagttt 420

ttcgcgatgg cggaaaagta cgagcgaaaa cggttcacag agaagtacaa ctttgatatt 480

gttagagatt tgccgttgga gggtcgctac cagtgggttc gtttacattg a 531

<210> 129
<211> 201
<212> PRT
<213> Glycine sp.

<400> 129

Met Gly Lys Tyr Met Lys Lys Ser Lys Ile Ala Gly Asp Val Ala Ala
1 5 10 15

Val Ile Met Glu Ala Pro Pro Pro His Ser His Leu Gly Val Arg Thr
20 25 30

Arg Ala Lys Thr Leu Ala Leu Gln Asn Asn Thr Thr Ser Pro Asp Pro
35 40 45

Ser Ala Tyr Leu Gln Leu Arg Ser Arg Arg Leu Leu Lys Leu Pro Pro
50 55 60

Thr Pro Pro Glu Asn Pro Arg Arg Ser Ser Ala Glu Thr Ala Ala Asn
65 70 75 80

Phe Arg Leu Ala Asn Ala Gln Lys Leu Ala Ser Phe Glu Asp Asp Asn
85 90 95

Asn Thr Glu Cys Ser Phe Gly Glu Asn Phe Leu Asp Ala Glu Pro Arg
100 105 110

Glu Glu Arg Ser Thr Arg Glu Gly Thr Pro Cys Ser Leu Ile Arg Asp
115 120 125

Ser Asn Ala Ile His Thr Pro Gly Ser Thr Thr Arg Pro Arg Thr Arg
130 135 140

Gln Ile Ile His Glu His Val Gln Arg Asn Ile Pro Thr Ala Tyr Glu
145 150 155 160

Met Glu Glu Phe Phe Ala Tyr Ala Glu Lys Gln Gln Gln Thr Ile Phe
165 170 175

Met Asp Lys Tyr Asn Phe Asp Ile Val Asn Asp Val Pro Leu Pro Gly
180 185 190

Arg Tyr Glu Trp Val Pro Val Leu His
195 200

<210> 130
<211> 205
<212> PRT
<213> Glycine sp.

<400> 130

Met Gly Lys Tyr Met Lys Lys Ser Lys Ile Ala Gly Asp Val Ala Ala
1 5 10 15

Val Ile Met Glu Ala Pro Pro Pro His Ser His Leu Gly Val Arg Thr
20 25 30

Arg Ala Lys Thr Leu Ala Leu Gln Asn Thr Ser Pro Asp Ser Ser Ala
35 40 45

Tyr Leu Gln Leu Arg Ser Arg Arg Leu Leu Lys Leu Pro Pro Thr Pro
50 55 60

```
Pro Glu Asn Pro Arg Arg Ser Ala Ala Glu Thr Ala Ala Asn Ser Arg
65                  70                  75                  80

Leu Ala Lys Thr Thr Ser Ser Arg Asn Ala Glu Lys Phe Ala Ser Phe
                85                  90                  95

Asp Asp Asp Asn Asn Thr Glu Cys Ser Phe Gly Glu Asn Phe Leu Asp
            100                 105                 110

Ala Glu Pro Arg Glu Glu Arg Ser Thr Arg Glu Ser Thr Pro Cys Ser
        115                 120                 125

Phe Ile Arg Asp Ser Asn Ala Ile His Thr Pro Gly Ser Thr Thr Arg
    130                 135                 140

Pro Arg Thr Arg Gln Ile Ile His Glu His Ile Gln Arg Asn Ile Pro
145                 150                 155                 160

Thr Ala Tyr Glu Met Glu Glu Phe Phe Ala Tyr Ala Glu Lys Gln Gln
                165                 170                 175

Gln Thr Ile Phe Met Asp Lys Tyr Asn Phe Asp Ile Val Asn Glu Val
            180                 185                 190

Pro Leu Pro Gly Arg Tyr Glu Trp Val Pro Val Leu His
        195                 200                 205
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<210> 131
<211> 162
<212> PRT
<213> Glycine sp.

<400> 131

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Met Glu Met Ala Gln Val Lys Ala Arg Ala Arg Thr Ala Leu Ala Met
1               5                   10                  15

Ala Ala Ser Ala Ser Ser Arg Lys Arg Arg Lys Ile Ser Ile Asn Asn
            20                  25                  30

Asn Phe Val Gln Ile Lys Ser Leu Ser Asn Ala Thr Val Pro Ala Thr
        35                  40                  45

Gly Glu Arg Ile Ser Gly Glu Ser Pro Ala Ser Cys Cys Ser Ser Asn
    50                  55                  60

Gly Ser Val Asp Asp Glu Asn Arg Ile Ile Lys Phe Ser Asp Leu Glu
65                  70                  75                  80
```

Val Glu Ser Thr Arg Val Val Thr Ser Thr Cys Asp Cys Gly Glu Gln
85 90 95

Gln Gln Gln Ile Arg Arg Glu Met Ser Leu Thr Ser Glu Leu Arg Ile
100 105 110

Thr Asn Ser Ser Ser Gln Glu Val Asp Ser Ala Glu Glu Gln Ile Thr
115 120 125

Gln Thr Lys Ser Leu Pro Pro Gln Lys Met Pro Thr Glu Leu Glu Leu
130 135 140

Asp Glu Phe Phe Ala Ala Ala Glu Lys Asp Ile Arg Lys Arg Phe Ser
145 150 155 160

Asp Lys

<210> 132
<211> 144
<212> PRT
<213> Glycine sp.

<400> 132

Met Ala Gln Val Lys Ala Arg Ala Arg Thr Ala Leu Ala Met Ala Ala
1 5 10 15

Ser Ala Thr Ser Pro Lys Arg Arg Lys Ile Ser Phe Val Gln Ile Lys
20 25 30

Ser Leu Ser Asn Ala Thr Ser Pro Thr Thr Glu Glu Arg Ile Ser Gly
35 40 45

Glu Ser Pro Ala Ser Cys Cys Ser Ser Asn Gly Ser Phe Asp Asn Glu
50 55 60

Asn Arg Ile Ile Lys Ser Ser Asp Leu Glu Val Glu Ser Ala Gln Val
65 70 75 80

Glu Thr Trp Thr Cys Asn Cys Gly Glu Gln Gln Gln Gln Lys Ile Arg
85 90 95

Arg Glu Met Ser Leu Thr Arg Glu Val Asp Ser Thr Glu Glu His Ile
100 105 110

Thr Lys Thr Lys Ser Arg Cys Val Pro Thr Glu Ser Glu Leu Glu Asp
115 120 125

Phe Phe Ala Ala Ala Glu Lys Asp Ile Gln Lys Arg Phe Thr Asp Lys
130 135 140

<210> 133
<211> 198
<212> PRT
<213> Glycine sp.

<400> 133

Met Ser Ser Gln Val Gly Val Arg Thr Arg Ala Arg Ala Ala Leu Ala
1 5 10 15

Met Glu Ala Ala Thr Ala Ser Ser Ala Gln Pro Ser Ser Lys Arg Lys
20 25 30

Lys Ile Tyr Asp Thr Asn His Val Ala Lys Leu Ser Lys Thr Pro Arg
35 40 45

Thr Ser Ser Ser Ser Phe Phe Ile Pro Ala Thr Val Thr Glu Ile Val
50 55 60

Gln Glu Arg Cys Leu Ser Pro Thr Ser Ser Glu Ile Pro Ala Ser Cys
65 70 75 80

Cys Ser Ser Asn Gly Ser Ile Gly Leu Asp Glu Asp Arg Ile Lys Leu
85 90 95

Leu Asp Leu Glu Val Glu Ser Ala Gln Val Glu Thr Ser Thr Cys Asn
100 105 110

Gly Gly Gln Glu Ile Glu Arg Arg Glu Met Lys Ser Ser Ser Glu Leu
115 120 125

Arg Glu Asn Ser Gln Glu Pro Glu Pro Met Glu Ile Asn Ser His Arg
130 135 140

Ala Leu Ser Lys Ala Lys Ala Met Pro Thr Glu Leu Glu Leu Glu Glu
145 150 155 160

Phe Phe Val Ala Ala Glu Lys Asp Ile Gln Lys Arg Phe Gln Asp Lys
165 170 175

Tyr Asn Tyr Asp Ile Val Lys Asp Val Pro Leu Glu Gly Arg Tyr Glu
180 185 190

Trp Val Gln Leu Lys Pro
195

<210> 134
<211> 205
<212> PRT
<213> Glycine sp.

<400> 134

Met Ser Ala Gln Val Gly Val Arg Thr Arg Ala Gln Ala Ala Leu Ala
1 5 10 15

Met Glu Ala Val Ser Ser Ala Glu Pro Ser Ser Lys Arg Lys Lys Ile
20 25 30

Ser Asn Ser Thr Asn Gln Glu Pro Lys Leu Ser Lys Thr Pro Arg Thr
35 40 45

Ser Ser Ser Ser Ala Val Lys Pro Ala Thr Val Thr Glu Met Val Gln
50 55 60

Pro Val Ser Pro Glu Met Val Gln Gln Arg Cys Leu Ser Pro Thr Ser
65 70 75 80

Ser Glu Ile Pro Ala Ser Cys Cys Ser Ser Asn Gly Ser Ile Gly Leu
85 90 95

Asp Gln Asp Arg Ile Lys Leu Leu Asp Leu Glu Val Glu Ser Ala Gln
100 105 110

Val Glu Thr Ser Thr Cys Asn Gly Gly His Glu Ile Glu Arg Arg Glu
115 120 125

Met Lys Arg Ser Ser Glu Leu Arg Glu Asn Ser Gln Glu Pro Glu Pro
130 135 140

Met Glu Ile Asn Ser His Arg Val Leu Ser Lys Ala Lys Ala Met Pro
145 150 155 160

Thr Glu Leu Glu Leu Glu Glu Phe Phe Ala Ala Ser Glu Lys Asp Ile
165 170 175

Gln Lys Arg Phe Gln Asp Arg Tyr Asn Tyr Asp Ile Val Lys Asp Val
180 185 190

Pro Leu Glu Gly Arg Tyr Glu Trp Val Gln Leu Lys Pro
195 200 205

<210> 135
<211> 224

<212> PRT
<213> Glycine sp.

<400> 135

Met Gly Lys Tyr Met Lys Lys Ala Lys Pro Lys Gly Glu Leu Ala Leu
1 5 10 15

Val Glu Ser Thr Thr Ser Asn Thr Thr Thr Ser Tyr Met Gly Val Arg
20 25 30

Thr Arg Ala Lys Thr Leu Ala Leu Gln Lys Ser His Ala Gln Gln His
35 40 45

Glu Leu Ala Ala Thr Ser Asp Ser Tyr Leu Gln Leu Arg Ser Arg Arg
50 55 60

Leu Gln Lys Pro Pro Ile Leu Val His Ser Pro Lys Arg Pro Lys His
65 70 75 80

Pro Asn Pro Lys Ser Pro Ile Pro Glu Pro Pro Arg Leu Gly Leu Ala
85 90 95

Ser Glu Arg Asp Ala Thr Leu Asn His Asn Lys Asp Asn Thr Leu His
100 105 110

Glu Asn Ala Glu Pro Gln Glu Ala Ser Phe Gly Glu Asn Val Leu Asp
115 120 125

Phe Glu Gly Arg Glu Arg Ser Thr Arg Glu Ser Thr Pro Cys Ser Leu
130 135 140

Ile Arg Asp Ser Asp Thr Val Arg Thr Pro Gly Ser Thr Thr Arg Pro
145 150 155 160

Thr Cys Ser Ala Glu Ala Tyr Arg Arg Thr Glu His Ala Ala Arg Arg
165 170 175

Gln Ile Pro Thr Ser Arg Glu Met Asp Glu Phe Phe Ala Glu Ile Glu
180 185 190

Glu Ala Gln Gln Lys Lys Phe Ile Glu Lys Tyr Asn Phe Asp Pro Val
195 200 205

Asn Glu Lys Pro Leu Ser Gly Arg Tyr Glu Trp Glu Lys Leu Lys Pro
210 215 220

<210> 136
<211> 187

<212> PRT
<213> Glycine sp.

<400> 136

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Met Gly Glu Cys Lys Arg Cys Cys Ser Leu Thr Val Leu Ala Met Glu
1               5                  10                  15

Glu Pro Ser Ser Ser Gln His Ser Ile Phe Lys Lys Arg Lys Thr Thr
            20                  25                  30

Ala Thr Ala Ala His Ser Thr Ser Phe Gln Leu Cys Ser Ser Asp Met
        35                  40                  45

Gln Phe Pro His Thr Ile Val Ser Pro Glu Val Ser Phe Ser Ser Ala
    50                  55                  60

Cys Thr Val Val Ser Gly Glu Phe Cys Ser Asp Arg Ser Cys Cys Ser
65                  70                  75                  80

Ser Ser His Val Lys Asp Leu His Ser Val Pro Ser Asp Leu Gln Thr
                85                  90                  95

Lys Gly Phe Glu Thr Val Glu Asp Ser Thr Ser Leu Asn Phe Lys Ser
            100                 105                 110

Phe Ser Leu Leu Ser Glu Phe Ser Gly Asp Ser Glu Glu Ser Ala Met
        115                 120                 125

Ile Pro Ala Lys Ser Ser Ala Ala Val Leu Lys Val Lys Thr Pro Pro
    130                 135                 140

Lys Ala Glu Ile Glu Glu Phe Phe Ala Met Ala Glu Lys Tyr Glu Gln
145                 150                 155                 160

Lys Arg Phe Thr Glu Lys Tyr Asn Phe Asp Ile Val Arg Asp Leu Pro
                165                 170                 175

Leu Glu Gly Arg Tyr Gln Trp Val Arg Leu His
            180                 185
```

<210> 137
<211> 176
<212> PRT
<213> Glycine sp.

<400> 137

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Met Gly Glu Cys Lys Arg Cys Ser Leu Thr Ile Ala Ala Ile Glu Gln
1               5                  10                  15
```

Pro Ser Ser Ser Gln His Ser Ile Ser Lys Lys Arg Lys Thr Thr Ala
20 25 30

Ser Phe Gln Leu Arg Ser Ser Asp Thr Gln Phe Pro Asp Thr Ile Val
35 40 45

Ser Pro Glu Ala Ser Val Ser Ser Thr Gly Thr Val Val Ser Gly Asp
50 55 60

Phe Cys Ser Asp Arg Ser Cys Cys Ser Ser Ser His Phe Lys Asp Leu
65 70 75 80

His Ser Val Pro Ser Asp Leu Gln Thr Lys Gly Phe Gln Thr Val Glu
85 90 95

Asp Ser Thr Asn Arg Tyr Phe Lys Pro Phe Ser Leu Leu Ser Glu Phe
100 105 110

Ser Gly Asp Ser Glu Glu Ser Ala Lys Ser Ser Ala Ala Val Arg Lys
115 120 125

Leu Lys Thr Pro Pro Gln Ala Glu Ile Glu Glu Phe Phe Ala Met Ala
130 135 140

Glu Lys Tyr Glu Arg Lys Arg Phe Thr Glu Lys Tyr Asn Phe Asp Ile
145 150 155 160

Val Arg Asp Leu Pro Leu Glu Gly Arg Tyr Gln Trp Val Arg Leu His
165 170 175

<210> 138
<211> 1678
<212> DNA
<213> Triticum sp.

<400> 138
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agaacaggaa gagaaggaag aaagaaaaaa gccgatggcc gccaccgccg cggccaccgt 120

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cgccgacttg tcagtctcca gctctccctc tccctgcctt ccttccttcc ttctctcctc 240

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ggagatggag gccttcttcg ctgcggcgga gggcgacgtc gcgcggcgct tcgctgccaa 420

gtgagtaccg cgcacatgca tgagtcaggc aacagcttcc tcctatcctc ttggggaaaa 480

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ccaaaaaagt agttttgggg atcttggtct ttaaaaaaaa actggaacgg accggaagat 840

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aagcaattat atttccagaa ataaaaagtg aagttggcag tgcagtacta ctcctgttct 960

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cgccgcagcc agtcaagtgt cagaggcccg cacagacaga ccacgttgtg tccttttaa 1140

tcattctttt gtagttacct tgtcatgctt tattagctgt aattattgct caccagatgc 1200

ctaatcatgc actgtattag ctcaccatgt aagtcgccag tgtattgttc cacctgtagc 1260

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aagtcattca gagccggact agacaaatac gaccctaggc gtctgtagac gcacaggaca 1620

tgtccgggac agtgtgccct caaatgttct actgcacatc acacccttcg tatcaaaa 1678

<210> 139
<211> 264
<212> DNA
<213> Triticum sp.

<400> 139
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tcgccgccgg cggaggagat ggaggccttc ttcgctgcgg cggagggcga cgtcgcgcgg 180

cgcttcgctg ccaagtacaa ctatgacgtc gtcacagacg ctcccatgga tgggcggtac 240

gagtgggtcc gagtgaggcc gtag 264

<210> 140
<211> 87
<212> PRT
<213> Triticum sp.

<400> 140

Met Ala Ala Thr Ala Ala Ala Thr Val Thr Ala Thr Ala Ala Ala Ser
1 5 10 15

Ser Cys Ser Lys Gly Glu Ser Val Gly Ile Ala Ala Pro Ala Asp Leu
20 25 30

Thr Lys Lys Ala Lys Lys Gly Arg Ser Pro Pro Ala Glu Glu Met Glu
35 40 45

Ala Phe Phe Ala Ala Ala Glu Gly Asp Val Ala Arg Arg Phe Ala Ala
50 55 60

Lys Tyr Asn Tyr Asp Val Val Thr Asp Ala Pro Met Asp Gly Arg Tyr
65 70 75 80

Glu Trp Val Arg Val Arg Pro
85

<210> 141
<211> 1834
<212> DNA
<213> Triticum sp.

<400> 141
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cacacactca cacagtaaaa aaaacgctag catgtgtttt tctgttgctg ttttcttttt 720

ctcttgaaaa ggccagctgc tggtgctagt gtatgaagga atttgaagaa aatcactaca 780

cagataggga gtttttgagc aaacaaaaaa agaaaaatgg ggatcttggt cttttgtaaa 840

aacccacaca cgcaacgggg acggactggg agaattcaga gcagcaaagg aaaaaatgat 900

ttcaactcaa ttatggattt tggcaaatca gctcaagcaa ttatacacta ctatttccaa 960

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Claims

1. A plant cell, plant part, plant tissue culture or whole plant comprising at least one *Kinase Inhibitor Protein (KIP) Related Protein (KRP)* gene, wherein the function of one or more copies of the *KRP* gene is disrupted, and wherein the disruption is due to one or more nucleotide changes of a wild type KRP gene as set forth in Table 14.

2. A plant cell, plant part, plant tissue culture or whole plant comprising at least one *Kinase Inhibitor Protein (KIP) Related Protein (KRP)* gene, wherein the function of one or more copies of the *KRP* gene is disrupted, and wherein the disruption is due to one or more nucleotide changes of a wild type KRP gene as set forth in Table 13.

3. A plant cell, plant part, plant tissue culture or whole plant comprising at least one *Kinase Inhibitor Protein (KIP) Related Protein (KRP)* gene, wherein the function of one or more copies of the *KRP* gene is disrupted, and wherein the disruption is due to one or more nucleotide changes of a wild type KRP gene as set forth in Table 22.

4. The plant cell, plant part, plant tissue culture or whole plant of any of claims 1-3, wherein the plant is a monocotyledonous plant.

5. The plant cell, plant part, plant tissue culture or whole plant of claim 4, wherein the monocotyledonous plant is a species in the *Triticeae* tribe, preferably wherein the plant in the *Triticeae* tribe is a plant in the *Triticum* genus, preferably wherein the plant in the *Triticum* genus is wheat, and preferably wherein the wheat plant is hexaploid.

6. A wheat plant cell, plant part, plant tissue culture or whole plant comprising at least one *Kinase Inhibitor Protein (KIP) Related Protein (KRP)* gene, wherein the function of one or more copies of the *KRP* gene is disrupted, and wherein the disruption is due to one or more nucleotide changes of a wild type KRP gene as set forth in Tables 14, 13 or 22, wherein the disruption is selected from the group consisting of *TaKRP1, TaKRP2, TaKRP4, TaKRP5* and *TaKRP6,* preferably wherein the wheat plant is hexaploid and the disrupted KRP gene is *TaKRP2A, TaKRP2B or TaKRP2D*.

7. A method of increasing organ weight, organ size, organ number and/or yield of a wheat plant in the *Triticeae* tribe, comprising disrupting one or more *KRP* genes as set forth in Tables 14, 13 or 22 in the plant.

8. The method of claim 7, wherein the organ is seed, preferably wherein the plant in the *Triticeae* tribe is a plant in the

Triticum genus.

9. The method of claim 7 or claim 8, wherein the *KRP* in the wheat is *TaKRP1, TaKRP2, TaKRP4, TaKRP5,* or *TaKRP6,* preferably wherein the wheat is hexaploid wheat and the disrupted KRP gene is *TaKRP2A, TaKRP2B* or *TaKRP2D,*

10. The method of claim any of claims 7 to 9, preferably wherein the *KRP* gene function is disrupted by nucleotide substitution, deletion, insertion, homologous recombination, T-DNA, transposon, antisense oligonucleotide, double stranded oligonucleotide, siRNA, shRNA, inverted oligonucleotide repeat, or combination thereof.

11. A method of producing a plant with increased organ size, organ weight, organ number and/or yield compared to a wild type plant, comprising

i) making a cross between a first plant to a second plant to produce a F1 plant, wherein the first plant is in the *Triticeae* tribe comprising one or more disrupted *KRP* genes as set forth in Tables 14, 13 or 22.

12. A method according to claim 11, wherein the method further comprises

ii) backcrossing the F1 plant to the first or the second plant; and
iii) repeating the backcrossing step to generate a near isogenic line,

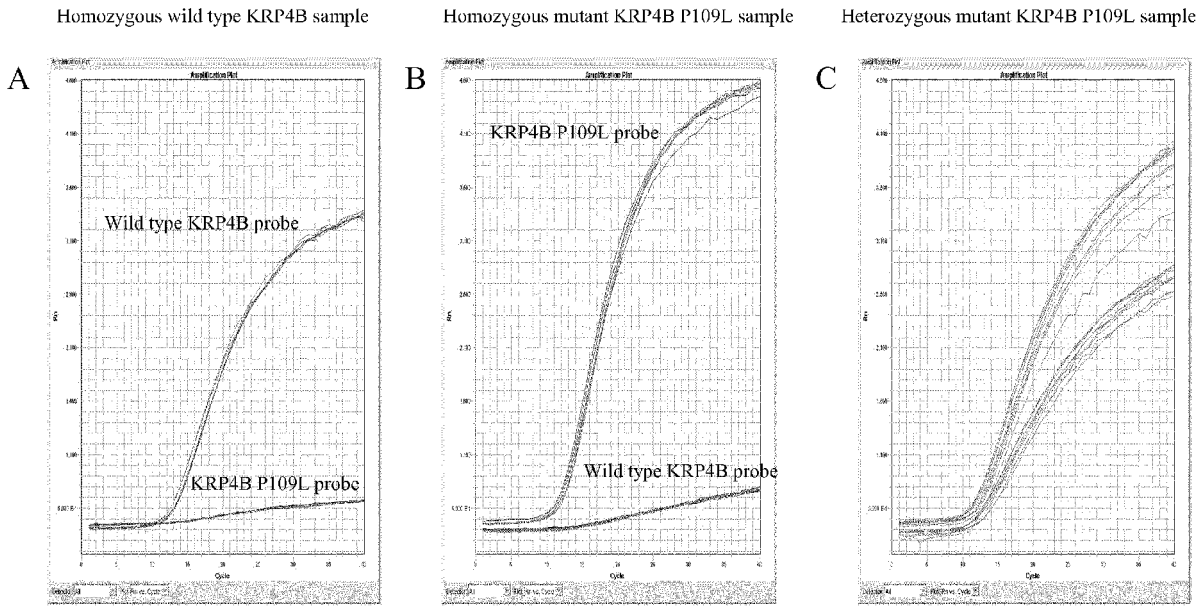
wherein the one or more disrupted *KRP*s in the first plant are integrated into the genome of the near isogenic line.

13. The method of claims 11 or 12, wherein the plant in the *Triticeae* tribe is a plant in the *Triticum* genus, preferably wherein the plant in the *Triticum* genus is a wheat plant.

14. The method of any of claims 11 to 13, wherein the *KRP* in the wheat is *TaKRP1, TaKRP2, TaKRP4, TaKRP5,* or *TaKRP6,* preferably wherein the wheat is hexaploid wheat and the disrupted KRP gene is *TaKRP2A, TaKRP2B or TaKRP2D*.

15. The method of any of claims 11 to 14, wherein the *KRP* gene function is disrupted by nucleotide substitution, deletion, insertion, homologous recombination, T-DNA, transposon, antisense oligonucleotide, double stranded oligonucleotide, siRNA, shRNA, inverted oligonucleotide repeat, or combination thereof.

Figure 1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A
B
C
Wild type KRP4B probe
KRP4B P109L probe
KRP4B P109L probe
Wild type KRP4B probe

Heterozygous mutant KRP4B P109L sample

D

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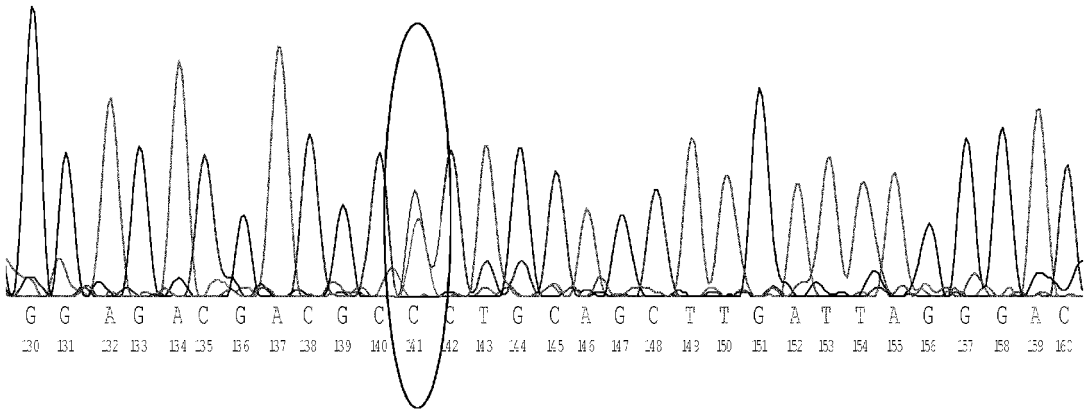
Heterozygous C401T
mutation (P109L)



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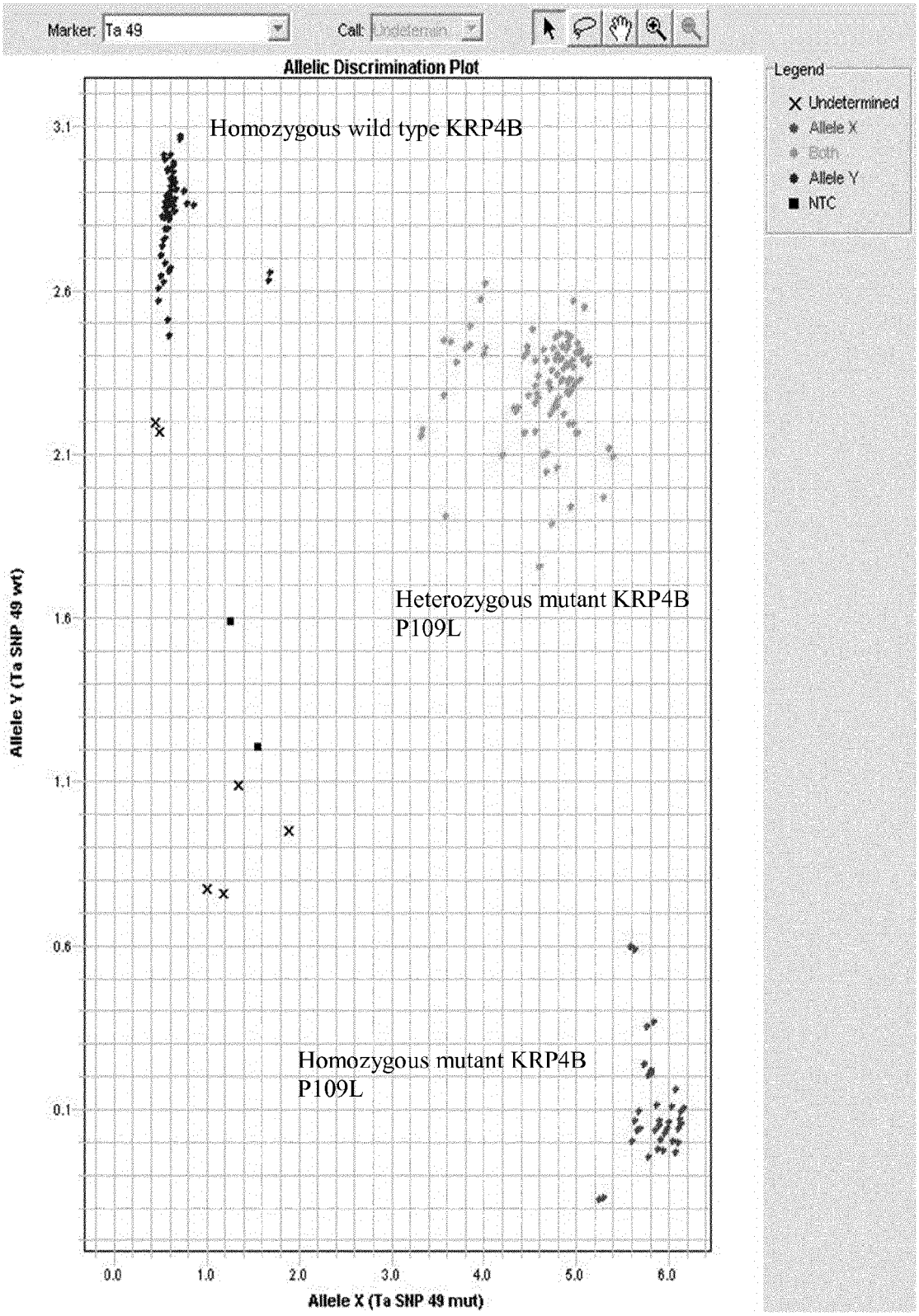
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Allelic Discrimination Plot
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Allele X (Ta SNP 49 mut)
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2.6
2.1
1.6
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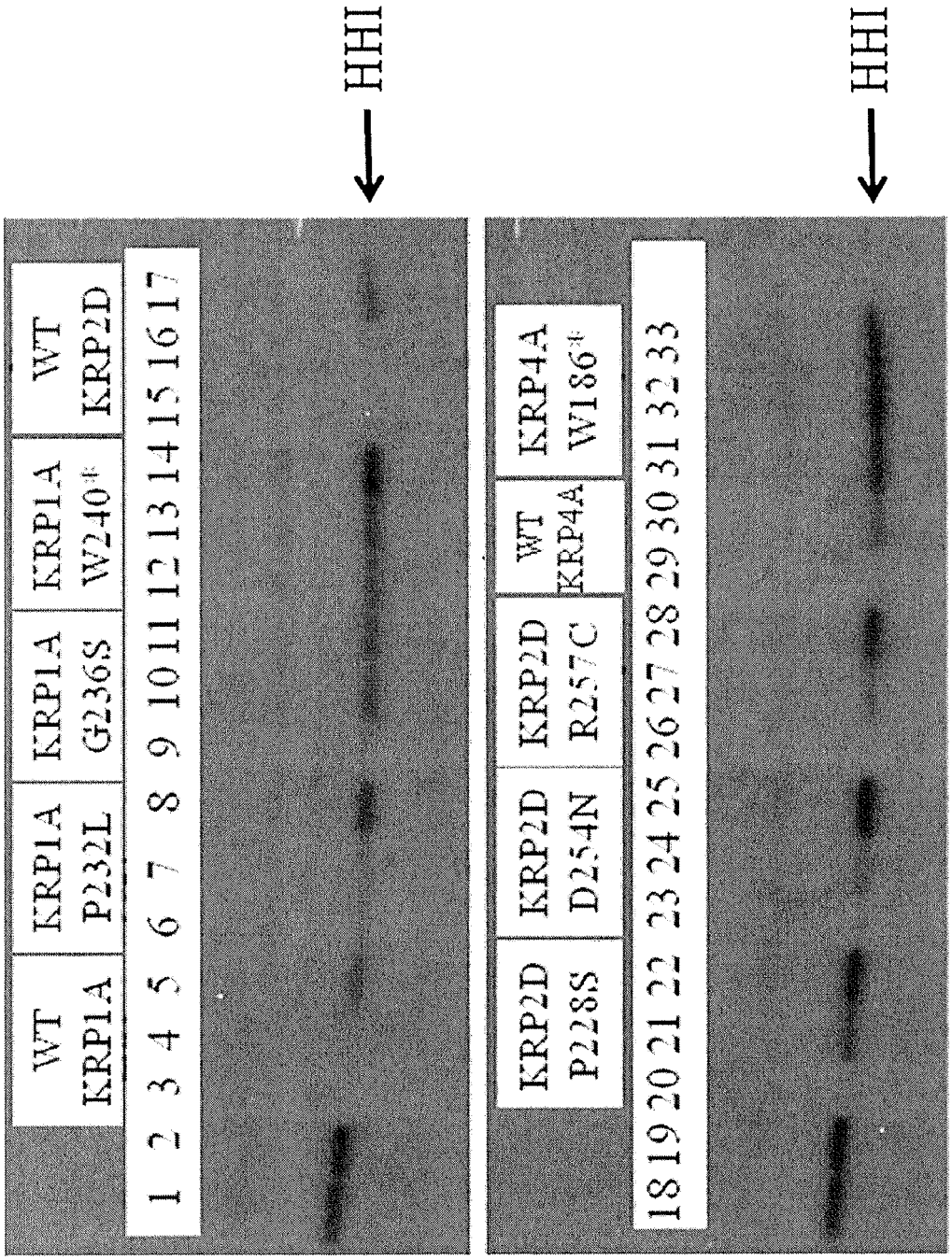
4.0
5.0
6.0

Figure 3

EP 2 927 323 A2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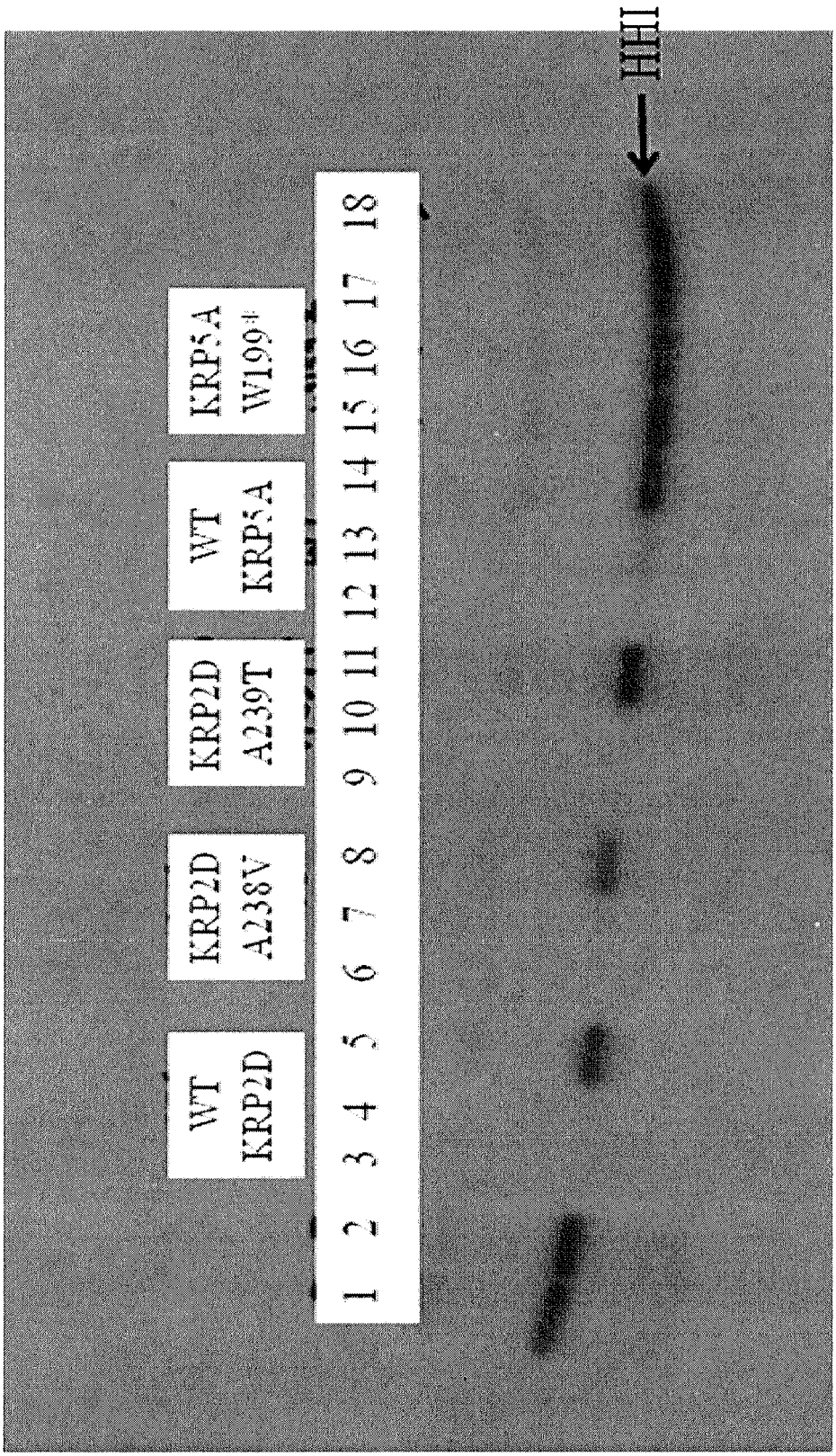
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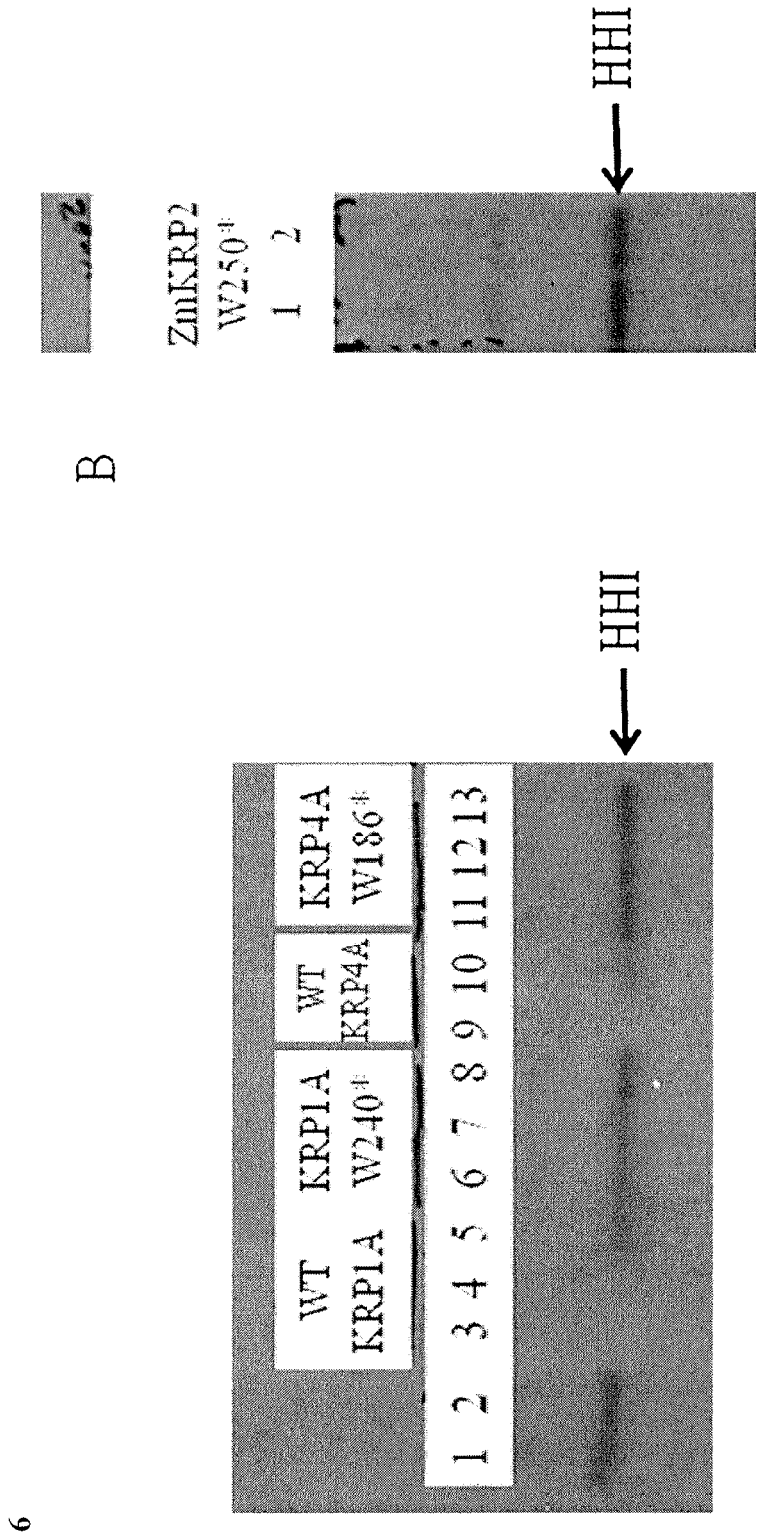
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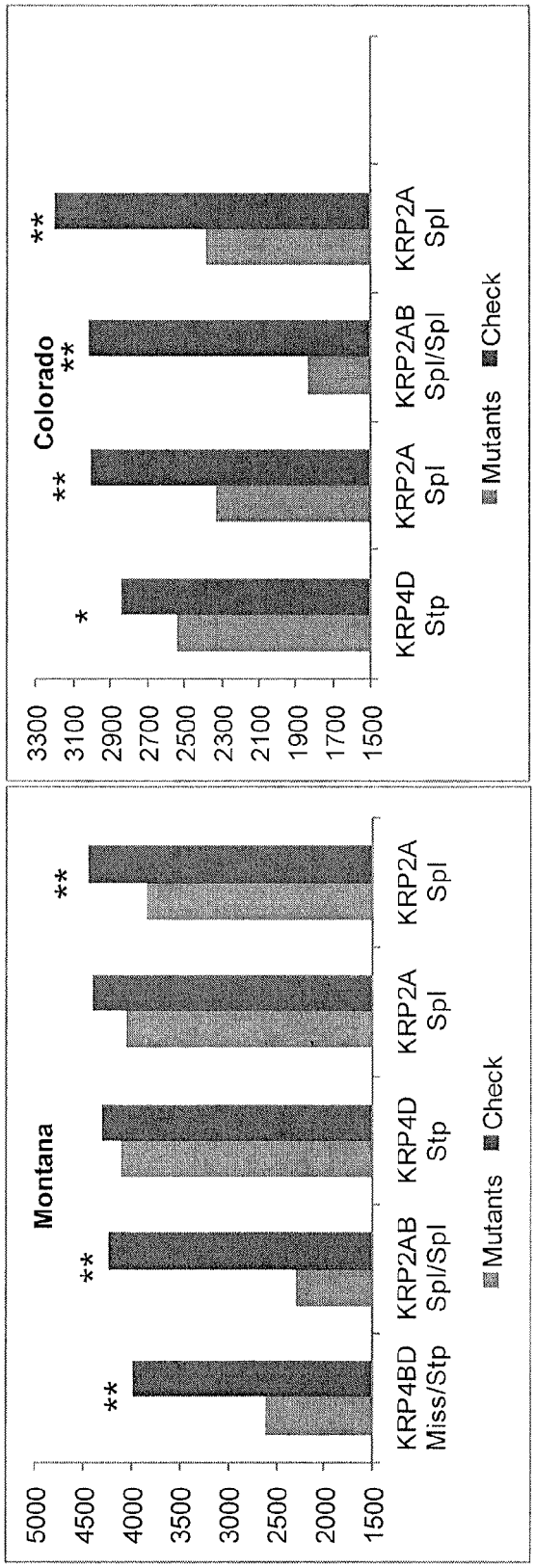
HHI

Figure 6

Figure 7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4000
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3000
2500
2000
1500
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KRP2AB Spl/Spl
KRP4D Stp
KRP2A Spl
KRP2A Spl
Mutants
Check
Colorado
3300
3100
2900
2700
2500
2300
2100
1900
1700
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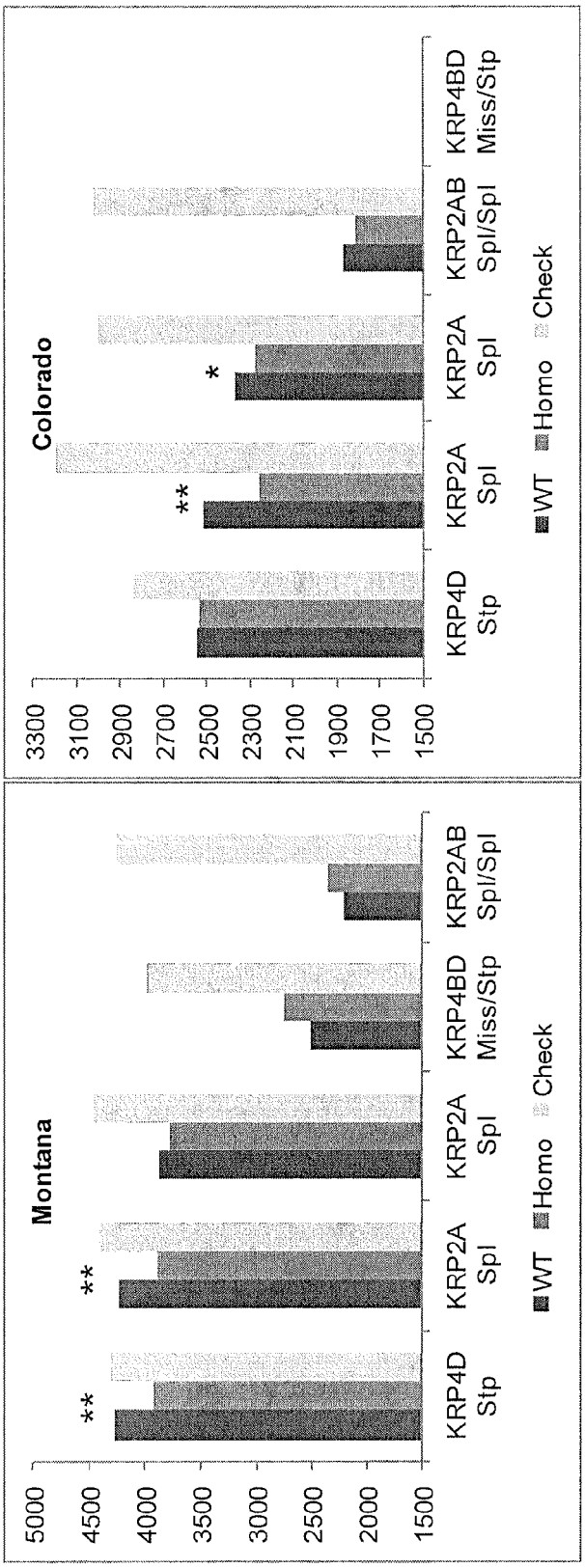
Mutants
Check

Figure 8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Colorado
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1900
1700
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KRP2A Spl
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KRP2AB Spl/Spl
WT
Homo
Check

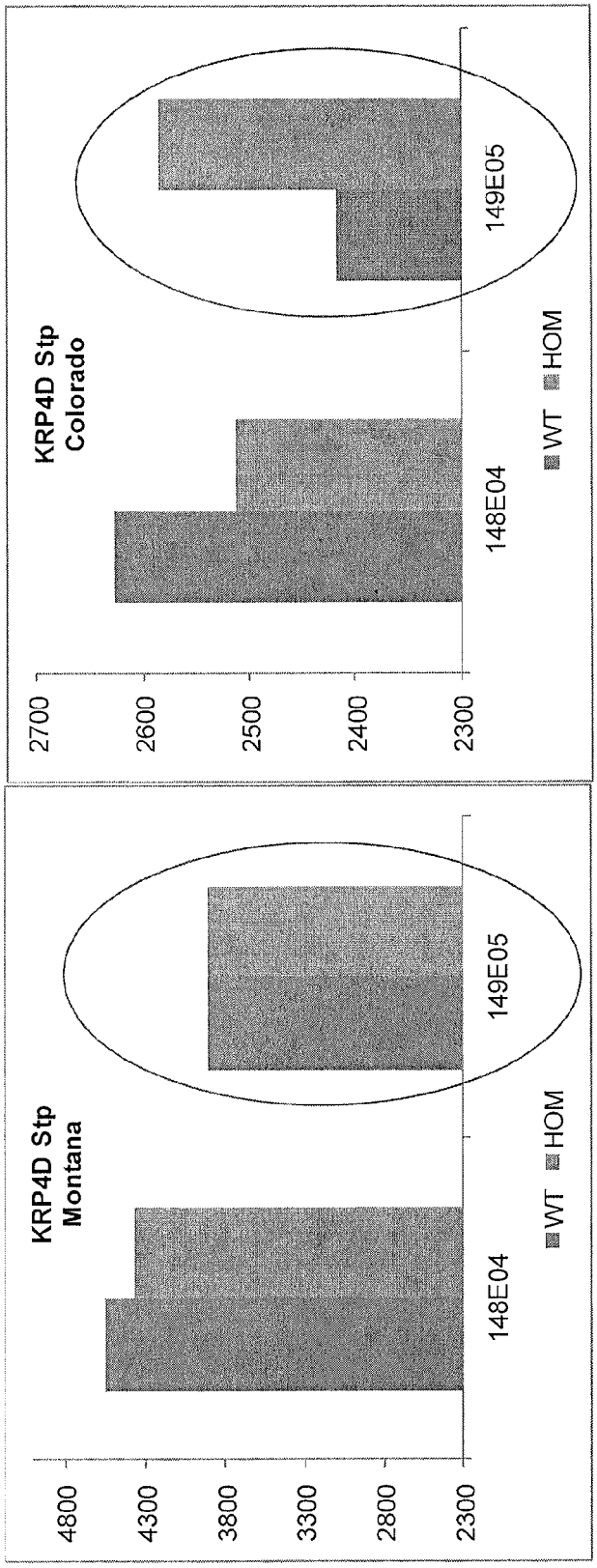
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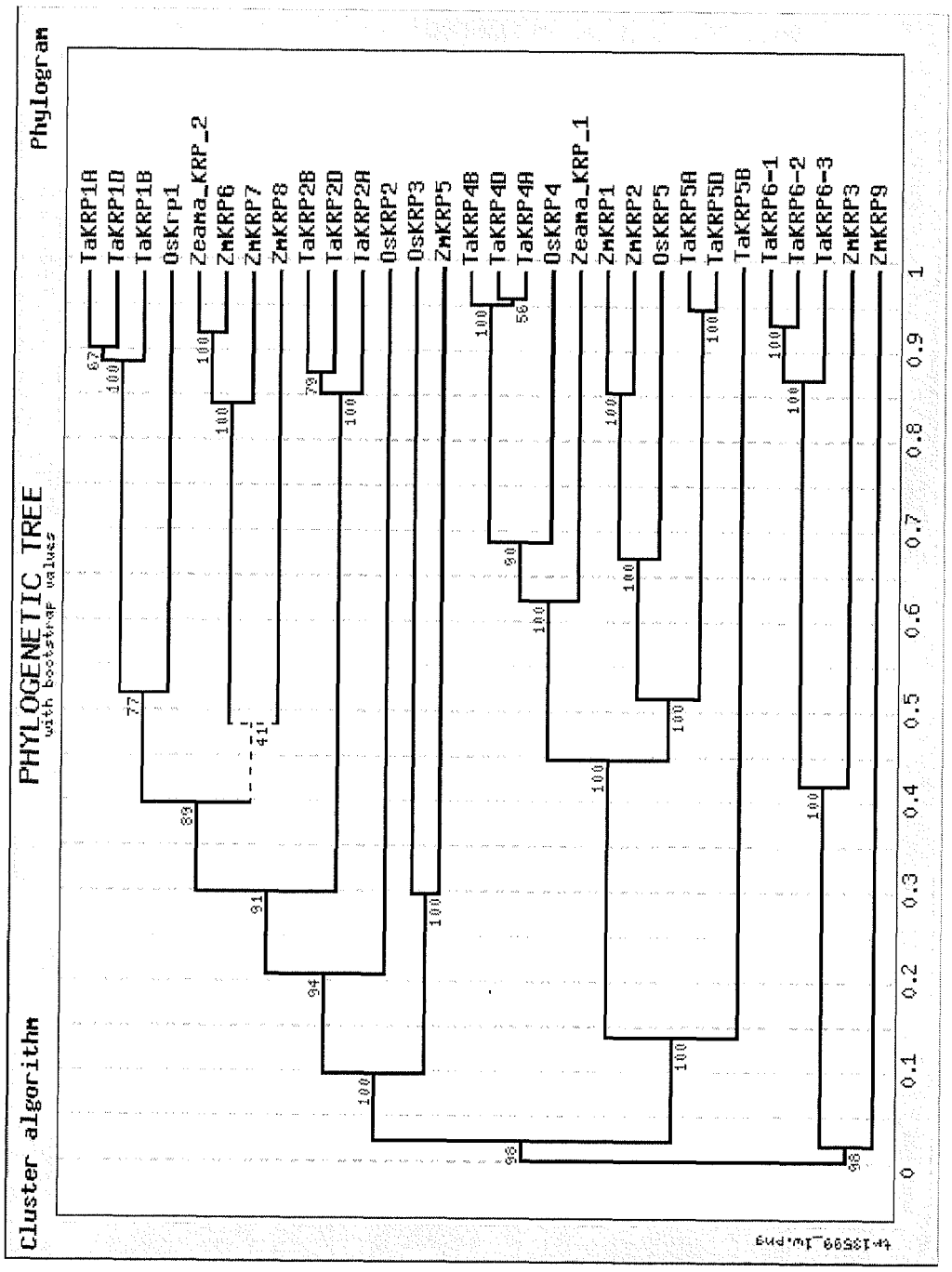
Figure 9

Figure 10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Cluster algorithm
PhyLogram
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OsKrp1
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ZmKRP7
ZmKRP8
TaKRP2B
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TaKRP2A
OsKRP2
OsKRP3
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Figure 11

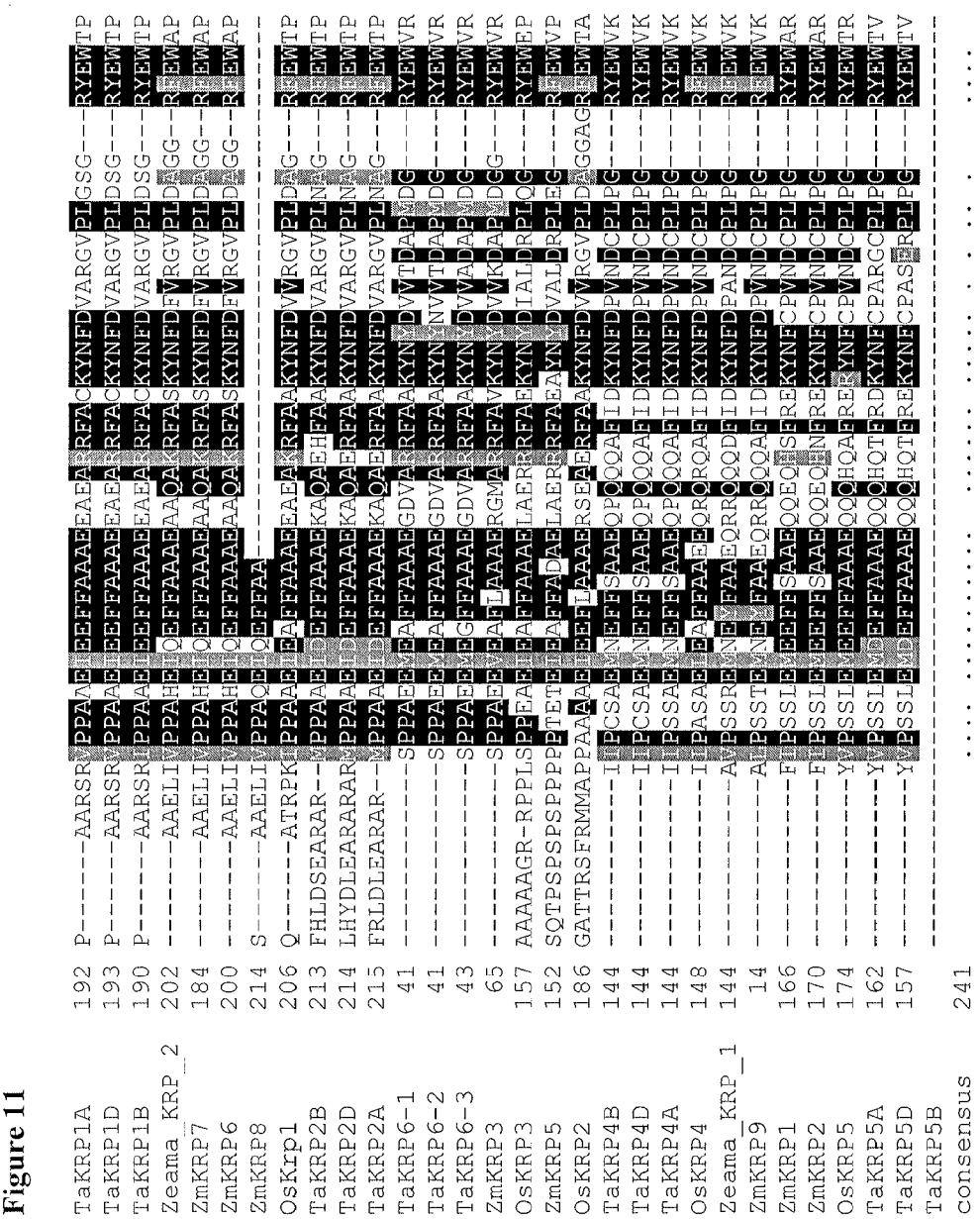

Figure 11 (Continued)

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ZmKRP3           110 RPG------
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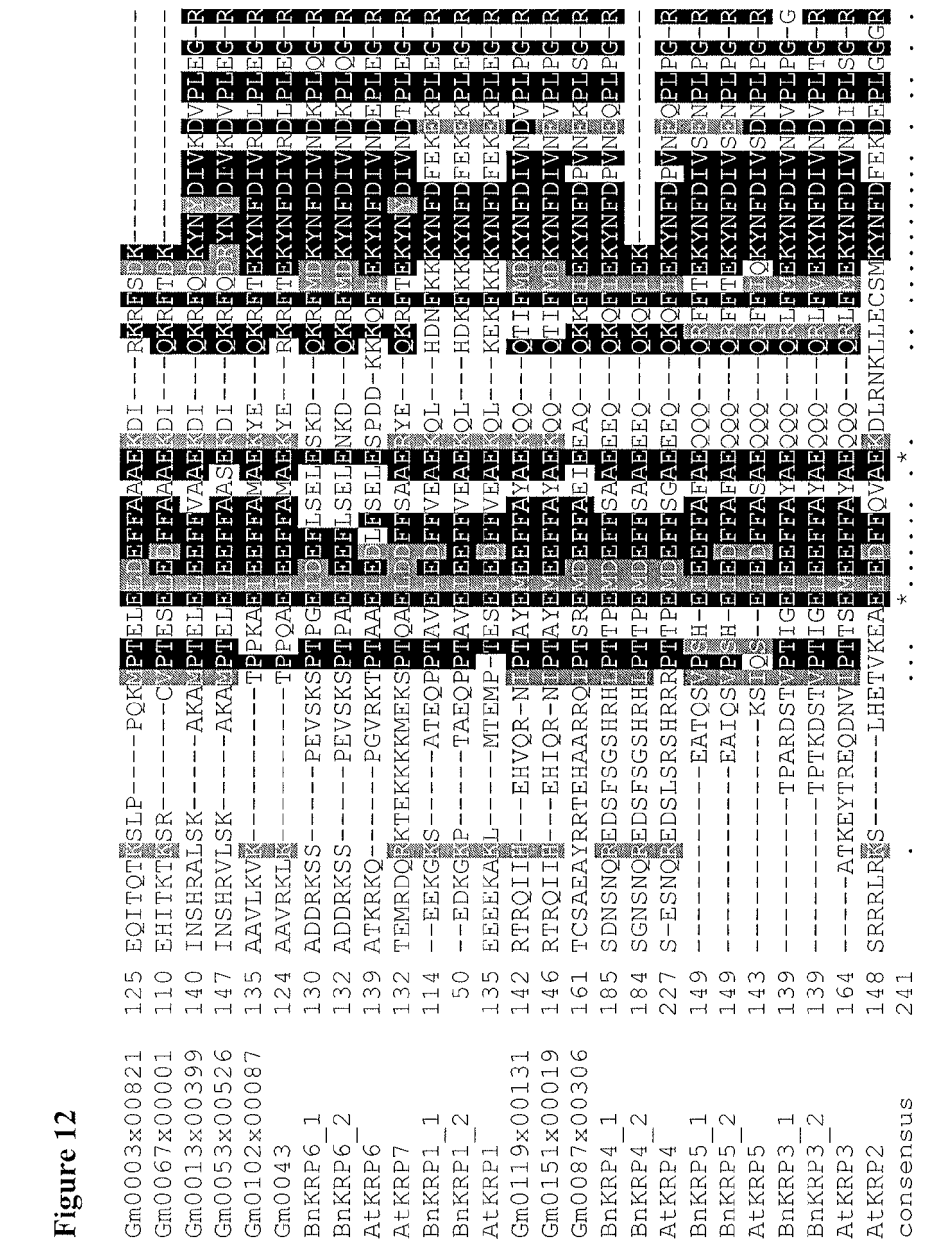

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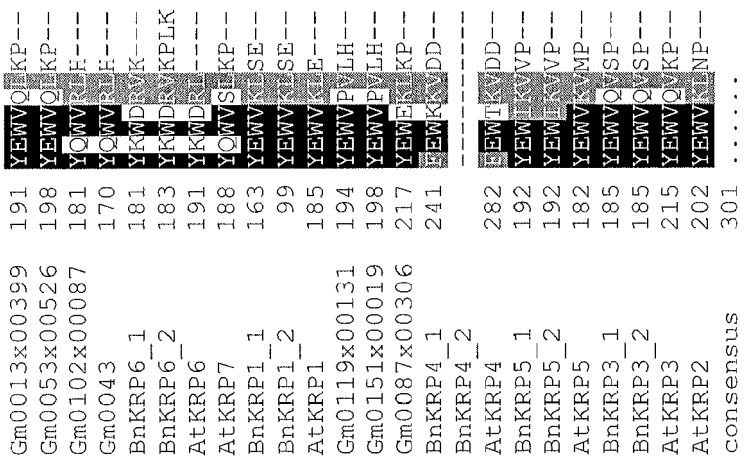

REFERENCES CITED IN THE DESCRIPTION

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- US 7528297 B **[0146]**
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- US 20090320151 A **[0146]**
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