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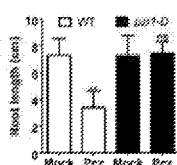


FIG. 1E

(57) **Abstract:** Compositions and methods for increasing plant yield, either directly or through selective inhibition of competing weeds, are disclosed herein. Particular focus is paid to cell cycle signaling permutations such as mutations in the DP-E2F-Rb cell cycle regulatory pathway, and to permutations in brassinolide pathways as transgenic or small-molecule targets for increasing plant yield.

TRANSGENIC PLANTS WITH INCREASED YIELDS

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/710,603, filed February 16, 2018, which application is incorporated herein by reference in its entirety for all purposes.

BACKGROUND

[0002] Plant yield can be increased through the manipulation of the plant's genetic background. For example, manipulation of a plant's genetic makeup can affect seed yield, plant height, leaf erectness, the number of tillers and panicles per plant, and seed size can be affected by manipulation of a plant DNA. In the case of crop plants, increases in plant yield achieved through genetic modification can offer commercial and societal advantages. Some success in increasing seed yield has been achieved through the manipulation of the *DWF4* gene pathway or the *BR11* gene pathway, but more robust strategies are needed.

SUMMARY

[0003] Described herein are methods of engineering a trait of interest in a plant cell. Some methods disclosed herein comprise increasing yield in a crop field comprising a plant having a mutation affecting a signaling pathway selected from the group consisting of: a cell cycle pathway, a hormone pathway, and a metabolic pathway. In some cases, the mutation affects hormone signaling. In some cases, the mutation affects BR signaling. In some cases, the mutation causes increased BR signaling. The mutation affects a cell cycle pathway, in some embodiments. In some cases, the mutation affects Rb signaling. In some cases, the mutation results in a downregulation of Rb signaling activity. In some cases, the mutation alters the expression of at least one gene implicated in DP-E2F signaling. In some cases, the mutation causes overexpression of at least one gene implicated in DP-E2F signaling. In some cases, the mutation comprises an exogenous nucleic acid sequence. In some cases, the mutation affects the expression of the *PZRI* gene. In some cases, the mutation causes the overexpression of the *PZRI* gene. In some cases, the mutation is a *pzr1-D* mutation. In some cases, the plant is homozygous for the *pzr1-D* mutation. In some cases, the at least one gene is selected from the group consisting of: OS11G0549665, OS10G0381601, OS11G0573100, OS11G0549675, OS11G0549680, OS11G0639300, OS01G0146101, OS03G0629800, OS11G0636050, OS07G0159200, OS12G0100100, OS11G0606400, OS03G0223301, OS07G0486000, OS02G0129000, OS11G0540600, OS07G0531900,

OS12G0431300, OS03G0576200, OS08G0203350, OS11G0562100, OS11G0701700, OS12G0222650, OS12G0172150, OS12G0257450, OS12G0247700, OS12G0222300, OS12G0255200, OS12G0250900, OS12G0254400, OS10G0175500, OS05G0202800, OS11G0518900, OS08G0255500, OS11G0689800, OS07G0543500, OS12G0209800, OS11G0255300, OS12G0211500, OS06G0254300, OS11G0134300, OS09G0467700, OS01G0146101, OS01G0148100, OS07G0187001, OS11G0640300, OS12G0257400, OS07G0159200, OS01G0845950, OS11G0691100, OS07G0153150, OS11G0605100, OS03G0223301, OS08G0367300, OS11G0618700, OS07G0162450, OS02G0129000, OS03G0299700, OS11G0549680, OS07G0486000, OS11G0569800, OS09G0467700, OS12G0250900, OS12G0406000, OS11G0696600, OS11G0532600, OS01G0520180, OS07G0297400, OS07G0535200, OS12G0425800, OS11G0685200, OS12G0204600, OS12G0239300, OS07G0677100, OS07G0103000, OS05G0414400, OS11G0693800, OS05G0369900, OS11G0687100, OS12G0425500, OS08G0255500, OS11G0689800, Flowering-promoting factor 1-like protein 1, Cyclin-B1-1, Similar to Lipid transfer protein, Similar to Cyclin-dependent kinase B1-1, Cyclin-dependent kinase A-2, Cyclin-dependent kinase G-1, Proliferating cell nuclear antigen, Cyclin-dependent kinases regulatory subunit 1, Cyclin-dependent kinase G-2, Cyclin-B2-1, Cyclin-P1-1, Similar to Cyclin-D3-1, Cyclin-B2-2, Cyclin-D6-1, Cyclin-D2-2, Cyclin-dependent kinase B2-1, Cyclin-dependent kinase B2-1, and Cyclin-A3-2. In some cases, at least one aspect of the plant is increased, the aspect being selected from the group consisting of: plant weight, tiller number, panicle number, total length, root length, and coleoptile length. In some cases, the yield of the crop field is at least 5% greater than that of a crop field planted with a reference line and grown under similar conditions as the field comprising the plant. In some cases, the yield of the crop field is at least 5% greater than that of a crop field planted with a reference line and grown under similar conditions as the crop field comprising the plant. In some cases, the mutation of the plant of the crop field is a *pzr1-D* mutation. In some cases, the crop field is treated with a herbicide. In some cases, the crop field comprises a weed, and in some cases the herbicide inhibits brassinosteroid synthesis in the weed prior to harvest. In some cases, the herbicide is selected from the group consisting of Brassinazole and propiconazole. In some cases, the mutation affects the expression of the *PZRI* gene. In some cases, the mutation causes overexpression of the *PZRI* gene. In some cases, the mutation is a *pzr1-D* mutation. Some methods disclosed herein comprise clearing a weed from a field comprising: planting the field using a plant having a mutation affecting a signaling pathway selected from the group consisting of: a cell cycle pathway, a hormone signaling pathway, and a metabolic pathway; and administering a herbicide that impacts brassinosteroid signaling. In some cases, the mutation affects a hormone signaling pathway. In

some cases, the mutation affects BR signaling. In some cases, the mutation affects a cell cycle pathway. In some cases, the mutation affects Rb signaling. In some cases, the mutation alters the expression of at least one gene implicated in DP-E2F signaling. In some cases, the mutation affects the expression of the *PZR1* gene. In some cases, the mutation causes the overexpression of the *PZR1* gene. In some cases, the mutation is a *pzr1-D* mutation. In some cases, the herbicide is selected from the group consisting of Brassinazole and propiconazole.

BRIEF DESCRIPTION OF THE DRAWINGS

[0004] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0005] Some understanding of the features and advantages of the subject matter disclosed herein will be obtained by reference to the following detailed description that sets forth illustrative embodiments.

[0006] **FIG. 1A-FIG. 1E** illustrate propiconazole resistance of *pzr1-D* mutant seedlings. FIG. 1A shows representative morphologies of 10-day-old rice seedlings under mock or 30 μ M Pcz treatment in darkness. FIG. 1B shows total lengths and root lengths of seedlings shown in FIG. 1A. FIG. 1C shows RT-PCR comparing *DWF4* expression in mock and Pcz treatments, with actin used as an internal control. FIG. 1D shows representative morphologies of 10-day-old wild-type and mutant rice seedlings under mock or 30 μ M Pcz treatment. FIG. 1E shows quantified root lengths of wild-type and *pzr1-D* seedlings under each treatment.

[0007] **FIG. 2A-FIG. 2I** illustrate phenotypes of *pzr1-D* adult plants. FIG. 2A shows a comparison of plant height between wild type and *pzr1-D* rice plants. FIG. 2B shows a comparison of plant weight between wild type and *pzr1-D* rice plants. FIG. 2C shows a comparison of tiller number between wild type and *pzr1-D* rice plants. FIG. 2D shows a comparison of the number of panicles observed in wild type and *pzr1-D* plants. FIG. 2E shows a comparison of adult plant morphology between wild type and *pzr1-D* rice plants. FIG. 2F shows a comparison of panicle morphology between wild type and *pzr1-D* rice plants. FIG. 2G shows a comparison of seed weight (grams per plant) between wild type and *pzr1-D* rice plants. FIG. 2H shows a comparison of numbers of primary branches observed in wild type and *pzr1-D* rice plants. FIG. 2I shows a comparison of the number of secondary branches observed in wild type and *pzr1-D* rice plants.

[0008] **FIG. 3A-FIG. 3H** illustrate BR-related phenotypes of *pzr1-D* mutant seedlings. FIG. 3A shows representative images of inclination of the segment corresponding to the second leaf in wild-type and *pzr1-D* mutant plants. FIG. 3B shows a quantification of segment inclination angle for wild type and *pzr1-D* plants. FIG. 3C shows representative images of wild type and *pzr1-D* plants

in lamina inclination bioassay testing under mock or 1 μ M brassinolide (BL) treatment. FIG. 3D shows inclination angle quantification results from lamina inclination bioassay testing. FIG. 3E shows a comparison of inclination angle in wild type (circles) and *pzr1-D* (squares) plants when treated with 0 μ M, 0.1 μ M, or 1 μ M BL. FIG. 3F shows a comparison of total plant length for wild-type and *pzr1-D* seedlings grown in darkness. FIG. 3G shows BL sensitivity tested by root inhibition in wild type and *pzr1-D* plants in the presence of BL (1 μ M) and in darkness. FIG. 3H shows a comparison of coleoptile growth in wild type and *pzr1-D* seedlings in response to BL and dark treatment.

[0009] FIG. 4A-FIG. 4F illustrate microscopy analysis of *pzr1-D* and morphology of calli-derived from mutant and wild-type seeds. FIG. 4A shows a schematic representation of a root. The red square in FIG. 4A (60 mm²) shows the point at which the images shown in FIG. 4B were taken. FIG. 4C shows cell counts obtained by analyzing images of calli-derived *pzr1-D* mutant seeds and calli-derived wild-type seeds. FIG. 4D shows cell size measurements obtained by analyzing images of calli-derived *pzr1-D* mutant seeds and calli-derived wild-type seeds. FIG. 4B shows a section of the meristematic zone of PI-stained roots of 7-day-old wild-type and *pzr1-D* seedlings. FIG. 4C shows a quantification of the average number of cells in four samples of wild type and *pzr1-D* seedlings. FIG. 4D shows a comparison of average cell size in four different samples per genotype (wild type vs. *pzr1-D* mutant). FIG. 4E shows morphologies of calli derived from wild-type and *pzr1-D* plants at 16 days, 24 days, and 32 days. FIG. 4F shows growth profiles of wild type and *pzr1-D* calli, illustrating effects of the *pzr1-D* mutation on callus development.

[0010] FIG. 5A-FIG. 5H illustrate activation-tagging T-DNA insertion in *pzr1-D* and *PZR1* overexpression rice lines. FIG. 5A shows a schematic representation of *PZR1* wild-type (WT) and mutant (*pzr1-D*) alleles, including pGA2715 insert comprising tetramer CaMV 35S promoter cassette. FIG. 5B shows RT-qPCR analysis result of three genes in close proximity to the T-DNA insertion (03g05750; 03g05760, *PZR1*; and 03g05770). FIG. 5C shows transcription levels of *PZR1* in plants comprising wild-type (Dongjin), segregating wild-type (*PZR1*), heterozygous (*PZR1/pzr1-D*), and mutant (*pzr1-D*) genotypes. FIG. 5D shows RT-qPCR analysis results of *PZR1* expression in wild type (WT) plants after propiconazole (30 μ M) treatment. FIG. 5E shows representative images of morphologies of 1-month-old plants from wild type (WT) plants, and plants derived from overexpression lines (35S::*PZR1*), OX 1, OX 2, OX 3, OX 9, OX 10, OX 13, OX 16. FIG. 5F shows a comparison of *PZR1* expression in non-transformed (wild type) plants and plants derived from *PZR1* overexpression lines. FIG. 5G shows a comparison of the number of tillers in non-transformed (wild type) plants and plants derived from *PZR1* overexpression lines. FIG. 5G shows

a comparison of the number of panicles in non-transformed (wild type) plants and plants derived from *PZR1* overexpression lines.

[0011] FIG. 6A-FIG. 6E illustrate phylogenetic and expression analysis of *PZR1*. FIG. 6A shows a phylogenetic tree constructed using DP protein sequences from human (UniProt protein ID Q14186 and Q14188), wheat (Q9FET1), Arabidopsis (Q9FNY2 and Q9FNY3), and the putative rice homologs (Q84VA0, Q84VF4 and Q84VD5). FIG. 6B shows a representative image of morphology of 7-day-old rice seedlings showing shoot and root area. FIG. 6C shows RT-qPCR analysis of *PZR1* expression in the shoot and root of wild-type seedlings. FIG. 6D shows a representative image of morphology of adult rice plant. FIG. 6E shows RT-qPCR analysis results comparing the expression of *PZR1* in 7-day-old seedlings in different tissues from adult wild type and *pzr1-D* plants.

[0012] FIG. 7A-FIG. 7E illustrate differentially expressed genes (DEGs) and enriched GO terms for DEGs in mutant and wild-type seedlings. FIG. 7A shows a Venn diagram of genes found to be differentially expressed between wild type and mutant plants under light and dark conditions. FIG. 7B shows a comparison of changes in gene expression in response to light or dark conditions among DEGs. FIG. 7C shows a quantification of DEGs (evaluated in seedlings under dark conditions) separated by gene ontology (GO) category. FIG. 7D shows a quantification of DEGs (evaluated in seedlings under light conditions) separated by gene ontology (GO) category. FIG. 7E shows a quantification of DEGs (observed in seedlings under both light and dark conditions) separated by gene ontology (GO) category.

[0013] FIG. 8A-FIG. 8D illustrate dose response and light studies of propiconazole effect in rice. FIG. 8A shows morphologies of plants after 10 days of treatment with 0 (Mock), 0.1 μ M, 1 μ M, 20 μ M, and 40 μ M of the Brassinosteroid (BR) inhibitor Pcz. FIG. 8B shows a comparison of root length of plants after 10 days of treatment with 0 (Mock), 0.1 μ M, 1 μ M, 20 μ M, and 40 μ M of the Brassinosteroid (BR) inhibitor Pcz. FIG. 8C shows morphologies of wild type and *pzr1-D* plants after 10 days of treatment with under light conditions. FIG. 8D shows a comparison of root lengths of seedlings after 10 days of treatment with 30 μ M Pcz under normal light conditions.

[0014] FIG. 9A-FIG. 9F illustrate propiconazole sensitivity and phenotypes of *pzr1-D* progeny. FIG. 9A shows morphologies of wild type and *pzr1-D* plants. FIG. 9B shows representative images of plants of a variety of genotypes, including: wild type Dongjin (w/w), segregating wild type or *PZR1* (w/w), heterozygous *PZR1/pzr1-D* (w/T), and homozygous mutant *pzr1-D* (T/T). FIG. 9C is a graph comparing plant height in wild-type plants (WT) and plants heterozygous (*PZR1/pzr1-D*) or homozygous (*pzr1-D*) for a *pzr1-D* overexpression mutation. FIG. 9D shows a comparison of panicle number in wild type, heterozygous (*PZR1/pzr1-D*), and homozygous (*pzr1-D*) plants. FIG.

9E shows a comparison of tiller number in wild type, heterozygous (*PZRI/pzr1-D*), and homozygous (*pzr1-D*) plants. FIG. 9F shows RT-qPCR analysis of *PZRI* expression in wild type (DongJin and *PZRI*), heterozygous (*PZRI/pzr1-D* #2 and *PZRI/pzr1-D* #3), and homozygous (*pzr1-D*) plants.

[0015] FIG. 10A-FIG. 10D illustrate analysis of *pzr1-D* seeds. FIG. 10A shows representative images of wild-type and mutant seeds. FIG. 10B shows a comparison of average seed length of wild type and *pzr1-D* seeds. FIG. 10C shows a comparison of average relative area of wild type and *pzr1-D* seeds. FIG. 10D shows a comparison of average weight of wild type and *pzr1-D* seeds.

[0016] FIG. 11A-FIG. 11C illustrate microscopy analysis of leaves from wild type and *pzr1-D* mutant seedlings. FIG. 11A shows images of leaves from 7-day-old seedlings wild-type and mutant seedlings dissected transversally down the middle observed under 10X magnification. FIG. 11B shows images of samples observed under 20X magnification. FIG. 11C shows cell sizes measured using images of samples observed under 20X magnification.

[0017] FIG. 12A-FIG. 12E illustrates phenotypes and gene expression levels of Arabidopsis plants heterologously expressing rice *PZRI*. FIG. 12A shows morphologies of non-transformed Col-0 wild-type plant and representative seedlings from three independent transgenic lines. FIG. 12B shows RNA blots produced from RNA samples collected from seedlings of independent transgenic lines to measure the expression levels of rice *PZRI*. FIG. 12C shows RT-qPCR analysis of *PZRI* expression in transgenic plants using overexpression line OX 8 as a reference. FIG. 12D shows a quantification of primary root length in four overexpression lines (n=10). FIG. 12E shows a quantification of number of lateral roots are shown as the average value (n=10).

[0018] FIG. 13 illustrates promoter regions of the differentially expressed genes (DEGs).

[0019] FIG. 14 illustrates multiple sequence alignment of DP proteins.

DETAILED DESCRIPTION

[0020] The present disclosure provides high-yielding plants and methods for producing high-yielding plants. The present disclosure also provides plants exhibiting resistance to a herbicide and methods for producing plants that exhibit resistance to a herbicide. High-yielding plants can be achieved by increasing plant height, plant weight, tiller number, panicle number, seed number, seed weight, seed size, number of primary branches, number of secondary branches, and/or growth rate as compared to a second plant or second plant line. Herbicide-resistant plants can be achieved by improving at least one of plant height, plant weight, tiller number, panicle number, seed number, seed weight, seed size, number of primary branches, number of secondary branches, growth rate, and/or plant survival in the presence of a herbicide compared to a second plant or second plant line in the presence of the same or a similar herbicide at the same or a similar concentration. High-

yielding plants or herbicide-resistant plants may be produced by modifying the expression of at least one gene or to altering the activity of at least one signaling pathway (e.g., a cell cycle pathway, a hormone regulation pathway, or a metabolic pathway) by using methods disclosed herein, which may comprise genetic engineering techniques. A second plant or second plant line may be a reference plant or reference plant line. A reference plant or reference plant line may be isogenic to a high-yielding plant or a herbicide-resistant plant, as disclosed herein, at a genomic location (or a plurality of genomic locations) other than those modified in the high-yielding plant or herbicide-resistant plant, as disclosed herein. For example, a reference plant or reference plant line may be genetically identical to a high-yielding plant or herbicide-resistant plant described herein except for a genetic modification in the high-yielding plant or herbicide-resistant plant. In some cases, a reference plant or reference plant line, such as a wild type plant or wild type plant line, may lack at least one genetic modification (e.g., an inserted or deleted nucleic acid sequence) that is present in a high-yielding plant or herbicide-resistant plant described herein.

[0021] Methods for producing a high-yielding plant described herein can result in a plant that is resistant to a herbicide. In some cases, methods for producing a herbicide-resistant plant described herein can result in a high-yielding plant.

[0022] Methods disclosed herein include altering expression of a gene or the activity of a biological signaling pathway, such as genes and signaling pathways related to plant growth, metabolic processes, or cellular processes (e.g., cell cycle regulation) to produce a high-yielding plant or a herbicide resistant plant. Methods for producing a high-yielding plant or a herbicide-resistant plant can comprise modulating the expression of a gene or the activity of a signaling pathway that may impact plant yield, that may affect a plant hormone signaling pathway, or that may be implicated in herbicide resistance. For example, altering expression of the E2F Dimerization Partner (DP) gene, the E2F gene, or a gene involved in the DP-E2F-Rb signaling pathway or the brassinosteroid (BR) signaling pathway in a plant cell can produce a high-yielding plant or a herbicide-resistant plant. As described herein, altering expression of the DP gene, the E2F gene, a gene involved in the DP-E2F-Rb signaling pathway, or a gene involved in the BR signaling pathway can be useful in producing a high-yielding plant or a herbicide-resistant plant that exhibits increased height, weight, tiller number, panicle number, seed number, seed weight, seed size, number of primary branches, number of secondary branches, and/or growth rate under normal growing conditions or in the presence of an exogenous agent as compared to second plant, such as a wild-type plant, grown under the same or similar conditions.

[0023] In some cases, the manipulation of expression of at least one gene in a plant or plant cell or the modulation of at least one signaling pathway in a plant or plant cell is used to produce a high-

yield crop field or in methods for clearing weeds from a field. For example, overexpression of the *PZR1* gene in a plant cell is useful for increasing plant yield and for improving the plant's resistance to herbicides, such as propiconazole and Brassinazole.

[0024] Modulation of a gene's expression in a plant or plant cell or modulation of a signaling pathway's activity in a plant or a plant cell, as described herein, can be evaluated with respect to a reference plant or reference plant line. In some cases, a reference plant, plant cell, or plant line can comprise a wild type plant or plant cell. A reference plant, plant cell, or plant line can be an untreated plant or plant cell. It is contemplated that a plant or plant line that would be considered wild type by a person of skill in the art can comprise at least one mutation. In some cases, a reference plant or reference plant line may lack a genetic modification of a high-yielding plant or herbicide-resistant plant, as described herein, but may be otherwise genetically similar to or identical to the high-yielding plant or herbicide-resistant plant. For example, a reference plant or reference plant line used to evaluate the yield or herbicide resistance traits of a high-yielding plant or herbicide-resistant plant described herein may be isogenic to the high-yielding plant or herbicide-resistant plant at a plurality of genetic locations other than the location(s) at which the high-yielding plant or herbicide-resistant plant has been modified according to methods described herein.

Modulation of Gene Expression

[0025] One method of increasing plant yield or resistance of a plant to a herbicide is the modulation of at least one gene's expression in the plant or plant cell (e.g., relative to a reference plant or plant cell). Modulation of a gene's expression in a plant or plant cell, as described herein, can modulate activity of at least one signaling pathway (e.g., a hormone signaling pathway, a cell cycle regulation pathway, a pathway related to cellular processes, or a pathway related to metabolic processes). A number of beneficial effects described herein can be conferred upon a plant as a result of modulating expression at least one gene in the plant, such as increased plant height, plant weight, tiller number, panicle number, seed number, seed weight, seed size, number of primary branches, number of secondary branches, growth rate and/or resistance to a herbicide. In some cases, modifying expression of a gene comprises overexpressing the gene. Methods of modifying expression of a gene comprising downregulation or repression of a gene's expression are also consistent with embodiments described herein.

[0026] One approach for producing a high-yielding plant or a herbicide-resistant plant is overexpressing a gene in a plant or plant cell. Overexpression of a gene can be accomplished in a number of ways, including increasing transcription of at least one DNA sequence, decreasing repression of a DNA sequence, increasing translation of an RNA molecule into a protein, or a

combination thereof. Increasing transcription of a DNA sequence can comprise introducing an exogenous DNA sequence into a cell's genomic DNA. An exogenous DNA sequence can be stably or transiently introduced into a cell's genomic DNA.

[0027] Methods of producing a high-yielding plant or a herbicide-resistant plant can include downregulating a gene in a plant or plant cell. Methods of downregulating a gene's expression include repressing a gene's expression or deleting at least one allele of the gene from a cell's genome (e.g., through the introduction of a nucleic acid sequence into a cell). Repression of a gene of interest can include upregulation of a gene that inhibits expression of the gene of interest. Deletion of at least one allele of a gene is accomplished by removing a DNA sequence from a cell's genome. In some cases, downregulation of a gene's expression comprises introducing a genetic sequence into a cell's DNA that encodes an RNA molecule or protein that is non-functional or partially functional. For example, an RNA molecule that lacks a 5' cap or a poly-A tail can be a non-functional or a partially functional RNA. In some cases, downregulation of a gene's expression can comprise introducing a nucleic acid sequence into a cell that encodes a truncated protein.

[0028] A gene's expression can be modulated directly or indirectly. In some cases, direct modulation of a gene's expression results in a greater degree of modulation of a gene's expression than indirect modulation of a gene's expression. Indirect modulation of a gene's expression can reduce the risk of off-target effects possible with some methods of direct modulation of gene expression.

[0029] Direct modulation of a gene's expression can comprise insertion of a nucleic acid sequence into a cell's genome. For example, a gene's expression in a plant can be modulated by inserting of a nucleic acid encoding a gene or a portion of a gene into a cell's genomic DNA. Additionally or alternatively, direct modulation of a gene's expression can comprise removal of all or a portion of a nucleic acid sequence encoding a gene or a portion of a gene from a cell's genomic DNA. Direct modulation of a gene's expression can result in increased expression of the gene (e.g., overexpression) or decreased expression of the gene (e.g., underexpression, knock-down, silencing, or deletion). Insertion of at least one nucleic acid sequence into a cell's genomic DNA can result in increased expression. For example, insertion of a nucleic acid sequence encoding an additional copy of an endogenous gene into a cell's genomic DNA can result in increased overall expression of the gene in the cell. Insertion of at least one nucleic acid sequence into the sequence of an endogenous gene can cause decreased expression of the gene. For example, insertion of at least one nucleic acid sequence into the sequence of an endogenous gene can interfere with transcription of the endogenous gene.

[0030] In some cases, direct modulation of a gene's expression can comprise removing (e.g., deleting) a nucleic acid sequence from a cell's genomic DNA. Removing a nucleic acid sequence (e.g., removing all or a portion of a nucleic acid sequence encoding a gene of interest) from a cell's genomic DNA can result in decreased expression of a gene's expression. In some cases, indirect modulation of a gene's expression can comprise removing (e.g., deleting) a nucleic acid sequence from a cell's genomic DNA. For example, removing a nucleic acid sequence that influences the expression of a gene of interest (e.g., an enhancer sequence, a promoter sequence, or a silencer sequence) can modulate the expression of the gene of interest.

[0031] Indirect modulation of a gene's expression can also result in increased expression of the gene or decreased expression of the gene. For example, insertion of a nucleic acid sequence that aids in transcription of a gene (e.g., a promoter sequence or an enhancer sequence) into a cell's genomic DNA can comprise indirect modulation of the gene's expression. Insertion of a nucleic acid sequence that inhibits transcription of a gene (e.g., a silencer sequence) into a cell's genomic DNA can comprise indirect modulation of the gene's expression.

[0032] Modulation of one gene's expression in a plant (e.g., direct or indirect modulation of gene expression) or plant cell can be sufficient to increase yield or herbicide resistance in a plant or a field in which the plant is grown. A gene related to cell cycle modulation, hormone signaling, cellular processes, or metabolic processes can be modulated to increase yield or herbicide resistance in a plant or a field in which the plant is grown. As a representative example, altering expression of a gene involved in DP, E2F, or BR signaling may alter expression of at least one additional gene. For example, altering expression of a first gene in the BR signaling pathway can alter expression of a second gene in the BR signaling pathway. In some cases, altering first gene involved in a first signaling pathway can affect expression of a second gene involved in a second signaling pathway.

[0033] Modulation of a plurality of genes' expression in a plant or plant cell can offer improved control over cellular signaling pathways. For example, production of a high-yield plant can comprise modulation of a first gene's expression and modulation of a second gene's expression. In some cases, expression of a first gene and expression of a second gene are both increased in methods described herein. In some cases, expression of a first gene and expression of a second gene are both decreased in methods described herein. Increasing expression of a first gene and decreasing expression of a second gene can also be advantageous for methods described herein. For example, increasing the expression of a first gene (e.g., DP1) while decreasing the expression of a second gene (e.g., Rb) that encodes an RNA or protein capable of antagonizing the expression of the first gene is a strategy that can be used in methods disclosed herein. In some cases, strategies

for increasing expression of a plurality of genes can be used to activate or enhance the activity of a first signaling pathway while inhibiting the activity of a second signaling pathway.

[0034] As such, expression of 2, 3, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 1000, from 2 to 50, from 50 to 100, from 100 to 200, from 200 to 500, from 500 to 1000 or more than 1000 genes can be altered.

[0035] In some cases, methods described herein can cause a change in a gene's expression of 1.1 fold to 1.2 fold, 1.2 fold to 1.3 fold, 1.3 fold to 1.4 fold, 1.4 fold to 1.5 fold, 1.5 fold to 1.6 fold, 1.6 fold to 1.7 fold, 1.7 fold to 1.8 fold, 1.8 fold to 1.9 fold, 1.9 fold to 2.0 fold, 2.0 fold to 2.4 fold, 2.4 fold to 2.6 fold, 2.6 fold to 2.8 fold, 2.8 fold to 3 fold, 3 fold to 3.5 fold, 3.5 fold to 4 fold, 4 fold to 5 fold, 5 fold to 10 fold, 10 fold to 20 fold, 20 fold to 30 fold, 30 fold to 40 fold, 40 fold to 50 fold, 50 fold to 60 fold, 60 fold to 70 fold, 70 fold to 80 fold, 80 fold to 90 fold, 90 fold to 100 fold, 100 fold to 200 fold, 200 fold to 500 fold, 500 fold to 1000 fold, 1000 fold to 2000 fold, 2000 fold to 5000 fold, 5000 fold to 10000 fold, more than 10000 fold, from 5 fold to 10 fold, from 1 fold to 20 fold, or from 1 fold to 40 fold relative to that of a gene in a plant (e.g., a reference plant or reference plant line) that has not been subjected to the same method(s). In some cases, methods described herein can cause an increase in a gene's expression of 1.1 fold to 1.2 fold, 1.2 fold to 1.3 fold, 1.3 fold to 1.4 fold, 1.4 fold to 1.5 fold, 1.5 fold to 1.6 fold, 1.6 fold to 1.7 fold, 1.7 fold to 1.8 fold, 1.8 fold to 1.9 fold, 1.9 fold to 2.0 fold, 2.0 fold to 2.4 fold, 2.4 fold to 2.6 fold, 2.6 fold to 2.8 fold, 2.8 fold to 3 fold, 3 fold to 3.5 fold, 3.5 fold to 4 fold, 4 fold to 5 fold, 5 fold to 10 fold, 10 fold to 20 fold, 20 fold to 30 fold, 30 fold to 40 fold, 40 fold to 50 fold, 50 fold to 60 fold, 60 fold to 70 fold, 70 fold to 80 fold, 80 fold to 90 fold, 90 fold to 100 fold, 100 fold to 200 fold, 200 fold to 500 fold, 500 fold to 1000 fold, 1000 fold to 2000 fold, 2000 fold to 5000 fold, 5000 fold to 10000 fold, more than 10000 fold, from 5 fold to 10 fold, from 1 fold to 20 fold, or from 1 fold to 40 fold relative to that of a gene in a plant (e.g., a reference plant or reference plant line) that has not been subjected to the same method(s).

[0036] Plants and methods disclosed herein can comprise modulating expression of at least one gene or modulating activity of at least one signaling pathway. For example, producing a high-yielding plant or a herbicide resistant plant can comprise altering activity or expression of a gene involved in cellular processes, metabolic processes, hormone signaling, or cell cycle regulation, such as genes involved in DP, E2F, or BR signaling. Representative examples of genes that can be affected by altering expression of genes involved in DP, E2F, or BR signaling include: Flowering-promoting factor 1-like protein 1, Cyclin-B1-1, Similar to Lipid transfer protein, Similar to Cyclin-dependent kinase B1-1, Cyclin-dependent kinase A-2, Cyclin-dependent kinase G-1, Proliferating cell nuclear antigen, Cyclin-dependent kinases regulatory subunit 1, Cyclin-dependent kinase G-2,

Cyclin-B2-1, Cyclin-P1-1, Similar to Cyclin-D3-1, Cyclin-B2-2, Cyclin-D6-1, Cyclin-D2-2, Cyclin-dependent kinase B2-1, Cyclin-dependent kinase B2-1, Cyclin-A3-2, and the following representative examples of rice genes: OS11G0549665, OS10G0381601, OS11G0573100, OS11G0549675, OS11G0549680, OS11G0639300, OS01G0146101, OS03G0629800, OS11G0636050, OS07G0159200, OS12G0100100, OS11G0606400, OS03G0223301, OS07G0486000, OS02G0129000, OS11G0540600, OS07G0531900, OS12G0431300, OS03G0576200, OS08G0203350, OS11G0562100, OS11G0701700, OS12G0222650, OS12G0172150, OS12G0257450, OS12G0247700, OS12G0222300, OS12G0255200, OS12G0250900, OS12G0254400, OS10G0175500, OS05G0202800, OS11G0518900, OS08G0255500, OS11G0689800, OS07G0543500, OS12G0209800, OS11G0255300, OS12G0211500, OS06G0254300, OS11G0134300, OS09G0467700, OS01G0146101, OS01G0148100, OS07G0187001, OS11G0640300, OS12G0257400, OS07G0159200, OS01G0845950, OS11G0691100, OS07G0153150, OS11G0605100, OS03G0223301, OS08G0367300, OS11G0618700, OS07G0162450, OS02G0129000, OS03G0299700, OS11G0549680, OS07G0486000, OS11G0569800, OS09G0467700, OS12G0250900, OS12G0406000, OS11G0696600, OS11G0532600, OS01G0520180, OS07G0297400, OS07G0535200, OS12G0425800, OS11G0685200, OS12G0204600, OS12G0239300, OS07G0677100, OS07G0103000, OS05G0414400, OS11G0693800, OS05G0369900, OS11G0687100, OS12G0425500, OS08G0255500, OS11G0689800.

[0037] Methods for producing a high-yielding plant or a herbicide-resistant plant can comprise modulation of a gene involved in a hormone signaling pathway (e.g., comprising hormone synthesis, hormone degradation, or hormone regulation), such as the *PZRI* gene. For example, overexpression of the *PZRI* gene in a rice plant cell can produce a high-yielding plant or a herbicide-resistant plant. Modulation of the *PZRI* gene can comprise incorporation of all or a portion of a vector's nucleic acid sequence to a genomic location within a distance of 0 kilobases (KB) to 0.5 KB, from 0.5 KB to 1.0 KB, from 1.8 KB to 3.8 KB, from 1.0 KB to 5.0 KB, from 5.0 KB to 10 KB, from 0 KB to 2.0 KB, from 1.0 KB to 4.0 KB, from 0 KB to 5 KB, or more than 10 KB of a *PZRI* gene can result in the modulation of the expression of the *PZRI* gene. In some cases, a high-yielding plant can be produced by introducing a T-DNA vector to a genomic location within a distance of 0 KB to 0.5 KB, from 0.5 KB to 1.0 KB, from 1.8 KB to 3.8 KB, from 1.0 KB to 5.0 KB, from 5.0 KB to 10 KB, from 0 KB to 2.0 KB, from 1.0 KB to 4.0 KB, from 0 KB to 5 KB, or more than 10 KB of a *PZRI* gene can result in modulation of the expression of the *PZRI* gene. In some cases, at least one CaMV 35 S promoter can be introduced to a genomic location with a distance of 0 KB to 0.5 KB, from 0.5 KB to 1.0 KB, from 1.8 KB to 3.8 KB, from 1.0 KB to 5.0

KB, from 5.0 KB to 10 KB, from 0 KB to 2.0 KB, from 1.0 KB to 4.0 KB, from 0 KB to 5 KB, or more than 10 KB of a *PZRI* gene to cause the modulation of the expression of the *PZRI* gene.

Genes can be overexpressed as described above by functionally linking the gene to an exogenous promoter, such as cauliflower mosaic virus promoter (CaMV 35S). In some cases, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 copies of a CaMV 35 S promoter can be introduced to a genomic location with a distance of 0 KB to 5 KB of a *PZRI* gene to cause the modulation of the expression of the *PZRI* gene.

[0038] An example of a plant with altered expression of a gene that is functionally linked to an exogenous promoter is *pzr1-D*. A *pzr1-D* mutant plant or plant cell can comprise overexpression of at least one gene. Many embodiments of a *pzr1-D* plant or plant cell can comprise overexpression of the *PZRI* gene. The expression of a putative transcription factor gene homologous to Arabidopsis Dimerization Partner (*DPb*) is activated in *pzr1-D*. *pzr1-D* plants (e.g., *pzr1-D* mutants) also exhibit phenotypes including increased seed yields, reduced height, increased tiller number, and increased BR sensitivity. *pzr1-D* plants may display altered cell division phenotypes, including the production of small calli. In addition, the cell number and size in mutant roots and leaves differed from those in wild-type plants of the same age. A rice *propiconazole resistant1-D* (*pzr1-D*) mutant can be isolated by screening an activation-tagging mutant population of rice in the presence of the BR biosynthesis inhibitor propiconazole (Pcz). *PZRI* expression may thus be manipulated to increase seed yield in economically important rice varieties.

[0039] Like Arabidopsis *DPb*, rice *PZRI* is expressed differentially in the different tissues of a plant.

[0040] Analogous genes from other plant species (e.g., which may be modulated in methods for producing high-yielding plants or herbicide-resistant plants) can be identified by running a BLAST search and a backBLAST search using the accession number(s) or sequence(s) of genes disclosed herein (e.g., rice genes disclosed herein) and comparing the results of those searches. Genes returning the strongest correlation in both directions (e.g., the closest matches from both BLAST and backBLAST searches) can reasonably be assumed to be homologous to the genes disclosed herein. Genes found to be homologous or potentially homologous to genes disclosed herein may be useful in producing high-yielding plants or herbicide-resistant plants.

[0041] Throughout the disclosure, reference is made to particular genes, loci, transcripts, or encoded gene products. Reference is often made to a particular allele of a particular species. However, it is understood by one of skill in the art that there are a number of approaches for identifying homologous genes, loci, transcripts, or encoded gene products in related species, members of a related genus, common family members, or members of a common or related

division in plant phylogeny. For example, a gene sequence may be blasted against a sequence database comprising sequence of another species, such that strongest hits may be identified. Such hits may be scrutinized for presence of particular motifs indicative of the homologue of interest and/or may be searched against the genome or other sequence information of the source organism, such that a 'back-blast' which identifies the original starting sequence indicates that the second species hit is likely a homologue. Alternate approaches for identifying homologous genes or proteins across species are known to those of skill in the art, such that disclosure of a particular sequence, gene name, protein name or allele enables one to find a homologous gene, protein or allele in any number of crop species, monocot or dicot relatives, or other flowering or nonflowering plant.

Modulation of Signaling Pathways

[0042] Alternatively to, in addition to, in combination with, or as a consequence of modulation of gene expression in a plant or plant cell, activity of a signaling pathway in a plant or plant cell can be modulated in methods of producing a high-yielding plant or a herbicide-resistant plant.

Modulation of a signaling pathway can comprise altering a cellular process, a metabolic process, hormone signaling, or a cell cycle pathway. In some cases, a method of producing a high-yielding plant or a herbicide-resistant plant alters activity of a signaling pathway (e.g., relative to that of a reference plant) without altering the level at which a gene is expressed. For example, methods of genetic engineering described herein can result in a non-functional RNA or protein (e.g., an immature RNA or a truncated protein) without altering the transcription of a gene from which the RNA or protein is produced.

[0043] The yield of a plant and a plant's robustness in response to environmental changes are largely dependent on genes controlling the plant's growth characteristics. For example, plants respond to environmental cues by altering their growth in many cases. Many growth characteristics of plants described herein are dependent on regulation of the plant's cell cycle.

[0044] The regulation of the cell cycle can be important in strategies for producing high-yield plants and high-yielding crop fields. Many aspects of plant growth depend on both cell elongation and division, which are processes regulated by the cell cycle. The progression of the cell cycle has two major checkpoints: the transition from G₁ to S phase and from G₂ to M phase. The E2F family of transcription factors regulates the transcription of genes involved in the G₁-to-S phase transition. The DNA-binding activity of E2F is stimulated by binding to Dimerization Partner (DP) proteins; E2F-DP heterodimeric transcription factors activate the expression of genes responsible for cell cycle control, the initiation of replication, and enzymes required for DNA synthesis during S phase (Kosugi and Ohashi, 2002). The E2F-DP pathway is conserved in animals and plants. In Arabidopsis, at least three E2Fs (E2Fa, E2Fb, and E2Fc) and two DPs (DPa and DPb) have been

identified, along with their target genes (Magyar et al., 2000; Kosugi and Ohashi, 2002; Vandepoele et al., 2005). The overexpression of *E2F* and *DPa* in Arabidopsis produced dwarfed plants with curled leaves and cotyledons (De Veylder et al., 2002). A genome-wide analysis identified several core cell cycle genes in rice, including four E2Fs and three DPs (*OsDP1*, *OsDP2*, and *OsDP3*). However, only *DP1* transcripts have been detected by RT-PCR, while attempts to detect *DP2* and *DP3* transcripts were not successful (Guo et al., 2007).

[0045] DP proteins are widely conserved proteins and are involved in regulation of the cell cycle. DP complexed with E2F is capable of initiating transcription of S-phase specific genes. DP proteins comprise a characteristic DNA-binding domain and a dimerization domain. Controlling expression of DP-encoding nucleic acids can provide a differential growth characteristic. For example, using a seed-specific promoter can stimulate cell division rate and result in increased seed biomass. Similarly, using a root-specific promoter can result in larger roots and faster growth (e.g., more biomass accumulation). DP-E2F activity is negatively regulated by the Retinoblastoma protein Rb. Thus, in some cases downregulation of Rb and upregulation of DP yield similar outcomes.

[0046] A change in expression of genes implicated in yield or in herbicide resistance, such as, for example, a gene or genes involved in Rb signaling, DP/E2F signaling and/or BR signaling, variously results in an increase in yield of a plant or plant cell subject to the change in gene expression or signaling pathway activity relative to corresponding a plant (e.g., a reference plant or reference plant line) not subject to the change in gene expression. An increase in yield can include an improvement (e.g., an increase) in at least one of the following: root length, plant weight, tiller number, panicle number, plant height, seed weight, seed number, seed size, number of primary branches, number of secondary branches, or leaf angle relative to a reference plant or reference plant line. The increase in yield can be from 1% to 5%, from 5% to 10%, from 10% to 30%, from 30% to 50%, from 25% to 75%, from 50% to 80%, from 80% to 100%, from 100% to 150%, from 150% to 160%, from 150% to 200%, from 125% to 175%, from 100% to 200%, from 50% to 150%, at least 1%, at least 2%, at least 3% , at least 5%, at least 10%, at least 15%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, at least 110%, at least 120%, at least 130%, at least 140%, at least 150%, at least 160%, at least 170%, at least 180%, at least 190%, at least 200%, or more than 200% relative to a corresponding plant (e.g., a reference plant or reference plant line) not subject to the change in gene expression. In some examples, increase in yield can be at least 5%. In other examples, increase in yield can be at least 10% or more.

[0047] In some cases, alteration of expression of genes using methods for genetic modifications disclosed herein may result in change in expression of genes involved in Rb signaling, DP/E2F

signaling, and/or BR signaling. Brassinosteroids (BRs) play crucial roles in controlling plant architecture (Clouse et al., 1996; Choe et al., 1998; Yamamuro et al., 2000; Sakamoto et al., 2006). In some cases, plant growth characteristics can be improved by altering expression of at least one gene involved in the DP signaling pathway, the E2F signaling pathway, or the BR signaling pathway. Plant growth characteristics may comprise increased biomass, or other features related to growth. Biomass can refer to the amount of biological material produced. An increase in biomass can be in one or more parts of a plant relative to the biomass of corresponding reference plants, for example relative to the biomass of corresponding wild-type plants. Increased biomass can be used to describe increased yield, particularly seed yield.

[0048] Following introduction of the genetic modification, plants with increased activity of a DP polypeptide may be selected. The increased activity of a DP polypeptide can be correlated with improved growth characteristics, high seed yield, for example. Increased signaling activity can be brought about by increasing DP expression levels or activity. Increasing expression levels or activity of a DP agonist, such as E2F, (e.g., inside a plant cell) can also increase activity of a DP polypeptide. Alternatively or additionally, decreasing expression levels or activity of a DP antagonist, such as Rb, can result in increased activity of a DP polypeptide. Expression or activity of cell cycle regulators such as cyclins or CDKs may be modulated in methods for producing high-yielding plants or herbicide-resistant plants, as disclosed herein.

[0049] The DP-encoding nucleic acid or functional variant thereof may be derived from a number of natural or artificial sources, such as eukaryotic genomes. The source may be a microbial source, such as yeast or fungi, or plant, algal or animal (including human) source. The nucleic acid may be modified from its native form in composition and/or genomic environment through deliberate human manipulation or random mutagenesis. The nucleic acid is preferably of plant origin, whether from the same plant species (for example to the one in which it is to be introduced) or whether from a different plant species. The nucleic acid may be isolated from a dicotyledonous species, *Arabidopsis thaliana*, for example. The nucleic acid may be isolated from a monocotyledonous species, *Oryza sativa* or *Zea mays*, for example.

[0050] Additional disclosures relating to altering expression of DP or E2F in plants can be found in PCT publications WO 2005/117568, which published on December 15, 2005, and WO 00/47614, which published on August 17, 2000. Both WO 2005/117568 and WO 00/47614 are incorporated by reference herein in their entireties and for all purposes.

[0051] Mutants defective in brassinosteroid (BR) biosynthesis or signaling pathways often display semi-dwarfism, as do the highly productive gibberellin mutants that enabled the Green Revolution.

However, reduced vegetative growth in BR mutants does not necessarily correspond to increased seed yields.

[0052] Typical BR-deficient mutants in rice display dwarf phenotypes, including dark-green, erect leaves and shortened leaf sheaths in the early vegetative stage of growth. After flowering, the mutant plants are only ~40% the height of wild-type plants, and internode elongation, especially the second internode, differs from that of the wild type, with malformed panicles and a reduced number of branches and spikelets (Hong et al., 2003; Tanabe et al., 2005; Nakamura et al., 2006). By contrast, plants overexpressing BR biosynthesis genes or plants with increased BR sensitivity often have a large stature, with increased numbers of flowers and seeds and lamina with increased bending from the vertical axis of the leaf towards the abaxial side (Wu et al., 2008; Tanaka et al., 2009; Zhang et al., 2009). Regulating the expression of genes involved in modulating endogenous BR levels or affecting sensitivity to BR is a promising technique for improving agricultural traits. To date, the expression of the key BR biosynthetic gene, *DWF4*, has been altered in *Arabidopsis thaliana*, *Oryza sativa* (rice), tomato, *Brassica napus* and maize (Choe et al., 2001; Sakamoto et al., 2006; Liu et al., 2007; Li et al., 2016; Sahni et al., 2016). Similarly, studies in the BR receptor BRASSINOSTEROID INSENSITIVE 1 (*BRI1*) mutant *d61* in rice and *zmbri1*-RNAi plants in maize have been reported (Morinaka et al., 2006; Kir et al., 2015). In these examples, the overexpression or disruption of *DWF4* or *BRI1* resulted in plants with desirable traits like increased seed yield. Thus, identifying novel mutants related to BR biosynthesis or action could reveal novel ways to enhance seed yield phenotype.

[0053] Methods of altering gene expression can be increased in any one or more of the following, relative to corresponding plants lacking the gene expression alteration: (i) increased biomass (weight) of one or more parts of a plant, particularly aboveground (harvestable) parts, increased root biomass or increased biomass of any other harvestable part; (ii) increased seed yield, which may result from an increase in seed biomass (seed weight) and which may be an increase in the seed weight per plant or on an individual seed basis, and which increase in seed weight may be due to altered seed dimensions, such as seed length and/or seed width and/or seed area; (iii) increased number of (filled) seeds; (iv) increased seed size, which may also influence the composition of seeds; (v) increased seed volume, which may also influence the composition of seeds; (vi) increased harvest index, which is expressed as a ratio of the yield of harvestable parts, such as seeds, over the total biomass; and (vii) increased thousand kernel weight (TKW), which is extrapolated from the number of filled seeds counted and their total weight.

[0054] Taking rice as an example, a yield increase may be manifested by an increase in one or more of the following: number of plants per hectare or acre, number of panicles per plant, number

of spikelets per panicle, number of flowers per panicle, increase in the seed filling rate, increase in thousand kernel weight, among others. For example, thousand kernel weight in a mutant plant can be 20 g, 24 g, 28 g, 30 g, 33 g, 36 g, 40 g, 45 g, 50 g, 55 g, 60 g, from 20 g to 24 g, from 24 g to 28 g, from 30 g to 33 g, from 33 g to 36 g, from 36 g to 40 g, from 40 g to 45 g, from 45 g to 50 g, from 50 g to 55 g, from 55 g to 60 g, or more than 60 g. An increase in yield may also result in modified architecture, or may occur as a result of modified architecture.

[0055] The high-yielding plants and/or herbicide resistant plants disclosed herein can have increased yield relative to corresponding wild type plants, to near isogenic lines differing in expression of the identified gene or genes, or differing in activity of a related protein or pathway. Increased yield may be accompanied by an increased growth rate (during at least part of the life cycle), relative to the growth rate of corresponding wild type plants at a corresponding stage in the life cycle. The increased growth rate may be specific to one or more parts of a plant (including seeds), or may be throughout substantially the whole plant. The increase in growth rate can be from 1% to 5%, from 5% to 10%, from 10% to 30%, from 30% to 50%, from 25% to 75%, from 50% to 80%, from 80% to 100%, from 100% to 150%, from 150% to 160%, from 150% to 200%, from 125% to 175%, from 100% to 200%, from 50% to 150%, at least 1%, at least 2%, at least 3% , at least 5%, at least 10%, at least 15%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, at least 110%, at least 120%, at least 130%, at least 140%, at least 150%, at least 160%, at least 170%, at least 180%, at least 190%, at least 200%, or more than 200% relative to a corresponding plant (e.g., a reference plant or reference plant line) not subject to a change in gene expression or to a change in signaling pathway activity, as described herein. A plant having an increased growth rate may exhibit early flowering. The increase in growth rate may take place at one or more stages in the life cycle of a plant or during substantially the whole plant life cycle. Increased growth rate during the early stages in the life cycle of a plant may reflect enhanced vigor. The increase in growth rate may alter the harvest cycle of a plant allowing plants to be sown later and/or harvested sooner than would otherwise be possible. If the growth rate is sufficiently increased, it may allow for the sowing of further seeds of the same plant species (for example sowing and harvesting of rice plants followed by sowing and harvesting of further rice plants all within one conventional growing period). Similarly, if the growth rate is sufficiently increased, it may allow for the sowing of further seeds of different plants species (for example the sowing and harvesting of rice plants followed by, for example, the sowing and optional harvesting of soy bean, potatoes or any other suitable plant). Harvesting additional times from the same rootstock in the case of some plants may also be possible. Altering the harvest cycle of a plant may lead to an increase in annual biomass production per acre (due to an increase

in the number of times that any particular plant may be grown and harvested). An increase in growth rate may also allow for the cultivation of transgenic plants in a wider geographical area than their wild-type counterparts, since the territorial limitations for growing a crop are often determined by adverse environmental conditions either at the time of planting (early season) or at the time of harvesting (late season). Such adverse conditions may be avoided if the harvest cycle is shortened. The growth rate may be determined by deriving various parameters from growth curves plotting growth experiments, such parameters may be: T-Mid (the time taken for plants to reach 50% of their maximal size) and T-90 (time taken for plants to reach 90% of their maximal size), amongst others.

[0056] An improvement of any of the growth characteristics may provide plants with improved stress tolerance. These stresses may be biotic and/or abiotic (environmental) stresses to which a plant is exposed. Typical abiotic or environmental stresses include temperature stresses caused by atypical hot or cold/freezing temperatures; salt stress; water stress (drought or excess water). Abiotic stresses may also be caused by chemicals. Biotic stresses are typically those stresses caused by pathogens, such as bacteria, viruses, fungi and insects.

Methods of Modifying Gene Expression

[0057] An efficient method for altering expression of a gene or a signaling pathway in a plant is the introduction of a genetic sequence into at least one cell of the plant. In some cases, genetic modification is used for altering the expression of a gene or a plurality of genes involved in Rb signaling, DP/E2F signaling, and/or BR signaling. For example, the introduction of a nucleic acid sequence, such as a promoter sequence, into a plant cell can cause an increase in the expression of a gene in the plant cell or in a plant or plurality of plants arising from the plant cell. As described herein, methods of genetic modification can be used to produce a high-yielding plant, to increase seed yield in a plant, to improve yield in a crop field treated with a herbicide, and in methods for clearing a weed from a field.

[0058] A nucleic acid sequence may be stably or transiently present in a cell into which it is introduced. A nucleic acid sequence that is incorporated into a cell's genomic DNA is stably present in a cell. Nucleic acids that are subject to degradation with a cell or export from a cell are examples of nucleic acids that are transiently present in a cell.

[0059] A nucleic acid sequence can be stably introduced into a cell's genomic DNA using an integrating vector. Integrating vector can be viral or non-viral. Viral integrating vectors include retroviruses, adeno-associated viruses, and lentiviruses. Non-viral integrating strategies can include episomal vectors and injection of naked DNA.

[0060] A nucleic acid can be transiently present in a cell following introduction into the cell via a non-integrating vector. Non-integrating vectors can be viral or non-viral. Viral non-integrating vectors include adenoviruses, adeno-associated viruses, integration deficient retro-lentivirus, poxviruses, and Sendai virus. Non-viral non-integrating strategies for introducing a nucleic acid into a cell include introduction of episomal vector into a plant cell and injection of naked DNA into a plant cell.

[0061] Representative examples of methods of genetic modification include: activation tagging (e.g., T-DNA activation), Targeted Induced Local Lesions IN Genomes (TILLING), CRISPR-Cas system, site-directed mutagenesis, directed evolution, homologous recombination, or by introducing into a plant a DP-encoding nucleic acid or functional variant thereof encoding a DP polypeptide or a homologue of a DP polypeptide. In many cases, methods of genetic modification involve the use of a vector to deliver a nucleic acid sequence to the cell.

[0062] The use of a method for genetic modification to deliver a nucleic acid sequence to a cell can result in the incorporation of all or a portion of the sequence into the cell's genome. Delivery of a vector to a cell can result in the incorporation of all or a portion of a sequence of the vector into a gene of interest in the cell's genome and can cause modulation of the expression of the gene of interest. Incorporation of all or a portion of a vector's nucleic acid sequence to a genomic location within a distance of 0 kilobases (KB) to 0.5 KB, from 0.5 KB to 1.0 KB, from 1.8 KB to 3.8 KB, from 1.0 KB to 5.0 KB, from 5.0 KB to 10 KB, from 0 KB to 2.0 KB, from 1.0 KB to 4.0 KB, from 0 KB to 5 KB, or more than 10 KB of a gene of interest can result in modulation of the expression of the coding region of a gene of interest (e.g., a region of a cell's DNA comprising an exon of the gene of interest). A sequence of a vector can modify expression of a gene in a cell when introduced into a cell's genome upstream or downstream of the coding region of a gene (e.g., a gene of interest). In some cases, a translation enhancer or an intron may be used instead of or in addition to a promoter in the vector. Regulation of expression of the targeted gene by its natural promoter may be disrupted and the gene may be directed by the newly introduced promoter after insertion of a nucleic acid sequence into a cell.

[0063] Activation tagging can involve the insertion of a T-DNA sequence (e.g., via a vector such as a Ti plasmid containing a pGA2715 insert) or other insertional activation tagging vector into a cell. A vector for activation tagging often comprises a nucleic acid sequence and can comprise a promoter sequence or an enhancer sequence in the genomic vicinity of a gene of interest. Delivery of an activation tagging vector to a cell can result in the incorporation of all or a portion of a sequence of the activation tagging vector into the cell's genome. Delivery of all or a portion of an activation tagging vector's nucleic acid sequence to a genomic location within a distance of 0

kilobases (KB) to 0.5 KB, from 0.5 KB to 1.0 KB, from 1.0 KB to 5.0 KB, from 1.8 KB to 3.8 KB, from 5.0 KB to 10 KB, from 0 KB to 2.0 KB, from 1.0 KB to 4.0 KB, from 0 KB to 5 KB, or more than 10 KB of a gene of interest can result in modulation of the expression of the coding region of a gene of interest (e.g., a region of a cell's DNA comprising an exon of the gene of interest). A sequence of an activation tagging vector can modify expression of a gene in a cell when introduced into a cell's genome upstream or downstream of the coding region of a gene (e.g., a gene of interest). In some cases, a translation enhancer or an intron may be used instead of or in addition to a promoter in the activation tagging vector. Regulation of expression of the targeted gene by its natural promoter may be disrupted and the gene may be directed by the newly introduced promoter after insertion of a nucleic acid sequence into a cell. The promoter is typically encoded in the sequence of a T-DNA although other insertion fragments are suitable. This T-DNA or other segment is randomly inserted into the plant genome, for example, through *Agrobacterium* infection and leads to overexpression of genes near to the inserted T-DNA. The resulting transgenic plants can show dominant phenotypes due to overexpression of genes close to the introduced promoter. In some cases, insertion of a T-DNA vector in a genome can cause transcriptional activation of genes flanking the inserted T-DNA and can result in dominant gain-of-function mutations. The promoter to be introduced may be any promoter capable of directing expression of a gene in the desired organism, in this case a plant. For example, constitutive, tissue-preferred, cell type-preferred and inducible promoters are all suitable for use in T-DNA activation.

[0064] TILLING can be used to introduce a genetic modification in a DP locus. TILLING is a mutagenesis technology useful in generating and/or identifying, and isolating mutagenized variants of a DP encoding nucleic acid exhibiting DP activity. TILLING can also allow selection of plants carrying such mutant variants. These mutant variants may exhibit higher DP activity relative to the activity exhibited by the gene in its natural form. TILLING combines high-density mutagenesis with high-throughput screening methods. The steps in TILLING can include: (a) EMS mutagenesis; (b) DNA extraction and pooling of individuals; (c) PCR amplification of a region of interest; (d) denaturation and annealing to allow formation of heteroduplexes; (e) detection of a heteroduplex in a pool as an extra peak in chromatogram; (f) identification of the mutant individual; and (g) sequencing of the mutant PCR product.

[0065] A targeted mutagenesis system such as a CRISPR-Cas9 system may be used to generate variants of DP-encoding nucleic acids or functional variants thereof encoding active proteins. For example, a guide RNA can be designed to direct the dCas9-activator to promoter or regulatory regions of a gene of interest, such as DP-encoding gene. A transcriptional activator can be fused to dCas9 that in turn can activate expression of the gene of interest. Single and/or multiple different

activators can be used to amplify expression. CRISPR-Cas9 system can also be used to generate allelic variants with altered gene expression. For example, allelic variant of DP-encoding gene overexpressing DP can be produced using the CRISPR-Cas9 system.

[0066] Site-directed mutagenesis may be used to generate variants of DP-encoding nucleic acids or functional variants thereof encoding active proteins. Several methods are available to achieve site-directed mutagenesis; the most common being PCR based methods.

[0067] Directed evolution can be used to generate variants of DP-encoding nucleic acids. This consists of iterations of DNA shuffling followed by appropriate screening and/or selection to generate variants of DP-encoding nucleic acids or portions thereof encoding DP polypeptides or portions thereof having a modified biological activity.

[0068] Homologous recombination allows introduction of a selected nucleic acid in a genome at a defined position. The nucleic acid to be target, nucleic acid encoding for a DP or variants thereof may be introduced in, for example, regions of high expression. The nucleic acid to be targeted may be an improved allele used to replace the endogenous gene or may be introduced in addition or the endogenous gene.

[0069] A change in expression of at least one gene, such as genes implicated in yield or herbicide resistance, such as, for example, a gene or genes involved in cell cycle signaling, hormone regulation, or a metabolic pathway (e.g., a gene or genes involved in Rb signaling, DP/E2F signaling and/or BR signaling), variously results in an increase in yield of plants subject to the change in gene expression or signaling pathway activity relative to corresponding plants not subject to the change in expression. The increase in yield can be from 1% to 5%, from 5% to 10%, from 10% to 30%, from 30% to 50%, from 25% to 75%, from 50% to 80%, from 80% to 100%, from 100% to 150%, from 150% to 160%, from 150% to 200%, from 125% to 175%, from 100% to 200%, from 50% to 150%, at least 1%, at least 2%, at least 3% , at least 5%, at least 10%, at least 15%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, at least 110%, at least 120%, at least 130%, at least 140%, at least 150%, at least 160%, at least 170%, at least 180%, at least 190%, at least 200%, or more than 200% relative to a corresponding plant or plants (e.g., a reference plant or reference plant line) not subject to the change in expression. In some examples, increase in yield can be at least 5%. In other examples, increase in yield can be at least 10% or more.

[0070] A change in expression of at least one gene implicated in yield or in herbicide resistance, such as, for example, a gene or genes involved in Rb signaling, DP/E2F signaling and/or BR signaling, variously results in an increase in herbicide resistance in plants relative to corresponding plants not subject to the change in expression or signaling pathway activity. For example, a change

in expression of at least one gene, such as genes implicated in yield or herbicide resistance, such as, for example, a gene or genes involved in cell cycle signaling, hormone regulation, or a metabolic pathway (e.g., a gene or genes involve in Rb signaling, DP/E2F signaling and/or BR signaling), variously results in an improved root length, plant weight, tiller number, panicle number, plant height, seed weight, seed number, seed size, number of primary branches, number of secondary branches, or leaf angle relative to corresponding plants not subject to the change in expression, when both plants are exposed to a herbicide, such as Brassinazole or propiconazole (e.g., in similar concentrations).

Vectors

[0071] A vector can be used to introduce a nucleic acid sequence into a plant or a plant cell, e.g., in a method to produce a high-yielding plant or a herbicide-resistant plant. Methods described herein can comprise a number of different strategies for introducing a nucleic acid or polypeptide sequence into a cell. Introducing a sequence into a cell can comprise contacting the cell with a vector. In many cases, a vector comprises a virus, such as a lentivirus, a retrovirus, an adenovirus, an adeno-associated virus, a Herpesvirus, a poxvirus, a vaccinia virus, or a Sendai virus. In some cases, a vector is a non-viral vector, such as a naked plasmid or episome.

[0072] In many cases, a nucleic acid sequence introduced into a plant cell in methods for producing a high-yielding plant or a herbicide-resistant plant is an exogenous DNA sequence. A vector can comprise a linearized or circularized nucleic acid (DNA or RNA) sequence. A vector can also comprise an episome. In some cases, a naked nucleic acid (e.g., linearized or circularized DNA or RNA) or polypeptide introduced into a cell in the form of a plasmid. Delivery of naked sequences to a cell can comprise removal of a cell wall and/or strategies for membrane permeabilization, such as electroporation, sonoporation, ballistic nucleic acid introduction, and treatment of a cell membrane with chemicals such as polybrene or saponin.

Treatment of Plants with Chemicals

[0073] In accordance with some methods disclosed herein, a plant is treated with an exogenous agent, such as a chemical or a hormone. Chemicals useful in methods disclosed herein include chemicals that modulate the expression of a gene or the activity of a signaling pathway in a plant or plant cell. In some cases, chemicals useful for the production of a high-yielding plants or herbicide-resistant plants can modulate a cellular process, a metabolic process, hormone signaling pathway, or a cell cycle pathway. Herbicides and plant hormones are representative examples of chemicals useful in modulating expression of a gene or activity of a signaling pathway in a plant or plant cell.

[0074] A number of chemicals known to be valuable in weed control and crop growth can be useful in the production of high-yielding plants, high-yielding crop fields, or herbicide-resistant plants, as disclosed herein. In some cases, chemicals used in weed control (e.g., herbicides) or crop growth (e.g., hormones such as brassinosteroids) often impact cell cycle pathways or hormone regulation pathways. For example, modulatory chemicals such as hormones (e.g., brassinosteroids) can be used in crop growth. Yield of crop fields can be improved by removal of undesirable plants, such as weeds. Treatment of plants in a crop field with a chemical (e.g., a herbicide) or a plurality of chemicals often results in more efficient removal of undesirable plants from the crop field than manual removal of undesirable plants. In some case, the use of herbicides can have a deleterious effect on desirable plants in the field (e.g., crop plants such as rice or maize), reducing the benefits of their use in methods for removal of undesirable plants, such as weeds, from a crop field.

[0075] As described herein, methods of producing high-yield plant can confer resistance to a herbicide on plants produced using those methods. For example, a plant comprising a mutation affecting DP expression or DP-E2F signaling can confer resistance to certain herbicides upon the plant. Such strategies for producing high-yield plants can mitigate deleterious effects of herbicides in the maintenance of crop fields. Accordingly, methods for modulating the expression of DP or affecting DP-E2F signaling (e.g., through the introduction of a *pzr1-D* mutation) can be useful in methods for clearing a weed from a field.

[0076] Chemicals that can be used in crop or weed management in a field include Brassinazole (Brz), propiconazole (Pcz), and YCZ-18. Brassinazole (Brz) is a BR biosynthesis inhibitor that has been used to help identify novel components of the BR biosynthesis and signaling pathways in *Arabidopsis* (Wang et al., 2002; Kim et al., 2014; Maharjan et al., 2014). Propiconazole (Pcz) is a triazole-type inhibitor and can be used in BR sensitivity screening in rice (Corvalan and Choe, 2017). In contrast to Brz, which can be costly, Pcz is a commercially-used fungicide that is readily accessible and inexpensive, allowing it to be used in large-scale chemical genomics and field-testing. Propiconazole (Pcz) is a potent, specific BR inhibitor, and its use in maize and *Brachypodium* has been demonstrated previously (Hartwig et al., 2012; Corvalan and Choe, 2017). In contrast to its costly counterpart, brassinazole (Brz), the low cost of Pcz allows the performance of large-scale tests to screen a T-DNA activation-tagging mutant population. Pcz treatment produces typical BR-deficient phenotypes, such as epinastically growing, dark-green cotyledons, and reduced growth of hypocotyls and primary roots (Hartwig et al., 2012). Pcz treatment of genetically disrupted *dwf4-1* mutant seedlings suggested that DWF4 is likely a target of Pcz (Asami et al., 2001; Chung et al., 2011; Hartwig et al., 2012).

[0077] Alteration of expression in genes involved in Rb, DP, E2F, or BR signaling may confer plants with resistance to herbicide, fungicide, nematicide, bactericide, or inhibitors of biosynthetic pathways. For example, plants with elevated expression of DP can exhibit increased resistance to BR biosynthetic inhibitors, such as Pcz, brassinazole, or YCZ-18, compared to corresponding unaltered plants. For example, a change in expression of at least one gene, such as genes implicated in yield or herbicide resistance, such as, for example, a gene or genes involved in cell cycle signaling, hormone regulation, or a metabolic pathway (e.g., a gene or genes involve in Rb signaling, DP/E2F signaling and/or BR signaling), variously results in an improved root length, plant weight, tiller number, panicle number, plant height, seed weight, seed number, seed size, number of primary branches, number of secondary branches, or leaf angle relative to corresponding plants not subject to the change in expression, when both plants are exposed to a herbicide, such as Brassinazole or propiconazole (e.g., in similar concentrations). In some cases, an improvement in any of these outcomes or in a combination of these outcomes compared to a reference plant or reference plant line can be considered to be an increase in resistance to a herbicide. The increase in resistance to a given chemical can be from 1% to 5%, from 5% to 10%, from 10% to 30%, from 30% to 50%, from 25% to 75%, from 50% to 80%, from 80% to 100%, from 100% to 150%, from 150% to 160%, from 150% to 200%, from 125% to 175%, from 100% to 200%, from 50% to 150%, at least 1%, at least 2%, at least 3% , at least 5%, at least 10%, at least 15%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, at least 110%, at least 120%, at least 130%, at least 140%, at least 150%, at least 160%, at least 170%, at least 180%, at least 190%, at least 200%, or more than 200% compared to a corresponding plants (e.g., a reference plant or reference plant line, such as a wild type plant). In some examples, increase in resistance can be at least 5%. In other examples, an increase in resistance can be at least 10%.

[0078] In some cases, an increase in resistance to a herbicide can be measured with respect to the percentage of non-crop plants (e.g., weeds) that are killed in the presence of a herbicide while crop plants (e.g., crop plants present in the same field treated and/or treated with the same herbicide and/or at the same herbicide concentration) are not killed. In some cases, at least 100%, at least 99.9%, at least 99.5%, at least 99.0%, at least 98%, at least 97%, at least 96%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, at least 50%, at least 45%, at least 40%, from 100% to 95%, from 95% to 90%, from 90% to 85%, from 85% to 80%, from 80% to 70%, from 70% to 60%, from 60% to 50%, from 50% to 40%, from 40% to 30%, from 30% to 20%, from 20% to 10%, from 20% to 1% of non-crop plants (e.g., weeds) may be killed in the presence of a herbicide while crop plants (e.g., crop plants present

in the same field treated and/or treated with the same herbicide and/or at the same herbicide concentration) are not killed.

[0079] An increase in resistance to a herbicide of a plant or plant line can result in increased yield in a plant or crop field. In some cases, a herbicide-resistant plant, as described herein, can survive in the presence of a herbicide whereas a plant that is not herbicide resistant (e.g., a weed) does not survive in the presence of the herbicide. In some cases, a herbicide-resistant plant or a crop field comprising a herbicide-resistant plant exhibits increased yield because the herbicide-resistant plant is able to outcompete a plant that is not herbicide resistant (e.g., a weed) in the presence of a herbicide.

[0080] Some resistant plant lines do not exhibit substantial phenotypic differences between treated and untreated plants. For example, the Pcz-resistant lines can phenotypically be similar for both treated and untreated plants. Alternatively, other resistant plant lines do exhibit phenotypic differences between treated and untreated plants. In some cases, resistant lines may differ in terms of their growth characteristics between treated and untreated plants. For example, the resistant lines may exhibit differences in height between treated and untreated plants. Treated plants may be dwarf relative to untreated plants.

[0081] In some cases, resistant and non-resistant lines may be exposed to different dosages of a given chemical to produce different growth characteristics. For example, resistant and non-resistant lines of rice can be treated with Pcz having a concentration of 1 μM to 5 μM , 5 μM to 20 μM , 20 μM to 30 μM , 30 μM to 90 μM , 1 μM , 2 μM , 3 μM , 4 μM , 5 μM , 10 μM , 15 μM , 20 μM , 25 μM , 30 μM , 40 μM , 50 μM , 60 μM , 70 μM , 80 μM , 90 μM , 100 μM , 110 μM , 120 μM , 130 μM , 140 μM , 150 μM , higher than 150 μM , from 10 μM to 20 μM , from 20 μM to 30 μM , from 30 μM to 50 μM , from 50 μM to 90 μM , from 90 μM to 100 μM , from 100 μM to 110 μM , from 110 μM to 130 μM , or from 130 μM to 150 μM to produce differences in growth characteristics. In some examples, resistant lines relative to non-resistant lines can exhibit phenotypic differences, such as root growth, at about 10 μM Pcz. In some examples, resistant lines relative to non-resistant lines can exhibit phenotypic differences at about 20 μM Pcz. In some examples, resistant lines relative to non-resistant lines can exhibit phenotypic differences at about 30 μM Pcz. In some examples, resistant lines relative to non-resistant lines can exhibit phenotypic differences at about 40 μM Pcz. In some examples, resistant lines relative to non-resistant lines can exhibit phenotypic differences at about 50 μM Pcz. In some examples, resistant lines relative to non-resistant lines can exhibit phenotypic differences at about 60 μM Pcz. In some examples, resistant lines relative to non-resistant lines can exhibit phenotypic differences at about 70 μM Pcz. In some examples, resistant

lines relative to non-resistant lines can exhibit phenotypic differences at about 80 μM Pcz. In some examples, resistant lines relative to non-resistant lines can exhibit phenotypic differences at about 90 μM Pcz. In some examples, resistant lines relative to non-resistant lines can exhibit phenotypic differences at about 100 μM Pcz.

[0082] In some cases, differences in growth characteristics between resistant and non-resistant lines upon exposure to a given chemical can be exploited for clearing a weed in a field. In this case, the given chemical can serve as an herbicide. For example, mutations in DP, E2F, or BR signaling can confer resistance to BR inhibitors, such as Brassinazole, Pcz, or YCZ-18. For example, resistant and non-resistant lines of rice can be treated with Brassinazole having a concentration of 1 nM to 5 nM, 5 nM to 10 nM, 10 nM to 20 nM, 20 nM to 50 nM, 50 nM to 100 nM, 100 nM to 200 nM, 200 nM to 500 nM, 500 nM to 1 μM , from 1 μM to 2 μM , from 2 μM to 3 μM , from 3 μM to 5 μM , from 5 μM to 10 μM , or higher than 10 μM , to produce differences in growth characteristics.

Resistant lines in a field with at least one weed can be exposed to a BR inhibitor. Upon exposure to the BR inhibitor (e.g., prior to harvest of crops from a field), herbicide-resistant lines exhibit no substantial change in growth characteristics compared to a reference plant or reference plant line (e.g., a non-resistant plant line, such as a wild type plant like a weed). A reference plant or reference plant line (e.g., a wild type plant such as a weed), however, can exhibit substantially reduced growth characteristics, including slower root growth resulting in dwarf phenotype.

[0083] BR inhibitors alone or in combination with other chemicals, such as other herbicides, can be used. For example, a single BR inhibitor, such as Pcz, can be used in a field with at least one weed to clear the weed. In other examples, BR inhibitor in combination with other herbicides can be used. Combining or “stacking” a level of resistance to both BR inhibitor and other herbicide(s) can these herbicide combinations to be used for effective weed control without crop injury and/or reduction in crop yield. Further, combining BR inhibitor with other herbicides, such as glyphosate, can result in broader level of protection against the spectrum of weeds (e.g., annual and perennial grasses, smartweeds, nightshade, pigweed spp., morning glory spp., etc.). In such cases, both the BR inhibitor and the other herbicide can be co-administered for clearing at least one weed in a field.

[0084] Differences in growth characteristics between resistant crop lines (e.g., herbicide-resistant plants) and non-resistant weeds can be used for harvesting high-yielding crops without harvesting weeds. Crop lines resistant to BR inhibitor that shows an increase in yield compared to non-resistant weeds can be sprayed with BR inhibitor(s). The BR inhibitors can stunt the growth of the weeds conferring dwarf phenotype to weeds while the resistant high-yielding crops remain

unaffected. Harvesting methods can selectively harvest only the crops while leaving the weeds in the field.

[0085] Methods disclosed herein can also include treatment of a plant or a plant cell with a hormone, such as a brassinosteroid (BR). In some cases, treatment of a plant or plant cell can result in modulation of the expression of a gene or the activity of a signaling pathway (e.g., a gene or signaling pathway associated with growth, metabolic processes, or cellular processes (e.g., cell cycle regulation). For example, treatment of a plant with a brassinosteroid, such as brassinolide (BL), can result in increased expression of a gene or increased activity of a signaling pathway related to growth, metabolic processes, or cellular processes. In some cases, an effect of treating a plant or a plant cell with a steroid is an improvement in the treated plant's ability to outcompete an untreated second plant (e.g., a weed or a plant from a reference plant line). For example, a plant (e.g., plant cell) treated with a hormone, such as BL, can improve the plant's ability to outcompete a second, untreated, plant, such as a weed, e.g., through increased plant yield.

Plants

[0086] A number of plants are useful for the methods of producing high-yielding plants or herbicide-resistant plants disclosed herein. High-yielding plants can comprise a genetic modification. In various cases, a high-yielding plant can comprise an overexpressed gene, a downregulated gene, or a combination of an overexpressed gene and a downregulated gene.

[0087] Modification of gene expression, as described herein, can be accomplished in a variety of plant species. Plants and plant cells that are particularly useful in methods and plants disclosed herein include plants and plant cells which belong to the superfamily Viridiplantae. In particular, monocotyledonous and dicotyledonous plants can be useful in methods disclosed herein and to produce the plants described herein. Monocotyledonous and dicotyledonous plants include fodder legumes, forage legume, ornamental plants, food crops, trees, and shrubs. In some cases, a plant or plant cell useful in methods disclosed herein, including the production of a plants described herein, is *Acacia* spp., *Acer* spp., *Actinidia* spp., *Aesculus* spp., *Agathis australis*, *Albizia amara*, *Alsophila tricolor*, *Andropogon* spp., *Arachis* spp, *Areca catechu*, *Astelia fragrans*, *Astragalus cicer*, *Baikiaea plunjuga*, *Betula* spp., *Brassica* spp., *Bruguiera gymnorrhiza*, *Burkea africana*, *Butea frondosa*, *Cadaba farinosa*, *Calliandra* spp, *Camellia sinensis*, *Canna indica*, *Capsicum* spp., *Cassia* spp., *Centroema pubescens*, *Chaenomeles* spp., *Cinnamomum cassia*, *Coffea arabica*, *Colophospermum mopane*, *Coronillia varia*, *Cotoneaster serotina*, *Crataegus* spp., *Cucumis* spp., *Cupressus* spp., *Cyathea dealbata*, *Cydonia oblonga*, *Cryptomeria japonica*, *Cymbopogon* spp., *Cynthea dealbata*, *Cydonia oblonga*, *Dalbergia monetaria*, *Davallia divaricata*, *Desmodium* spp., *Dicksonia squarosa*, *Diheteropogon amplexans*, *Dioclea* spp, *Dolichos* spp., *Dorycnium rectum*, *Echinochloa*

pyramidalis, Ehrartia spp., Eleusine coracana, Eragrestis spp., Erythrina spp., Eucalyptus spp., Euclea schimperi, Eulalia villosa, Fagopyrum spp., Feijoa sellowiana, Fragaria spp., Flemingia spp., Freycinetia banksii, Geranium thunbergii, Ginkgo biloba, Glycine javanica, Gliricidia spp., Gossypium hirsutum, Grevillea spp., Guibourtia coleosperma, Hedysarum spp., Hemarthia altissima, Heteropogon contortus, Hordeum vulgare, Hyparrhenia rufa, Hypericum erectum, Hyperthelia dissoluta, Indigo incarnata, Iris spp., Leptarrhena pyrolifolia, Lespedeza spp., Lettuce spp., Leucaena leucocephala, Loudetia simplex, Lotonous bainesii, Lotus spp., Macrotyloma axillare, Malus spp., Manihot esculenta, Medicago sativa, Metasequoia glyptostroboides, Musa sapientum, Nicotianum spp., Onobrychis spp., Omithopus spp., Oryza spp., Peltophorum africanum, Pennisetum spp., Persea gratissima, Petunia spp., Phaseolus spp., Phoenix canariensis, Phormium cookianum, Photinia spp., Picea glauca, Pinus spp., Pisum sativum, Podocarpus totara, Pogonarthria fleckii, Pogonarthria squarrosa, Populus spp., Prosopis cineraria, Pseudotsuga menziesii, Pterolobium stellatum, Pyrus communis, Qualms spp., Rhabdolepsis umbellata, Rhopalostylis sapida, Rhus natalensis, Ribes grossularia, Ribes spp., Robinia pseudoacacia, Rosa spp., Rubus spp., Salix spp., Schyzachyrium sanguineum, Sciadopitys verticillata, Sequoia sempervirens, Sequoiadendron giganteum, Sorghum bicolor, Spinacia spp., Sporobolus fimbriatus, Stiburus alopecuroides, Stylosanthos humilis, Tadehagi spp., Taxodium distichum, Themeda triandra, Trifolium spp., Triticum spp., Tsuga heterophylla, Vaccinium spp., Vicia spp., Vitis vinifera, Watsonia pyramidata, Zantedeschia aethiopica, Zea mays, amaranth, artichoke, asparagus, broccoli, Brussels sprouts, cabbage, canola, carrot, cauliflower, celery, collard greens, flax, kale, lentil, oilseed rape, okra, onion, potato, rice, soybean, straw, sugar beet, sugar cane, sunflower, tomato, squash tea, trees, grasses (including forage grass) or algae.

[0088] In some cases, the plant is a grain plant. In some cases, the plant is a rice plant, such as a plant of the *Oryza* genus, such as *Oryza sativa* ssp. *indica* or *Oryza sativa* ssp. *japonica*. Rice serves as both a staple food and a model plant for molecular studies.

[0089] Rice plants can be grown in a paddy field or in a greenhouse. In some embodiments, 3-week-old seedlings in the field are transplanted in late May and harvested in mid to late October.

[0090] In some cases, plants can be surface sterilized before sowing. For example, wild-type plants and/or transgenic plants can be surface sterilized before sowing on 0.8% agar-solidified medium containing 0.5x Murashige and Skoog (MS) salts and 1% sucrose. Plants may be stratified for 48 h at 4°C in darkness. Plates may then be transferred to a growth room where plants can be grown. Plants may be grown at a temperature from 18°C to 25°C, 23°C to 25°C, 25°C to 27°C, 27°C to 30°C, 30°C to 35°C, or 35°C to 45°C. Plants may be maintained in total darkness or in the light (80-100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ intensity) under long-day conditions (14-h light/10-h dark photoperiod). For

example, plants may be grown at 22°C under a 16 h light/8 h dark photoperiod in white light (80 $\mu\text{mol m}^{-2} \text{s}^{-1}$). If needed, seedlings can be transferred to soil after 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 days of growth in MS medium.

[0091] Turning to the figures one sees the following:

[0092] FIG. 1A shows images of five rice plants from a mock treatment group, each plant having a shoot and a root. To the right of those images are five images rice plants from a 30 μM Pcz treatment group, each of which having a shoot shorter in length than any shoot of the mock treated group and a root shorter in length than any root of the Pcz-treated group. A scale bar is at the upper left corner of the figure with a label indicating that it represents 5 cm in the images.

[0093] FIG. 1B shows a bar graph of total plant length and root length for mock-treated and Pcz-treated plants. The y-axis indicates length in centimeters (cm) from 0 to 25 in increments of 5. Shown on the x-axis are the different groups tested, which are “Mock total,” “Pcz total,” “Mock root,” and “Pcz root” from left to right. Each bar in the bar graph includes an error bar, and the bars labeled “Pcz total” and “Pcz root” have three asterisks (***) above the error bars, while the bars labeled “Mock total” and “Mock root” do not have any asterisks above the error bars. Values shown in FIG. 1B represent results averaged from at least 10 samples, and error bars represent standard deviation.

[0094] FIG. 1C shows a gel blot with two rows, each row having one protein band in each of two columns. Shown above the gel at the top of the figure are the treatment conditions for each of the two columns. The left column is labeled, “Mock.” The right column is labeled “Pcz.” Shown to the left of the gel are the names of the proteins blotted in each row. The upper row is labeled, “OsDWF4.” The bottom row is labeled, “ACTIN.”

[0095] FIG. 1D shows two panels, each having images of plants separated into a left group, labeled “Mock,” and a right group, labeled “Pcz 30 μM .” Above the panel on the left is a label indicating that the plants shown therein have a WT genotype. Images of four plants are in the group labeled “Mock” in the left panel and images of three plants are in the group labeled “Pcz 30 μM ” in the left panel. Above the panel on the right is a label indicating that the plants shown therein have a *pzr1-D* genotype. Images of three plants are in the group labeled “Mock” in the right panel. Images of three plants are in the group labeled “Pcz 30 μM ” in the right panel. In the upper left of each panel is a scale bar indicating that it represents 2 cm.

[0096] FIG. 1E shows a bar graph having four bars, labeled “Mock,” “Pcz,” “Mock,” and “Pcz” from left to right along the x-axis. The two bars on the left of the graph (labeled “Mock” and “Pcz”) are white, while the two bars on the right of the graph (labeled “Mock” and Pcz”) are black. At the

top of the bar graph are two boxes, indicating that the white bars are “WT” and the black bars are “*pzr1-D*.” Each bar has an error bar at the top. Above the error bar on the white bar labeled “Pcz” are two asterisks (**). Above the error bar on the black bar labeled “Pcz” is “ns.” The y-axis indicates root length in centimeters (cm) from 0 to 10 in increments of 2. Values shown in FIG. 1E represent the mean of 12 samples per treatment. Error bars represent standard deviation. In both graphs, significant differences among treatments were determined using Student’s *t*-test. **, $P < 0.001$; ***, $P < 0.0001$; and ns, non-significant.

[0097] **FIG. 2A** shows a bar graph having two bars, labeled “WT” and “*pzr1-D*” from left to right along the x-axis. Each bar has an error bar. Above the error bar of the *pzr1-D* bar is one asterisk (*). The y-axis indicates plant height in centimeters (cm) from 0 to 100 in increments of 20.

[0098] **FIG. 2B** shows a bar graph having two bars, labeled “WT” and “*pzr1-D*” from left to right along the x-axis. Each bar has an error bar. Above the error bar of the *pzr1-D* bar are three asterisks (***). The y-axis indicates plant weight in grams (g) from 0 to 100 in increments of 20.

[0099] **FIG. 2C** shows a bar graph having two bars, labeled “WT” and “*pzr1-D*” from left to right along the x-axis. Each bar has an error bar. Above the error bar of the *pzr1-D* bar is one asterisk (*). The y-axis indicates tiller number per plant (plant^{-1}) from 0 to 50 in increments of 10.

[00100] **FIG. 2D** shows a bar graph having two bars, labeled “WT” and “*pzr1-D*” from left to right along the x-axis. Each bar has an error bar. Above the error bar of the *pzr1-D* bar is one asterisk (*). The y-axis indicates panicle number per plant (plant^{-1}) from 0 to 50 in increments of 10.

[00101] **FIG. 2E** shows images of adult wild type (WT) and *pzr1-D* mutant plants. A scale bar indicating 10 centimeters (cm) is located in the upper left corner of the image.

[00102] **FIG. 2F** shows images of panicles from wild type and *pzr1-D* plants. A scale bar indicating 2 centimeters (cm) is located in the upper left corner of the image.

[00103] **FIG. 2G** shows a bar graph having two bars, labeled “WT” and “*pzr1-D*” from left to right along the x-axis. Each bar has an error bar. Above the error bar of the *pzr1-D* bar are two asterisks (**). The y-axis indicates seed weight (g/plant) from 0 to 60 in increments of 20.

[00104] **FIG. 2H** shows a bar graph having two bars, labeled “WT” and “*pzr1-D*” from left to right along the x-axis. Each bar has an error bar. Above the error bar of the *pzr1-D* bar are three asterisks (***). The y-axis indicates the number of primary branches from 0 to 15 in increments of 5.

[00105] **FIG. 2I** shows a bar graph having two bars, labeled “WT” and “*pzr1-D*” from left to right along the x-axis. Each bar has an error bar. Above the error bar of the *pzr1-D* bar are three

asterisks (***)). The y-axis indicates the number of secondary branches from 0 to 30 in increments of 10.

[00106] The graphs of **FIG. 2A-FIG. 2D and FIG. 2G-FIG. 2I** represent average values ($n>7$), and error bars represent standard deviation among samples. Significant differences among treatments were determined using Student's *t*-test. *, $P<0.05$; **, $P<0.001$; and ***, $P<0.0001$.

[00107] **FIG. 3A** shows images of the inclination of segments corresponding to the second leaf in wild type and *pzr1-D* plants, labeled "WT" and "*pzr1-D*," respectively. A scale bar is present on the left side of the figure, indicating 1 centimeter (cm) in length.

[00108] **FIG. 3B** shows a bar graph having two bars, labeled "WT" and "*pzr1-D*" from left to right along the x-axis. Each bar has an error bar. Above the error bar of the *pzr1-D* bar are two asterisks (**). The y-axis indicates segment angle in degrees from 0 to 40 in increments of 10.

[00109] **FIG. 3C** shows representative images of wild type and *pzr1-D* plants in a lamina inclination bioassay testing under mock or 1 μ M brassinolide (BL) treatment.

[00110] **FIG. 3D** shows a bar graph having two bars, labeled "WT" and "*pzr1-D*" from left to right along the x-axis. Each bar has an error bar. Above the error bar of the *pzr1-D* bar are three asterisks (***). The y-axis indicates segment angle in degrees from 0 to 200 in increments of 50.

[00111] **FIG. 3E** shows a line graph having two lines. A legend identifies the line with circular dots as the wild type ("WT") group. The line with the square dots is identified as the "*pzr1-D*" group. The lower line is the line labeled "WT." Three concentrations of brassinolide at which segment angle measurements were taken are listed as "BL 0," "BL 0.1 μ M," and "BL 1 μ M," from left to right along the x-axis, with "BL 0" indicating that brassinolide concentration was 0 μ M. Each data point on each line has an error bar. The y-axis indicates segment angle in degrees from 0 to 200 in increments of 50. Graphs represent average values ($n=15$), and error bars represent standard deviation among samples.

[00112] **FIG. 3F** shows a bar graph having two bars, labeled "WT" and "*pzr1-D*" from left to right along the x-axis. Each bar has an error bar. Above the error bar of the *pzr1-D* bar are three asterisks (***). The y-axis indicates total length in millimeters (mm) from 0 to 150 in increments of 50.

[00113] **FIG. 3G** shows a bar graph having four bars. The first and second bars of the graph (from left to right along the x-axis) are labeled "WT" and third and four bars of the graph (from left to right along the x-axis) are labeled "*pzr1-D*". The first and third bars of the graph (from left to right along the x-axis) are white, and the second and fourth bars of the graph (from left to right along the x-axis) are black. A legend toward the top left of the graph area indicates that the white bars correspond to mock treatment, and the black bars correspond to BL treatment. Each bar has an

error bar. A bracket above the white and black bars labeled “WT” is labeled with two asterisks (**), indicating a significant difference between those two treatment groups. A bracket above the white and black bars labeled “*pzr-1D*” is labeled with three asterisks (***), indicating a significant difference between those two treatment groups. The y-axis indicates root length in millimeters (mm) from 0 to 50 in increments of 10.

[00114] **FIG. 3H** shows a bar graph having four bars. The first and second bars of the graph (from left to right along the x-axis) are labeled “WT” and third and fourth bars of the graph (from left to right along the x-axis) are labeled “*pzr-1D*”. The first and third bars of the graph (from left to right along the x-axis) are white, and the second and fourth bars of the graph (from left to right along the x-axis) are black. A legend toward the top left of the graph area indicates that the white bars correspond to mock treatment, and the black bars correspond to BL treatment. Each bar has an error bar. A bracket above the white and black bars labeled “WT” is labeled with “ns”, indicating no significant difference between those two treatment groups. A bracket above the white and black bars labeled “*pzr-1D*” is labeled with three asterisks (***), indicating a significant difference between those two treatment groups. The y-axis indicates root length in millimeters (mm) from 0 to 50 in increments of 10.

[00115] Significant differences among treatments shown in **FIG. 3B**, and **FIG. 3D** to **FIG. 3H** were determined using Student’s *t*-test. **, $P < 0.001$; ***, $P < 0.0001$; and ns, non-significant.

[00116] **FIG. 4A** shows a schematic of a plant root. The root tip is divided into three sections, with labels on the left side of the figure indicating that the sections are the Elongation zone, the Division zone, and the Root tip, from top to bottom. A red box representing an area of 60 μm is located just below the center of the image in the section marked “Division zone.” A distance from the red box to the lower end of the root tip section is indicated to be 300 μm .

[00117] **FIG. 4B** shows two representative micrograph images of plant cells taken from regions corresponding to the region indicated by the red box in **FIG. 4A**. Labels on top of the images indicate that the image on the left is of cells from a wild type (“WT”) plant, and the image on the right is of cells from a *pzr1-D* plant. Scale bars are present in the lower right of each image, indicating a distance of 25 μm .

[00118] **FIG. 4C** shows a bar graph having two bars, labeled “WT” and “*pzr-1D*” from left to right along the x-axis. Each bar has an error bar. Above the error bar of the *pzr-1D* bar are two asterisks (**). The y-axis indicates the average number of cells per 60 μm^2 from 0 to 80 in increments of 20.

[00119] **FIG. 4D** shows a bar graph having two bars, labeled “WT” and “*pzr-1D*” from left to right along the x-axis. Each bar has an error bar. Above the error bar of the *pzr-1D* bar are three

asterisks (***)). The y-axis indicates average cell size in microns (μm) from 0 to 20 in increments of 50. Cell size was measured in the vertical direction.

[00120] **FIG. 4E** shows images of calli arranged in two rows and four columns. The upper row is labeled “WT” on the left side of the figure, and the lower row is labeled “*pzr1-D*.” The first three columns of images are labeled, “16 d,” “24 d,” and “32 d,” from left to right. A scale bar in the lower left corner of indicates a length of 1 centimeter (cm). The upper and lower images in the fourth column, which are in separate subpanels, include labels indicating a magnification of 10x compared to the magnification of the first three columns.

[00121] **FIG. 4F** shows a line graph having two lines. A legend indicates that the circular data points comprising the upper line represent data from wild type (“WT”) calli and that the square data points comprising the lower line represent data from *pzr1-D* calli. The x-axis is labeled 8, 12, 16, 20, 24, 28, and 32 days, from left to right. The y-axis indicates callus weight in grams (g) from 0.0 to 0.4 in increments of 0.1.

[00122] In **FIGs. 4C, 4D, and 4F**, error bars represent standard deviation, and significant differences were determined using Student’s *t*-test. *, $P < 0.05$; **, $P < 0.001$; and ***, $P < 0.0001$.

[00123] **FIG. 5A** shows two schematics of sections of a plant genome. The upper schematic is labeled “WT” on the left and includes labels above and below the line of the schematic indicating sequences of interest. From left to right, the sequence labels are “3’ UTR,” “TGA,” “Os03go5750,” “ATG,” “5’ UTR,” “5’ UTR,” “ATG,” “*PZRI*, Os03g05760,” “TAG,” and “3’ UTR.” The lower schematic is labeled “*pzr1-D*.” A red arrow pointing from left to right labeled “F” and a second red arrow pointing from right to left labeled “R” are positioned above the upper schematic just to the left of the leftmost “ATG” and to the right of the leftmost “5’ UTR” labels, respectively. The leftmost portion of the lower schematic indicates a pGA2715 cassette comprising sequences labeled “4 x CaMV 35 S” and “LB.” White boxes represent 5’ and 3’ UTRs, blue boxes represent exons, red box denotes the tetramer CaMV 35S promoter in the T-DNA and black lines represent introns. Red arrowheads indicate the positions of forward and reverse primers used for genotyping. Gray boxes represent exons of the gene upstream of *PZRI*. Dotted lines lead from either side of the pGA2715 to a region just to the right of leftmost 5’ UTR sequence of the upper schematic, indicating that the pGA2715 sequence is present in the *pzr1-D* mutant in a locus corresponding to the position just to the right of the leftmost 5’ UTR of the upper schematic. The boxes on the remainder of the lower schematic (e.g., to the right of the “LB” segment of the pGA2715 cassette) match those of the upper schematic. A scale bar is present on the lower schematic, indicating a distance of 1.8 kb from the rightmost edge of the “LB” sequence of the pGA2715 cassette to the leftmost edge of the rightmost 5’ UTR sequence. A second scale bar is present on the lower

schematic, indicating a distance of 3.8 kb from the rightmost edge of the “LB” sequence of the pGA2715 cassette to the leftmost edge of the sequence symbol corresponding to the rightmost ATG of the upper schematic. At the lower right of the figure is a scale bar indicating 500 bp.

[00124] **FIG. 5B** is a bar graph with three groups of three bars each. Labels on the x-axis indicate that the leftmost group of three bars corresponds to expression of the 03g05750 gene, that the center group of three bars corresponds to expression of the 03g05760 gene, and that the rightmost group of three bars corresponds to expression of the 03g05770 gene. A legend at the top of the image indicates that the leftmost bar of each group of bars corresponds to wild type *PZR1* sample data, that the center bar of each group of bars corresponds to DongJin wild type sample data, and that the rightmost bar of each group of bars corresponds to *pzr1-D* sample data. The y-axis indicates relative expression in arbitrary units from 0 to 15 in increments of 5.

[00125] **FIG. 5C** is a bar graph of four bars, labeled DongJin, *PZR1*, *PZR1/pzr1-D*, and *pzr1-D* on the x-axis, from left to right. The y-axis indicates 03g05760 gene expression from 0 to 15 in increments of 5.

[00126] **FIG. 5D** shows a bar graph having two bars, labeled “Mock” and “Pcz” from left to right along the x-axis. The Pcz bar has an error bar. The y-axis indicates relative gene expression in arbitrary units from 0.0 to 1.5 in increments of 0.5.

[00127] **FIG. 5E** shows representative images of morphologies of 1-month-old plants from wild type (WT) plants, and plants derived from overexpression lines (35S::*PZR1*), OX 1, OX 2, OX 3, OX 9, OX 10, OX 13, OX 16. **FIG. 5F** shows a comparison of *PZR1* expression in non-transformed (wild type) plants and plants derived from *PZR1* overexpression lines. **FIG. 5G** shows a comparison of the number of tillers in non-transformed (wild type) plants and plants derived from *PZR1* overexpression lines. **FIG. 5G** shows a comparison of the number of panicles in non-transformed (wild type) plants and plants derived from *PZR1* overexpression lines.

[00128] **FIG. 5E** shows representative images of morphologies of 1-month-old plants from wild type (WT) plants, and plants derived from overexpression lines (35S::*PZR1*), OX 1, OX 2, OX 3, OX 9, OX 10, OX 13, OX 16.

[00129] **FIG. 5F** shows a bar graph having eight bars, labeled “WT,” “OX 1,” “OX 2,” “OX 3,” “OX 9,” “OX 10,” “OX 13,” and “OX 16” from left to right along the x-axis. Each bar has an error bar. The y-axis indicates relative *PZR1* gene expression in fold expression relative to WT from 0 to 80 in increments of 20. The bar heights are, from highest to lowest, “OX 2,” “OX 9,” “OX 3,” “OX 1,” “OX 10,” “OX 13,” “OX 16,” and “WT.”

[00130] **FIG. 5G** shows a bar graph having eight bars, labeled “WT,” “OX 1,” “OX 2,” “OX 3,” “OX 9,” “OX 10,” “OX 13,” and “OX 16” from left to right along the x-axis. Each bar has an

error bar. Above the error bar of each of the “OX 1,” “OX 2,” and “OX 3” bars is one asterisk (*). Above the error bars of each of the “OX 9” and “OX 16” bars is “ns,” indicating no significant difference relative to WT. The y-axis indicates tiller number in tillers per plant (plant⁻¹) from 0 to 20 in increments of 5. The bar heights are, from highest to lowest, “OX 10,” “OX 1,” “OX 3,” “OX 9,” “OX 2,” “OX 13,” “OX 16,” and “WT.”

[00131] **FIG. 5H** shows a bar graph having eight bars, labeled “WT,” “OX 1,” “OX 2,” “OX 3,” “OX 9,” “OX 10,” “OX 13,” and “OX 16” from left to right along the x-axis. Each bar has an error bar. Above the error bar of each of the “OX 1,” “OX 2,” and “OX 3” bars is one asterisk (*). Above the error bars of each of the “OX 9” and “OX 16” bars is “ns,” indicating no significant difference relative to WT. The y-axis indicates panicle number in panicles per plant (plant⁻¹) from 0 to 15 in increments of 5. The bar heights are, from highest to lowest, “OX 10,” “OX 1,” “OX 3,” “OX 9,” “OX 2,” “OX 13,” “OX 16,” and “WT.”

[00132] In **FIG. 5B-FIG. 5D** and **FIG. 5F-FIG. 5H**, error bars represent standard deviation, and significant differences were determined using Student’s *t*-test. *, *P*<0.05; ns, non-significant. Graphs show data from a representative experiment out of three biological replicates performed.

[00133] **FIG. 6A** shows a phylogenetic tree. The upper arm of the first dichotomy leads to a second dichotomy. The upper arm of the second dichotomy indicates At1g47870 E2F2. The lower arm of the first secondary dichotomy leads to the third dichotomy, the upper arm of which indicates At5g22220 E2F1 and the lower arm of which indicates At2g36010 E2F3. The lower arm of the first dichotomy leads to a fourth dichotomy. The lower arm of the fourth dichotomy leads to a fifth dichotomy, the upper arm of which indicates Wheat DP and the lower arm of which indicates LOC_Os01g48700. The upper arm of the fourth dichotomy leads to a sixth dichotomy. The upper arm of the sixth dichotomy leads to a seventh dichotomy, the upper arm of which indicates Human DP2, and the lower arm of which indicates Human DP1. The lower arm of the sixth dichotomy leads to an eighth dichotomy. The upper arm of the eighth dichotomy indicates At5g02470 DPa, and the lower arm of the eighth dichotomy leads to a ninth dichotomy. The upper arm of the ninth dichotomy indicates At5g03415 DPb, and the lower arm of the ninth dichotomy leads to a tenth dichotomy. The upper arm of the tenth dichotomy indicates LOC_Os10g30420. The lower arm of the tenth dichotomy indicates LOC_Os03g05760. A scale bar at the lower left of the image indicates a relative distance of 0.5.

[00134] **FIG. 6B** shows an image of a plant. A label on the right side of the image indicates a shoot portion of the plant, and a label on the left side of the image indicates a root portion of the plant.

[00135] FIG. 6C shows a bar graph having two bars, labeled “Shoot” and “Root” from left to right along the x-axis. Each bar has an error bar. The y-axis indicates relative *PZR1* expression from 0.0 to 1.5 in increments of 0.5.

[00136] FIG. 6D shows an image identifying portions of a plant analyzed in RT-qPCR analysis. Labels at the top of the image indicate a flag leaf, spikelets, and a leaf blade. The image comprises two inset images, with the upper inset indicating a sheath, and the lower inset indicating a node.

[00137] FIG. 6E shows a bar graph having twelve bars arranged in six groups of two bars each. Each group of two bars includes a white bar to the left of a black bar. The six groups of bars are labeled “Seedling,” “Leaf Blade,” “Sheath,” “Node,” “Spikelet,” and “Flag Leaf” from left to right along the x-axis. A label at the top of the graph indicates that the white bar represents data from wild type (“WT”) samples and the black bars represent data from *pzr1-D* samples. Each bar has an error bar. The y-axis indicates relative *PZR1* expression from 0 to 40 in increments of 10.

[00138] FIG. 7A is a Venn diagram of differentially expressed genes (DEGs). A label at the upper left of the figure indicates that the left circle represents 912 DEGs in dark conditions. A label at the upper right of the figure indicates that the right circle represents 463 DEGs in light conditions. Labels on the three regions of the diagram indicate that 234 genes are differentially expressed in both light and dark conditions, that 678 genes are differentially expressed in dark conditions but not light conditions, and that 229 genes are differentially expressed in light conditions but not dark conditions.

[00139] FIG. 7B a bar graph having two bars, labeled “Dark” and “Light” from left to right along the x-axis. Each bar is divided into an upper blue portion and a lower red portion. A legend shows that the lower red portion of each bar indicates upregulated genes and the upper blue portion indicates downregulated genes. The y-axis indicates number of DEGs from 0 to 1000 in increments of 200. Of the 678 DEGs identified under dark conditions, 481 genes were upregulated and 431 genes were downregulated. Under light conditions, 252 were upregulated and 211 were downregulated.

[00140] FIG. 7C shows a bar graph of DEGs in dark conditions. The bar graph has nine bars along the y-axis, labeled “response to stimulus,” “reproduction,” “multicellular organismal process,” “metabolic process,” “localization,” “developmental process,” “cellular process,” “cellular component organization,” “biological regulation,” from top to bottom. The y-axis label indicates that each bar represents a gene ontology term. The x-axis indicates DEG number in each GO Term group from 0 to 200 in increments of 50.

[00141] **FIG. 7D** shows a bar graph of DEGs in light conditions. The bar graph has nine bars along the y-axis, labeled “response to stimulus,” “reproduction,” “multicellular organismal process,” “metabolic process,” “localization,” “developmental process,” “cellular process,” “cellular component organization,” “biological regulation,” from top to bottom. The y-axis label indicates that each bar represents a gene ontology term. The x-axis indicates DEG number in each GO Term group from 0 to 30 in increments of 10.

[00142] **FIG. 7E** shows a bar graph of DEGs in both dark and light conditions. The bar graph has nine bars along the y-axis, labeled “response to stimulus,” “reproduction,” “multicellular organismal process,” “metabolic process,” “localization,” “developmental process,” “cellular process,” “cellular component organization,” “biological regulation,” from top to bottom. The y-axis label indicates that each bar represents a gene ontology term. The x-axis indicates DEG number in each GO Term group from 0 to 50 in increments of 10.

[00143] **FIG. 8A** shows images of five plants, labeled “Mock,” “0.1 Pcz (μM),” “1 Pcz (μM),” “20 Pcz (μM),” “40 Pcz (μM).” Seedlings in Fig. 8A are rice seedlings grown in darkness.

[00144] **FIG. 8B** shows a bar graph having five bars, labeled “Mock,” “0.1 Pcz (μM),” “1 Pcz (μM),” “20 Pcz (μM),” “40 Pcz (μM)” from left to right along the x-axis. Each bar has an error bar. The y-axis indicates root length in centimeters (cm) from 0 to 12 in increments of 2. Error bars represent standard deviation of 10 or more samples per treatment.

[00145] **FIG. 8C** shows images of four plants. At the upper left corner of the image is a scale bar indicating a length of 2 centimeters (cm). Seedlings in Fig. 8C are rice seedlings grown in light.

[00146] **FIG. 8D** shows a bar graph having two white bars, labeled “Mock” and “Pcz” from left to right, to the left of two black bars, labeled “Mock” and “Pcz” from left to right along the x-axis. Labels at the top of the figure indicate that the white bars indicate data from wild type samples and the black bars indicate data from *pzr1-D* samples. Each bar has an error bar. The y-axis indicates root length in centimeters (cm) from 0 to 15 in increments of 5. Error bars represent standard deviation of 10 or more samples per treatment.

[00147] **FIG. 9A** shows 14 images of 7-day-old seedlings grown in Pcz-supplemented medium., labeled “w/w” “w/w” “w/w” “T/T” “T/T” “T/T” “w/T” “T/T” “w/T” “w/T” “w/w” “w/T” “w/T” and “w/w” from left to right. Bars above the images indicate that the two leftmost images are of wild type (“WT”) plants and that the remaining 12 images are of *pzr1-D* plants. The different genotypes are represented as w/w for the wild type, T/T as homozygous mutant (where T represents a *pzr1-D* allele introduced via T-DNA), and w/T for the heterozygote

[00148] FIG. 9B shows images of four plants, labeled “DongJin (w/w)” “*PZRI* (w/w)” “*PZRI/prz1-D* (w/T)” “*prz1-D* (T/T)” from left to right. A scale bar in the upper left corner of the figure indicates a length of 10 centimeters (cm).

[00149] FIG. 9C shows a bar graph having three bars, labeled “WT,” “*PZRI/prz1-D*,” and “*prz1-D*” from left to right along the x-axis. Each bar has an error bar. A line between the “WT” and “*PZRI/prz1-D*” bars indicate that there is no significant difference (“ns”) between the groups. A line between the “WT” and “*prz1-D*” bars indicate that there is no significant difference (“ns”) between the groups. The y-axis indicates plant height in centimeters (cm) from 0 to 150 in increments of 50.

[00150] FIG. 9D shows a bar graph having three bars, labeled “WT,” “*PZRI/prz1-D*,” and “*prz1-D*” from left to right along the x-axis. Each bar has an error bar. A line between the “WT” and “*PZRI/prz1-D*” bars indicate that there is no significant difference (“ns”) between the groups. A line comparing the “WT” and “*prz1-D*” bars includes an asterisk (*). The y-axis indicates panicle number from 0 to 80 in increments of 20.

[00151] FIG. 9E shows a bar graph having three bars, labeled “WT,” “*PZRI/prz1-D*,” and “*prz1-D*” from left to right along the x-axis. Each bar has an error bar. A line comparing the “WT” and “*PZRI/prz1-D*” bars includes an asterisk (*). A line comparing the “WT” and “*prz1-D*” bars includes an asterisk (*). The y-axis indicates tiller number from 0 to 80 in increments of 20.

[00152] FIG. 9F shows a bar graph having five bars, labeled “DongJin,” “*PRZI*,” “*PRZI/prz1-D* #2,” “*PRZI/prz1-D* #3,” and “*prz1-D*” from left to right along the x-axis. Each bar has an error bar. The y-axis indicates relative *PZRI* expression from 0 to 6 in increments of 2.

[00153] FIG. 10A shows images of six wild type seeds (upper row) and six *prz1-D* seeds (lower row). A scale bar indicates a reference length of 5 mm.

[00154] FIG. 10B shows a bar graph having two bars, labeled “WT” and “*prz1-D*” from left to right along the x-axis. Each bar has an error bar. Above the error bar of the *prz1-D* bar is one asterisks (*). The y-axis indicates average seed length in centimeters (cm) from 0 to 1.0 in increments of 0.2.

[00155] FIG. 10C shows a bar graph having two bars, labeled “WT” and “*prz1-D*” from left to right along the x-axis. Each bar has an error bar. Above the error bar of the *prz1-D* bar are the letters “ns,” indicating no significant difference from the wild type data. The y-axis indicates relative seed area in arbitrary units from 0 to 50000 in increments of 10000.

[00156] FIG. 10D shows a bar graph having two bars, labeled “WT” and “*prz1-D*” from left to right along the x-axis. Each bar has an error bar. Above the error bar of the *prz1-D* bar are the

letters “ns” indicating no significant difference from the wild type data. The y-axis indicates seed weight (grams per 50 seeds) from 0 to 1.5 in increments of 0.5.

[00157] A total of 200 seeds per genotype were measured in FIG. 10B – FIG. 10D. Error bars in FIG. 10B – FIG. 10D represent standard deviation, and significant differences were determined using Student’s t-test. *, $P < 0.05$; ns, non-significant difference.

[00158] **FIG. 11A** shows light microscopy images of leaves from wild type seedlings (upper image) and *pzr1-D* seedlings (lower image) with 10x magnification.

[00159] **FIG. 11B** shows light microscopy images of leaves from wild type seedlings (left image) and *pzr1-D* seedlings (right image) with 20x magnification.

[00160] **FIG. 11C** shows a bar graph having two bars, labeled “WT” and “*pzr1-D*” from left to right along the x-axis. Each bar has an error bar. Above the error bar of the *pzr1-D* bar are three asterisks (***). The y-axis indicates average cell size in microns (μm) from 0 to 100 in increments of 20. Six slides, each containing 20 to 30 cells were analyzed per genotype in FIG. 11C. Graph bars of FIG. 11C represent averages and standard deviations of measured cells. Statistical significance between groups was determined using Student’s t-test. ***, $P < 0.0001$.

[00161] **FIG. 12A.** shows images of four plants, labeled Col-0, OX 2, OX 3, and OX 5 from left to right. A line under the OX 2, OX 3, and OX 5 plants labels these three plants as “35S::PZRI.” A scale bar in the upper left corner of the image indicates a distance of 1 centimeter (cm).

[00162] **FIG. 12B** shows two rows of an RNA blot. Above the image, the lanes of the RNA blot are labeled “OX 1,” “OX 2,” “OX 3,” “OX 5,” “OX 8,” “Col-0,” “+,” and “D.W.” from left to right. The upper row of the blot is labeled “PZRI” on the right side of the figure, and the lower row of the blot is labeled “UBQ10.” Positive and negative controls are represented with the sign + and D.W. (distilled water) respectively. Overexpression lines OX 1 and OX 8, which produced very low levels of the fragment corresponding to *PZRI*, were used as a reference.

[00163] **FIG. 12C** shows a bar graph having four bars, labeled “OX 8,” “OX 2,” “OX 3,” and “OX 5” from left to right along the x-axis. Each bar has an error bar. The y-axis indicates relative *PZRI* expression from 0 to 400 in increments of 100.

[00164] **FIG. 12D** shows a bar graph having four bars, labeled “Col-0,” “OX 2,” “OX 3,” “OX 5” from left to right along the x-axis. Each bar has an error bar. Above the error bar of the “OX 2” bar is one asterisk (*). Above the error bar of the “OX 3” bar is one asterisk. Above the error bar of the “OX 2” bar are two asterisks. The y-axis indicates main root length in centimeters (cm) from 0 to 4 in increments of 1.

[00165] **FIG. 12E** shows a bar graph having four bars, labeled “Col-0,” “OX 2,” “OX 3,” “OX 5” from left to right along the x-axis. Each bar has an error bar. Above the error bar of the “OX 2” bar is one asterisk (*). Above the error bar of the “OX 3” bar are two asterisks. Above the error bar of the “OX 2” bar are two asterisks. The y-axis indicates the average number of lateral roots from 0 to 10 in increments of 2.

[00166] Arabidopsis *UBIQUITIN 10* was used as an internal control. The error bars in FIG. 12C – FIG. 12E represent standard deviation, and significant differences were determined using Student’s t-test. *, $P < 0.05$; **, $P < 0.001$; and ***, $P < 0.0001$.

[00167] **FIG. 13** shows schematic representations of four different regions of the genome associated with differentially regulated genes and the locations of E2F/DP and BZR1/BES1 consensus cis-acting elements in those regions. A scale bar at the top of each schematic indicates relative genetic distances of -1000, -500, 1, and +100 basepairs from a transcription start site (labeled TSS on the scale bar). Labels at the bottom of each schematic indicate that red diamond symbols on the schematics represent locations of BZR1/BES1 sequences, that green triangles represent locations of E2F10PCNA sequences, and that gray squares represent E2FCONSENSUS sequences. The top schematic is labeled *LOC_Os10g2004* (up in light), indicating that the gene is upregulated in light conditions. The top schematic has three green triangles, one red diamond, one gray square, and one green triangle from left to right on the schematic map. The second schematic is labeled *LOC_Os11g39190* (up in dark), indicating that the gene is upregulated in dark conditions. The second schematic has one gray square, three green triangles, one red diamond, five green triangles, one red diamond, and one green triangle, from left to right on the schematic map. The third schematic is labeled *LOC_Os12g14840* (down in light), indicating that the gene is downregulated in light conditions. The third schematic has one gray square and one green triangle, one gray square, one red diamond, one gray square, one green diamond, one gray square and one green triangle, one gray square, and one green diamond, from left to right. The bottom schematic is labeled *LOC_Os11g32810* (down in dark), indicating that the gene is downregulated in dark conditions. The bottom schematic has one red diamond, one green triangle, one gray square, one green triangle, one gray square, and one green triangle. TSS defines the transcription start site, the red rhombus indicates position of BZR1/BES1 sites, the green triangle represents E2F10 PCNA and the purple square represents the E2F consensus sites.

[00168] **FIG. 14** shows multiple sequence analysis of Arabidopsis DPb and rice homolog PZR1 proteins. In DPb, the first black underline delimits DNA binding domain (amino acids 101-184) while the lower black line represents the heterodimerization domain (182-263). Asterisks indicate Serine and Threonine residues that follow the S/TxxxS/T pattern of phosphorylation by

BIN2 and homologs. The leftmost portion of each of the 7 alignment rows lists the sample identities, with the Q9FNY20/DPb sample appears on the top half of each row in the figure and Q84VA092/PZR1 appearing on the bottom half of each row in the figure. A first black line runs from Q9FNY20/DPb residue 101 and Q84VA092/PZR1 residue 122 to Q9FNY20/DPb residue 184 and Q84VA092/PZR1 residue 210. A lower black line runs from Q9FNY20/DPb residue 182 and Q84VA092/PZR1 residue 208 to Q9FNY20/DPb residue 263 and Q84VA092/PZR1 residue 2. Asterisks, appearing in alignment rows 1, 2, 3, 6, and 7, represent Serine and Threonine that follow the S/TxxxS/T pattern of phosphorylation by BIN2 and homologs.

Numbered Embodiments

[00169] The following embodiments recite permutations of combinations of features disclosed herein. In some cases, permutations of combinations of features disclosed herein are non-limiting. In other cases permutations of combinations of features disclosed herein are limiting. Other permutations of combinations of features are also contemplated. In particular, each of these numbered embodiments is contemplated as depending from or relating to every previous or subsequent numbered embodiment, independent of their order as listed. 1. A high yielding rice plant having a mutation at a DP locus, wherein the high yielding plant exhibits altered expression of at least one gene selected from the group consisting of: OS11G0549665, OS10G0381601, OS11G0573100, OS11G0549675, OS11G0549680, OS11G0639300, OS01G0146101, OS03G0629800, OS11G0636050, OS07G0159200, OS12G0100100, OS11G0606400, OS03G0223301, OS07G0486000, OS02G0129000, OS11G0540600, OS07G0531900, OS12G0431300, OS03G0576200, OS08G0203350, OS11G0562100, OS11G0701700, OS12G0222650, OS12G0172150, OS12G0257450, OS12G0247700, OS12G0222300, OS12G0255200, OS12G0250900, OS12G0254400, OS10G0175500, OS05G0202800, OS11G0518900, OS08G0255500, OS11G0689800, OS07G0543500, OS12G0209800, OS11G0255300, OS12G0211500, OS06G0254300, OS11G0134300, OS09G0467700, OS01G0146101, OS01G0148100, OS07G0187001, OS11G0640300, OS12G0257400, OS07G0159200, OS01G0845950, OS11G0691100, OS07G0153150, OS11G0605100, OS03G0223301, OS08G0367300, OS11G0618700, OS07G0162450, OS02G0129000, OS03G0299700, OS11G0549680, OS07G0486000, OS11G0569800, OS09G0467700, OS12G0250900, OS12G0406000, OS11G0696600, OS11G0532600, OS01G0520180, OS07G0297400, OS07G0535200, OS12G0425800, OS11G0685200, OS12G0204600, OS12G0239300, OS07G0677100, OS07G0103000, OS05G0414400, OS11G0693800, OS05G0369900, OS11G0687100, OS12G0425500, OS08G0255500, OS11G0689800, Flowering-promoting factor 1-like protein 1, Cyclin-B1-1, Similar to Lipid transfer protein, Similar to Cyclin-

dependent kinase B1-1, Cyclin-dependent kinase A-2, Cyclin-dependent kinase G-1, Proliferating cell nuclear antigen, Cyclin-dependent kinases regulatory subunit 1, Cyclin-dependent kinase G-2, Cyclin-B2-1, Cyclin-P1-1, Similar to Cyclin-D3-1, Cyclin-B2-2, Cyclin-D6-1, Cyclin-D2-2, Cyclin-dependent kinase B2-1, Cyclin-dependent kinase B2-1, and Cyclin-A3-2. 2. A method of increasing seed yield in a rice plant comprising altering expression of at least one gene of the rice plant implicated in DP-E2F signaling. 3. The method of embodiment 2, wherein the at least one gene is selected from the group consisting of: OS11G0549665, OS10G0381601, OS11G0573100, OS11G0549675, OS11G0549680, OS11G0639300, OS01G0146101, OS03G0629800, OS11G0636050, OS07G0159200, OS12G0100100, OS11G0606400, OS03G0223301, OS07G0486000, OS02G0129000, OS11G0540600, OS07G0531900, OS12G0431300, OS03G0576200, OS08G0203350, OS11G0562100, OS11G0701700, OS12G0222650, OS12G0172150, OS12G0257450, OS12G0247700, OS12G0222300, OS12G0255200, OS12G0250900, OS12G0254400, OS10G0175500, OS05G0202800, OS11G0518900, OS08G0255500, OS11G0689800, OS07G0543500, OS12G0209800, OS11G0255300, OS12G0211500, OS06G0254300, OS11G0134300, OS09G0467700, OS01G0146101, OS01G0148100, OS07G0187001, OS11G0640300, OS12G0257400, OS07G0159200, OS01G0845950, OS11G0691100, OS07G0153150, OS11G0605100, OS03G0223301, OS08G0367300, OS11G0618700, OS07G0162450, OS02G0129000, OS03G0299700, OS11G0549680, OS07G0486000, OS11G0569800, OS09G0467700, OS12G0250900, OS12G0406000, OS11G0696600, OS11G0532600, OS01G0520180, OS07G0297400, OS07G0535200, OS12G0425800, OS11G0685200, OS12G0204600, OS12G0239300, OS07G0677100, OS07G0103000, OS05G0414400, OS11G0693800, OS05G0369900, OS11G0687100, OS12G0425500, OS08G0255500, OS11G0689800, Flowering-promoting factor 1-like protein 1, Cyclin-B1-1, Similar to Lipid transfer protein, Similar to Cyclin-dependent kinase B1-1, Cyclin-dependent kinase A-2, Cyclin-dependent kinase G-1, Proliferating cell nuclear antigen, Cyclin-dependent kinases regulatory subunit 1, Cyclin-dependent kinase G-2, Cyclin-B2-1, Cyclin-P1-1, Similar to Cyclin-D3-1, Cyclin-B2-2, Cyclin-D6-1, Cyclin-D2-2, Cyclin-dependent kinase B2-1, Cyclin-dependent kinase B2-1, and Cyclin-A3-2. 4. A method of increasing seed yield in a rice plant comprising altering expression of at least one gene of the rice plant implicated in brassinosteroid signaling. 5. The method of embodiment 4, wherein the at least one gene is selected from the group consisting of: OS11G0549665, OS10G0381601, OS11G0573100, OS11G0549675, OS11G0549680, OS11G0639300, OS01G0146101, OS03G0629800, OS11G0636050, OS07G0159200, OS12G0100100, OS11G0606400, OS03G0223301, OS07G0486000, OS02G0129000, OS11G0540600, OS07G0531900, OS12G0431300, OS03G0576200,

OS08G0203350, OS11G0562100, OS11G0701700, OS12G0222650, OS12G0172150, OS12G0257450, OS12G0247700, OS12G0222300, OS12G0255200, OS12G0250900, OS12G0254400, OS10G0175500, OS05G0202800, OS11G0518900, OS08G0255500, OS11G0689800, OS07G0543500, OS12G0209800, OS11G0255300, OS12G0211500, OS06G0254300, OS11G0134300, OS09G0467700, OS01G0146101, OS01G0148100, OS07G0187001, OS11G0640300, OS12G0257400, OS07G0159200, OS01G0845950, OS11G0691100, OS07G0153150, OS11G0605100, OS03G0223301, OS08G0367300, OS11G0618700, OS07G0162450, OS02G0129000, OS03G0299700, OS11G0549680, OS07G0486000, OS11G0569800, OS09G0467700, OS12G0250900, OS12G0406000, OS11G0696600, OS11G0532600, OS01G0520180, OS07G0297400, OS07G0535200, OS12G0425800, OS11G0685200, OS12G0204600, OS12G0239300, OS07G0677100, OS07G0103000, OS05G0414400, OS11G0693800, OS05G0369900, OS11G0687100, OS12G0425500, OS08G0255500, OS11G0689800, Flowering-promoting factor 1-like protein 1, Cyclin-B1-1, Similar to Lipid transfer protein, Similar to Cyclin-dependent kinase B1-1, Cyclin-dependent kinase A-2, Cyclin-dependent kinase G-1, Proliferating cell nuclear antigen, Cyclin-dependent kinases regulatory subunit 1, Cyclin-dependent kinase G-2, Cyclin-B2-1, Cyclin-P1-1, Similar to Cyclin-D3-1, Cyclin-B2-2, Cyclin-D6-1, Cyclin-D2-2, Cyclin-dependent kinase B2-1, Cyclin-dependent kinase B2-1, and Cyclin-A3-2. 6. The method of embodiment 4, wherein the gene is implicated in DP-E2F signaling. 7. The method of embodiment 6, wherein the gene is regulated by DP. 8. The method of embodiment 6, wherein the gene exhibits altered expression in a DP mutant line. 9. A method of increasing yield in a rice field comprising planting the field using a rice line having a mutation affecting DP expression. 10. The method of embodiment 9, wherein the mutation is a CRISPR-directed mutation. 11. The method of embodiment 9, wherein the increasing yield is measured relative to a reference line lacking a mutated DP locus. 12. A plant comprising a *propiconazole resistant1-D (pzt1-D)* mutation. 13. The plant of embodiment 12, wherein the plant is heterozygous for the *pzt1-D* mutation. 14. The plant of embodiment 12, wherein the plant is homozygous for the *pzt1-D* mutation. 15. The plant of embodiment 12, wherein the plant overexpresses the *PZRI* gene compared to a plant lacking a *pzt1-D* mutation. 16. A high-yield crop field that yields at least 5% above a wild-type crop field grown under similar conditions as the high-yield rice field, wherein the high-yield crop field is treated with an herbicide inhibiting brassinosteroid activity prior to harvest. 17. The high-yield crop field of embodiment 16 wherein the herbicide comprises a brassinosteroid synthesis inhibitor. 18. The method of embodiment 16, wherein the herbicide is Brassinazole. 19. The method of embodiment 16, wherein the herbicide is propiconazole. 20. The high-yield crop field of embodiment 16, wherein the high-yield crop field

that yields at least 10% above a wild-type crop field grown under similar conditions as the high-yield crop field. 21. The high-yield crop field of embodiment 16, wherein the high-yield crop field comprises a rice plant. 22. The high-yield crop field of embodiment 21, wherein the rice plant comprises a *propiconazole resistant 1-D (pzt1-D)* mutation. 23. The high-yield crop field of embodiment 22, wherein the rice plant is heterozygous for the *pzt1-D* mutation. 24. The high-yield crop field of embodiment 22, wherein the rice plant is homozygous for the *pzt1-D* mutation. 25. The high-yield crop field of embodiment 21, wherein the rice plant overexpresses the *PZT1* gene compared to a plant lacking a *pzt1-D* mutation. 26. A method of clearing a weed from a field comprising planting the field using a plant line having a mutation affecting DP expression and administering an herbicide that impacts brassinosteroid signaling. 27. The method of embodiment 26, wherein the herbicide inhibits brassinosteroid signaling. 28. The method of embodiment 26, wherein the herbicide is a brassinosteroid synthesis inhibitor. 29. The method of embodiment 26, wherein the herbicide is Brassinazole. 30. The method of embodiment 26, wherein the herbicide is propiconazole. 31. The method of embodiment 26, using a rice plant line. 32. A method of clearing a weed from a field comprising planting the field using a plant line having a mutation affecting DP-E2F signaling and administering an herbicide that impacts brassinosteroid signaling. 33. The method of embodiment 32, wherein the herbicide inhibits brassinosteroid signaling. 34. The method of embodiment 32, wherein the herbicide is a brassinosteroid synthesis inhibitor. 35. The method of embodiment 32, wherein the herbicide is Brassinazole. 36. The method of embodiment 32, wherein the herbicide is propiconazole. 37. The method of embodiment 32, using a rice plant line. 38. A method of clearing a weed from a field comprising planting the field using a plant line having altered expression in a DP-E2F signaling pathway effector and administering an herbicide that impacts brassinosteroid signaling. 39. The method of embodiment 38, wherein the herbicide inhibits brassinosteroid signaling. 40. The method of embodiment 38, wherein the herbicide is a brassinosteroid synthesis inhibitor. 41. The method of embodiment 38, wherein the herbicide is Brassinazole. 42. The method of embodiment 38, wherein the herbicide is propiconazole. 43. The method of embodiment 38, using a rice plant line. 44. The method of embodiment 38, wherein the DP-E2F signaling pathway effector comprises at least one of OS11G0549665, OS10G0381601, OS11G0573100, OS11G0549675, OS11G0549680, OS11G0639300, OS01G0146101, OS03G0629800, OS11G0636050, OS07G0159200, OS12G0100100, OS11G0606400, OS03G0223301, OS07G0486000, OS02G0129000, OS11G0540600, OS07G0531900, OS12G0431300, OS03G0576200, OS08G0203350, OS11G0562100, OS11G0701700, OS12G0222650, OS12G0172150, OS12G0257450, OS12G0247700, OS12G0222300, OS12G0255200, OS12G0250900, OS12G0254400, OS10G0175500, OS05G0202800,

OS11G0518900, OS08G0255500, OS11G0689800, OS07G0543500, OS12G0209800, OS11G0255300, OS12G0211500, OS06G0254300, OS11G0134300, OS09G0467700, OS01G0146101, OS01G0148100, OS07G0187001, OS11G0640300, OS12G0257400, OS07G0159200, OS01G0845950, OS11G0691100, OS07G0153150, OS11G0605100, OS03G0223301, OS08G0367300, OS11G0618700, OS07G0162450, OS02G0129000, OS03G0299700, OS11G0549680, OS07G0486000, OS11G0569800, OS09G0467700, OS12G0250900, OS12G0406000, OS11G0696600, OS11G0532600, OS01G0520180, OS07G0297400, OS07G0535200, OS12G0425800, OS11G0685200, OS12G0204600, OS12G0239300, OS07G0677100, OS07G0103000, OS05G0414400, OS11G0693800, OS05G0369900, OS11G0687100, OS12G0425500, OS08G0255500, OS11G0689800, Flowering-promoting factor 1-like protein 1, Cyclin-B1-1, Similar to Lipid transfer protein, Similar to Cyclin-dependent kinase B1-1, Cyclin-dependent kinase A-2, Cyclin-dependent kinase G-1, Proliferating cell nuclear antigen, Cyclin-dependent kinases regulatory subunit 1, Cyclin-dependent kinase G-2, Cyclin-B2-1, Cyclin-P1-1, Similar to Cyclin-D3-1, Cyclin-B2-2, Cyclin-D6-1, Cyclin-D2-2, Cyclin-dependent kinase B2-1, Cyclin-dependent kinase B2-1, and Cyclin-A3-2. 45. The method of embodiment 38, wherein the DP-E2F signaling pathway effector is a gene selected from the group consisting of OS11G0549665, OS10G0381601, OS11G0573100, OS11G0549675, OS11G0549680, OS11G0639300, OS01G0146101, OS03G0629800, OS11G0636050, OS07G0159200, OS12G0100100, OS11G0606400, OS03G0223301, OS07G0486000, OS02G0129000, OS11G0540600, OS07G0531900, OS12G0431300, OS03G0576200, OS08G0203350, OS11G0562100, OS11G0701700, OS12G0222650, OS12G0172150, OS12G0257450, OS12G0247700, OS12G0222300, OS12G0255200, OS12G0250900, OS12G0254400, OS10G0175500, OS05G0202800, OS11G0518900, OS08G0255500, OS11G0689800, OS07G0543500, OS12G0209800, OS11G0255300, OS12G0211500, OS06G0254300, OS11G0134300, OS09G0467700, OS01G0146101, OS01G0148100, OS07G0187001, OS11G0640300, OS12G0257400, OS07G0159200, OS01G0845950, OS11G0691100, OS07G0153150, OS11G0605100, OS03G0223301, OS08G0367300, OS11G0618700, OS07G0162450, OS02G0129000, OS03G0299700, OS11G0549680, OS07G0486000, OS11G0569800, OS09G0467700, OS12G0250900, OS12G0406000, OS11G0696600, OS11G0532600, OS01G0520180, OS07G0297400, OS07G0535200, OS12G0425800, OS11G0685200, OS12G0204600, OS12G0239300, OS07G0677100, OS07G0103000, OS05G0414400, OS11G0693800, OS05G0369900, OS11G0687100, OS12G0425500, OS08G0255500, OS11G0689800, Flowering-promoting factor 1-like protein 1, Cyclin-B1-1, Similar to Lipid transfer protein, Similar to Cyclin-dependent kinase B1-1, Cyclin-

dependent kinase A-2, Cyclin-dependent kinase G-1, Proliferating cell nuclear antigen, Cyclin-dependent kinases regulatory subunit 1, Cyclin-dependent kinase G-2, Cyclin-B2-1, Cyclin-P1-1, Similar to Cyclin-D3-1, Cyclin-B2-2, Cyclin-D6-1, Cyclin-D2-2, Cyclin-dependent kinase B2-1, Cyclin-dependent kinase B2-1, and Cyclin-A3-2. 46. A method of increasing yield in a crop field comprising a plant having a mutation affecting a signaling pathway selected from the group consisting of: a cell cycle pathway, a hormone signaling pathway, and a metabolic pathway. 47. The method of embodiment 46, wherein the mutation affects a hormone signaling pathway. 48. The method of embodiment 47, wherein the hormone signaling pathway comprises BR signaling. 49. The method of embodiment 48, wherein the mutation causes increased BR signaling. 50. The method of embodiment 46, wherein the mutation affects a cell cycle pathway. 51. The method of embodiment 50, wherein the mutation affects Rb signaling. 52. The method of embodiment 51, wherein the mutation results in a downregulation of Rb signaling activity. 53. The method of embodiment 50, wherein the mutation alters the expression of at least one gene implicated in DP-E2F signaling. 54. The method of embodiment 53, wherein the mutation causes overexpression of at least one gene implicated in DP-E2F signaling. 55. The method of embodiment 54, wherein the mutation comprises an exogenous nucleic acid sequence. 56. The method of embodiment 54, wherein the mutation affects the expression of the *PZR1* gene. 57. The method of embodiment 56, wherein the mutation causes the overexpression of the *PZR1* gene. 58. The method of embodiment 57, wherein the mutation is a *pzr1-D* mutation. 59. The method of embodiment 58, wherein the plant is homozygous for the *pzr1-D* mutation. 60. The method of claim 50, wherein the at least one gene is selected from the group consisting of: OS11G0549665, OS10G0381601, OS11G0573100, OS11G0549675, OS11G0549680, OS11G0639300, OS01G0146101, OS03G0629800, OS11G0636050, OS07G0159200, OS12G0100100, OS11G0606400, OS03G0223301, OS07G0486000, OS02G0129000, OS11G0540600, OS07G0531900, OS12G0431300, OS03G0576200, OS08G0203350, OS11G0562100, OS11G0701700, OS12G0222650, OS12G0172150, OS12G0257450, OS12G0247700, OS12G0222300, OS12G0255200, OS12G0250900, OS12G0254400, OS10G0175500, OS05G0202800, OS11G0518900, OS08G0255500, OS11G0689800, OS07G0543500, OS12G0209800, OS11G0255300, OS12G0211500, OS06G0254300, OS11G0134300, OS09G0467700, OS01G0146101, OS01G0148100, OS07G0187001, OS11G0640300, OS12G0257400, OS07G0159200, OS01G0845950, OS11G0691100, OS07G0153150, OS11G0605100, OS03G0223301, OS08G0367300, OS11G0618700, OS07G0162450, OS02G0129000, OS03G0299700, OS11G0549680, OS07G0486000, OS11G0569800, OS09G0467700, OS12G0250900, OS12G0406000, OS11G0696600, OS11G0532600, OS01G0520180, OS07G0297400,

OS07G0535200, OS12G0425800, OS11G0685200, OS12G0204600, OS12G0239300, OS07G0677100, OS07G0103000, OS05G0414400, OS11G0693800, OS05G0369900, OS11G0687100, OS12G0425500, OS08G0255500, OS11G0689800, Flowering-promoting factor 1-like protein 1, Cyclin-B1-1, Similar to Lipid transfer protein, Similar to Cyclin-dependent kinase B1-1, Cyclin-dependent kinase A-2, Cyclin-dependent kinase G-1, Proliferating cell nuclear antigen, Cyclin-dependent kinases regulatory subunit 1, Cyclin-dependent kinase G-2, Cyclin-B2-1, Cyclin-P1-1, Similar to Cyclin-D3-1, Cyclin-B2-2, Cyclin-D6-1, Cyclin-D2-2, Cyclin-dependent kinase B2-1, Cyclin-dependent kinase B2-1, and Cyclin-A3-2. 61. The method of embodiment 46, wherein at least one aspect of the plant is increased, the aspect being selected from the group consisting of: plant weight, tiller number, panicle number, total length, root length, and coleoptile length. 62. The method of embodiment 46, wherein the yield of the crop field is at least 5% greater than that of a crop field planted with a reference line and grown under similar conditions as the crop field comprising the plant. 63. The method of embodiment 62, wherein the mutation is a *pzr1-D* mutation. 64. The method of embodiment 46, wherein the crop field is treated with a herbicide. 65. The method of embodiment 64, wherein the crop field comprises a weed, and the herbicide inhibits brassinosteroid synthesis in the weed prior to harvest. 66. The method of embodiment 65, wherein the herbicide is selected from the group consisting of Brassinazole and propiconazole. 66. The method of embodiment 65, wherein the mutation affects the expression of the *PZR1* gene. 67. The method of embodiment 66, wherein the mutation causes overexpression of the *PZR1* gene. 68. The method of embodiment 67, wherein the mutation is a *pzr1-D* mutation. 69. A method of clearing a weed from a field comprising: planting the field using a plant having a mutation affecting a signaling pathway selected from the group consisting of: a cell cycle pathway, a hormone signaling pathway, and a metabolic pathway; and administering a herbicide that impacts brassinosteroid signaling. 70. The method of embodiment 69, wherein the mutation affects a hormone signaling pathway. 71. The method of embodiment 70, wherein the mutation affects BR signaling. 72. The method of embodiment 69, wherein the mutation affects a cell cycle pathway. 73. The method of embodiment 72, wherein the mutation affects Rb signaling. 74. The method of embodiment 72, wherein the mutation alters the expression of at least one gene implicated in DP-E2F signaling. 73. The method of embodiment 72, wherein the mutation affects the expression of the *PZR1* gene. 74. The method of embodiment 73, wherein the mutation causes the overexpression of the *PZR1* gene. 75. The method of embodiment 74, wherein the mutation is a *pzr1-D* mutation. 76. The method of embodiment 69, wherein the herbicide is selected from the group consisting of Brassinazole and propiconazole.

[00170] The term “about” when used in the context of a scalar value refers to + or – 10% of the scalar value. The term “about” when used in the context of a range of values refers to a range that includes values from 10% lower than the lowest value of the range to values 10% higher than the highest value of the range.

[00171] The term “at least one of” followed by a list such as “A, B, C, or D” refers to a list comprising each member of the list, individually, or any combination of two or more members of the list, up to and including all members of the list and, optionally, including other elements not listed in the list.

[00172] As used herein, a plant can comprise whole plants, ancestors and progeny of the plants and plant parts, including seeds, shoots, stems, roots (including tubers), and plant cells, tissues and organs. The plant can also comprise suspension cultures, embryos, meristematic regions, callus tissue, leaves, seeds, roots, shoots, gametophytes, sporophytes, pollen, and microspores.

[00173] The term plant cell refers variously to constitutive parts of a plant or cells derived from plants. For example, a plant cell can be a protoplast or phytoplasm.

EXAMPLES

[00174] These examples are provided for illustrative purposes. Examples are not necessarily limiting on claimed subject matter, although particular elements may be drawn from any of these examples in support of claim amendments to clarify one or more of the pending claims in prosecution.

EXAMPLE 1

Production and screening of high-yielding plant lines

[00175] This example describes methods for creating mutant plant lines and screening for a high-yielding plant. Mutant plants produced by the methods of this example can exhibit increased growth characteristics, such as increased plant height, plant weight, tiller number, panicle number, seed number, seed weight, seed size, leaf angle, number of primary branches, number of secondary branches, and/or growth rate. As disclosed herein, modulation of genes related to DP-E2F signaling (e.g., overexpression of the *PZRI* gene in rice) using the methods of this example can produce a high-yielding plant.

[00176] **Cloning and plant transformation.** Vectors used to produce rice and Arabidopsis plants overexpressing rice *OsDPB/PZRI* under the control of a CaMV 35S promoter were constructed as follows: RNA was extracted, and cDNA was synthesized from 7-day-old rice

seedlings with specific primers (Table 5). The specific primers were used to amplify the full-length CDS of interest. The resulting products (696 bp) corresponding to the gene of interest was purified and cloned into the entry vector pENTR/SD/D-TOPO (Invitrogen), followed by cloning into the destination vector pEarleyGate101(C-YFP-HA), which is compatible with the Gateway system (Thermo Fisher Scientific). For *Arabidopsis* transformation, *Agrobacterium* strain GV3101 was transformed with the vectors, and the LBA4404 strain was used for rice. The constructs were transformed into plants using conventional *Agrobacterium*-mediated techniques, and transgenic seedlings were selected on MS medium supplemented with 20 mg/L BASTA. BASTA-resistant and -sensitive plants were identified, and a chi-square test was carried out to test monogenic segregation pattern.

[00177] A T-DNA activation-tagging mutant population was screened in the presence of Pcz treatment to identify Pcz-resistant lines. These mutants were developed using the pGA2715 T-DNA vector harboring four copies of the constitutive CaMV 35S enhancer. Insertion of a T-DNA vector in a genome can cause transcriptional activation of genes flanking the inserted T-DNA and can result in dominant gain-of-function mutations (Jeong et al., 2002).

[00178] Among the 17 propiconazole-resistant lines recovered, the *propiconazole-resistant 1* (*pzr1-D*) mutant was further investigated with the phenotypes of increased seed yields. This mutant exhibited several characteristic BR phenotypes, such as semi-dwarfism and a greater number of tillers along with increased sensitivity to BRs. Surprisingly, *pzr1-D* mutant plants exhibit normal seed size despite exhibiting semi-dwarfism. Molecular characterization and phylogenetic analysis showed that the T-DNA in *pzr1-D* inserted into a DP locus and activates the expression of a homolog of the *Arabidopsis DPb* transcription factor gene involved in cell cycle regulation. Both dominant mutants and transgenic lines overexpressing *PZR1* showed increased numbers of tillers, panicles, and branches in panicles, all contributing to a considerable increase in seed yield. These findings reveal a role for PZR1 in mediating BR-regulated cell division control and increasing seed yield in rice.

[00179] Genomic DNA and genotyping. Verification of successful nucleic acid cassette insertion in generated plant lines and evaluation of homozygosity in generated plant lines was performed. DNA extraction was performed using a DNA Prep Kit (BioFACT) following the manufacturer's recommendations. The DNA was quantified using a spectrophotometer system (BioTek) controlled with the Gen5 Data Analysis software interface. Genotyping was performed using 50 ng DNA template in two sets of PCR. In one set, specific primers for *LOC_Os03g05760* and the surrounding region were used to amplify the wild-type allele. The other set was performed using a primer specific to the left border of the T-DNA (pGA2715 LB) to amplify the mutant allele in which the

T-DNA was inserted. This analysis allowed identification of homozygous and heterozygous mutant plants for each line, as well as segregating wild-type plants. A line with only the wild-type allele was considered to be segregating wild type (w/w), and a line with only the mutant allele amplified was considered to be homozygous (T/T). Lines in which both alleles were amplified were considered to be heterozygous for the insertion (w/T). The sequences of the primers used for PCR are listed in Table 5.

Table 5. Primers used.

Gene	Locus ID	Direction	Sequence (5' to 3')	Size (bp)	Purpose
<i>PZR1</i>	<i>LOC_Os03g05760</i>	forward	CACC ATGAACTTGTTGCCGAATTTGC	693	cloning
		reverse	ATGCTCGTGCTTCACTCGC		
<i>pGA2715 LB</i>		forward	TTGGGATCCTCTAGAGTCGA		genotyping
		reverse			
<i>OsDPB/PZR1</i>	<i>LOC_Os03g05760</i>	forward	TGCGATCCCAGAGACAACCC	865	genotyping
		reverse	TGAGCCGTGGCCGCATAGAAA		
<i>OsDPB/PZR1</i>	<i>LOC_Os03g05760</i>	forward	AGCATTTTGCCACCAGATCC	149	real-time RT-PCR
		reverse	GCAACCCCTTCCACTGAATTTC		
<i>03g05750</i>	<i>LOC_Os03g05750</i>	forward	ACGAGAAGAAGAAGGACAAGGG	97	real-time RT-PCR
		reverse	TCGATGCTCTTGCTTTGTC		
<i>03g05770</i>	<i>LOC_Os03g05770</i>	forward	TCCTCTTCCACGACTGCTTC	144	real-time RT-PCR
		reverse	CCAGCTCCGTCTTGATCTCG		
<i>OsACTIN</i>	<i>LOC_Os03g50885</i>	forward	GCGTGGACAAAGTTTTCAACCG	152	real-time RT-PCR
		reverse	TCTGGTACCCTCATCAGGCATC		
<i>OsCPD</i>	<i>LOC_Os11g04710</i>	forward	TTCTTCTCCATCCCCTTTCCTCTCGCCA	211	real-time RT-PCR
		reverse	TTCTTCTCCATCCCCTTTCCTCTCGCCA		
<i>OsCYP85A1</i>	<i>LOC_Os03g40540</i>	forward	GAGGTGGCTGGAGAAGAACAT	99	real-time RT-PCR
		reverse	AGGAAGTAGGCACCGTAGAAAT		
<i>OsDWF4</i>	<i>LOC_Os03g12660</i>	forward	TGGGCTCTGAAACAATCTAACCT	118	real-time RT-PCR
		reverse	CAAGGAAGAAGATGGCGAGG		
<i>AtUBQ10</i>	<i>At4g05320</i>	forward	TCCAGGACAAGGAAGGTATTCC	205	real-time RT-PCR
		reverse	CCACCAAAGTTTTACATGAAACGAA		

EXAMPLE 2

Methods for conferring resistance to the BR biosynthesis inhibitor propiconazole on a plant line

[00180] This example describes methods of producing a plant or plant line resistant to a herbicide that impacts brassinosteroid signaling. Production of a plant resistant to a herbicide is useful in methods for producing a high-yield crop field. For example, a crop field comprising a

crop plant resistant to a herbicide can be treated with the herbicide to efficiently remove a weed or plurality of weeds from the crop field, allowing the crop plant to flourish and increasing the overall yield of the crop field.

[00181] The response of rice to various concentrations of the inhibitor Pcz was investigated to identify the optimal conditions for isolating Pcz-resistant mutants. Pcz affected rice plant growth and induced dwarfism in a dose-dependent manner (FIG. 8A and FIG. 8B). Treatment with 30 μ M Pcz reduced the total size of wild-type seedlings by up to 47%, and the response of roots was even more severe, with a 65% inhibition of growth (FIG. 1A and FIG. 1B). To ensure that Pcz was effectively inhibiting BRs, expression levels of a key BR biosynthetic gene, *OsDWF4*, was examined. *OsDWF4* mRNA levels increased in plants treated with Pcz, which is in accordance with the negative feedback regulation of BR biosynthetic genes (FIG. 1C). From these results, it should be possible to perform visual phenotypic screening for resistant seedlings, which would be identifiable by their longer roots and/or leaves compared to wild-type seedlings.

[00182] Seventeen lines were identified from mutant populations generated by activation-tagging, as described in Example 1, with various degrees of resistance to the treatment. The individual lines were designated PROPICONAZOLE RESISTANT (PZR) 1 to 17. Among these lines, a dominant mutant, *pzr1-D*, with visible resistant phenotypes that co-segregated with the T-DNA insertion (FIG. 9A) was isolated. The root lengths of homozygous mutant seedlings under 30 μ M Pcz treatment were almost identical to those under mock treatment conditions, unlike the wild type, which exhibited a 45% inhibition of root growth (FIG. 1D and FIG. 1E). Pcz treatment under dark conditions yielded similar resistant phenotype as to those obtained under the light (FIG. 8C and FIG. 8D).

[00183] **Chemical treatments and morphological analysis.** Rice seeds were sterilized for 30 min with 50% sodium hypochlorite and washed 4–5 times with water prior to planting. Before each treatment, the seeds were germinated on filter paper soaked with water for 2 days. Newly germinated seeds at similar growth stage were subjected to treatments at a planting depth of 1 cm. For Pcz treatment, the seeds were planted in coarse vermiculite soil soaked with water supplemented with the indicated Pcz concentrations. Pcz (100 mM) dissolved in DMSO was used as a working solution, and DMSO alone was added to water as a control or mock treatment. Plants were maintained in total darkness or in the light (80-100 μ mol m⁻² s⁻¹ intensity) under long-day conditions (14-h light/10-h dark photoperiod). After 7 days of treatment, images were taken, and growth parameters were measured from the images using ImageJ software. Overall plant height was measured from the end of the root to the highest leaf, whereas the length of the main root was used to determine root length. Seed length and area were calculated from the digital photographs

using ImageJ software. All statistical analyses were performed using GraphPad Prism 5 software. Significance was evaluated using Student's *t*-test.

EXAMPLE 3

Evaluation of plant architecture and yield in rice plants

[00184] This example describes methods of evaluating plant architecture and yield in plants generated by methods of genetic modification disclosed herein, such as those described in Example 1.

[00185] Phenotypes of adult plants in the field and under greenhouse conditions were examined and it was found that *pzr1-D* plants had higher yields than wild-type plants (FIG. 2A to FIG. 2I). The total plant weight was 33% higher in the mutant than in the wild type, although their total heights did not significantly differ. Increases in weight can be explained by the increased number of tillers in the mutant, as well as the increased number of panicles per plant (FIG. 2A to FIG. 2F). Seed weight per plant increased from 30 g in wild type to 50 g in the mutant, indicating an increase in yield of approximately 160%. The morphology of the panicles was examined and it was found that not only was the number of panicles increased in the mutant, but the number of primary and secondary branches per panicle was also higher in *pzr1-D* than in the wild type (FIG. 2F to FIG. 2I). A higher weight of total seeds in the activation-tagging mutants may be due to an increased number of seeds per plant rather than an increase in seed size. Indeed, the seed size, area, and weight of mutant seeds were not significantly different from those of wild-type seeds. In fact, the mutant seeds were slightly smaller than wild-type seeds (FIG. 10B).

[00186] **Mutant plants show increased sensitivity to BR.** Since the *pzr1-D* mutant was isolated based on its resistance to a BR inhibitor, the response of the mutant to exogenous treatment with the brassinosteroid, brassinolide (BL), was evaluated. Rice leaf bending is sensitive to active BRs, which forms the basis for the well-known lamina-joint inclination bioassay to investigate BR responses (Wada et al., 1981). Under conventional growth conditions, the bending angle of the leaves of *pzr1-D* seedlings was greater than that of wild-type plants (FIG. 3A and FIG. 3B). In the lamina bending assay, treatment with BL led to a dramatically increased leaf angle in *pzr1-D* plants, whereas wild-type plants exhibited a milder response (FIG. 3C and FIG. 3D). At greater BL concentrations, the difference in the leaf angle response was more pronounced (FIG. 3E). These results suggest that the *pzr1-D* mutant is more sensitive to exogenous BL treatment than are wild-type seedlings.

[00187] Root and coleoptile growth of seedlings were evaluated in response to BL treatment under dark conditions. In the absence of BL, there were significant differences in root growth between *pzr1-D* and its wild-type counterpart when grown in darkness (FIG. 3F). Moreover, when

the medium was supplemented with BL, the inhibition of root growth was more pronounced in *pzr1-D* than in the wild type, and the opposite response was observed in coleoptiles (e.g., increased growth) (FIG. 3G and FIG. 3H). Thus, the increased sensitivity of the mutant seedlings was confirmed based on their reduced root growth and increased coleoptile elongation in response to BL.

[00188] Callus induction. Rice seeds were sterilized and placed on filter paper until complete dry. The seeds (12–18) were germinated and cultured on 2N6 medium plates containing Duchefa's Chu (N6) powder (4 g L⁻¹), sucrose (30 g L⁻¹), L-proline (2.9 g L⁻¹), casein (0.3 g L⁻¹), myo-inositol (0.1 g L⁻¹), 2,4-D (2 mg L⁻¹), and Phytigel (4 g L⁻¹) at pH 5.8. The plates were incubated in a growth chamber under dark conditions at 32°C for the indicated number of days before being weighed and photographed for analysis with ImageJ software.

[00189] Microscopy. Root images were obtained under a Leica TCS SP9 confocal laser-scanning microscope. Root samples were excised from 7-day-old seedlings 1 cm above the root tip and submerged in 10 µg/mL propidium iodide (PI) solution for 3 minutes. The samples were rinsed twice in distilled water prior to observation. Images were compiled and analyzed using LAS X software version 3.0.2. For cell counting and size determination, cells inside a 60 µm² square drawn in the meristematic zone 300 µm above the root tip (FIG. 4A) were examined. Leaves were photographed under a Primo Vert inverted microscope (Zeiss), and ImageJ software was used for measurement. Each leaf of a 7-day-old seedling was dissected transversally down the middle, and images were taken and used to compare genotypes.

[00190] Lamina-joint bending assay. The lamina bending bioassay was performed as described (Wada et al., 1984; Zhang et al., 2012), with some modifications. Seeds were sterilized and germinated on filter papers and transferred to 0.5x MS medium, followed by incubation for 7 days in darkness. Segments containing the second-leaf lamina joint were cut from uniformly growing seedlings. The segments were floated on distilled water for 24 h in darkness to remove any chemical residues from the plant that might alter the experiment, and the samples were checked to ensure that all lamina angles were similar prior to treatment. Uniform samples with similar lamina angles were floated on distilled water containing the indicated concentration of brassinolide (BL). All procedures, including sectioning of the samples and transfer to various solutions, were performed in a dark room to avoid exposure to light as much as possible. The segments were incubated for 48 h in darkness under different treatments and photographed. The photographs were used to measure the angle between the lamina and the blade sheath using ImageJ software. All statistical analysis was performed using GraphPad Prism 5 software, and significance was evaluated using Student's *t*-test.

[00191] **The *pzr1-D* mutant shows altered cell number and size in different tissues.** Root and leaf tissues of wild-type and mutant seedlings were observed under a confocal microscope and noticed abnormalities in the *pzr1-D* samples. Roots of *pzr1-D* contained greater number of cells, most of which were smaller than those in wild-type roots (FIG. 4A to FIG. 4D). Similarly, mutant leaves were wider relative to wild-type leaves and had more but smaller cells (FIG. 11A to FIG. 11C). Because cell division appeared to be altered in the mutant, callus initiation and morphology were examined in wild-type and *pzr1-D* plants. Callus induced from mutant seeds was smaller than that derived from wild-type seeds, and it exhibited adventitious root formation, whereas wild-type callus did not (FIG. 4E and FIG. 4F).

[00192] These results support a role for *PZRI* in regulating cell division and growth in rice. The results also support the modulation of DP-E2F signaling pathway for producing high-yield rice plants and for producing a high-yield crop field.

EXAMPLE 4

Evaluation of gene expression for identification of high-yielding plants

[00193] This example describes methods of evaluating gene expression in a plant. In particular, the methods of this example can be used to determine the effect of a genetic modification on a gene, to identify a high-yielding plant or high-yielding plant line, or to select a high-yielding plant or high-yielding plant line from a plurality of plants or plant lines produced by methods described herein (e.g., methods comprising genetic modification).

[00194] **RNA isolation and gene expression analysis.** The total RNA used for RT-qPCR analysis was isolated from rice or Arabidopsis tissues using an RNeasy system (Qiagen) following the manufacturer's instructions. The cDNA was synthesized from 2 µg RNA using M-MLV reverse transcriptase (ELPIS). RT-qPCR analysis was performed on an Applied Biosystems StepOne Real-Time PCR System with Power SYBR Green PCR Master Mix as previously described (Corvalan and Choe, 2017) using the primers listed in Table 5. For the Arabidopsis OX lines, gene expression data in RT-qPCR were normalized against OX line 8, which showed low transcript levels in the RT-PCR analysis, as *Loc_Os03g05760* is a rice gene and is therefore not present in Col-0 control plants.

[00195] **Activation tagging and overexpression of the rice homolog of Arabidopsis *DPb* underlie the phenotypes observed in *pzr1-D*.** Previous analyses of the activation-tagging mutant population used in this study (Jeong et al., 2002) revealed that a gene, *Os03g05760*, is located 1.8 kb upstream of the T-DNA insertion (FIG. 5A). The expression level of *Os03g05760* and two other genes (*Os03g05750* and *Os03g05770*) near the insertion were investigated. The expression level of *Os03g05760* in the mutant was approximately 10-times that in the wild type, whereas the

expression levels of the other two genes were like those of wild-type samples (FIG. 5B). Thus, the increase in *Os03g05760* mRNA levels appears to be responsible for the mutant phenotypes in *pzr1-D*. The expression level of this gene was also examined in heterozygous and segregating wild-type plants and found that its expression increased in the mutant in a gene dose-dependent manner (FIG. 5C). This, along with the intermediate phenotypes observed in heterozygous plants, indicates that the mutation, which was designated *pzr1-D*, is dominant (FIG. 9B to FIG. 9F).

[00196] The question of whether *PZR1* expression is affected by BL levels was investigated by measuring transcript levels in seedlings following Pcz treatment. *PZR1* expression was downregulated in plants subjected to this treatment (FIG. 5D).

[00197] To confirm that the observed phenotypes were caused by the increased expression of *PZR1* in the *pzr1-D* mutant, wild-type rice and Arabidopsis plants were transformed with a vector expressing the *PZR1* coding sequence (CDS) under the control of the CaMV 35S promoter. In rice plants, like in *pzr1-D*, the tiller and panicle number increased in lines significantly overexpressing *PZR1* (OX 1–10), especially in OX 1, 2, 3, 9, and 10. The expression levels of this gene in lines OX 13 and OX 16 were not very different from those of the wild type, and their phenotypes were similar to those of Dongjin plants (FIG. 5E to FIG. 5H). For Arabidopsis *PZR1*-overexpressing plants, seedlings with high expression levels of *PZR1* (e.g., OX 2, 3, and 5) showed increased lateral root number (FIG. 12B and FIG. 12E), which could be attributed to the role of *DP* in the cell cycle.

[00198] **Phylogenetic analysis.** Protein sequences showing similarity to PZR1 were retrieved using the BLAST server on the Phytozome website (phytozome.jgi.doe.gov/pz/portal.html). Sequences of Arabidopsis, rice, wheat, and human proteins were obtained from the UniProt server (e.g., via the website uniprot.org), and phylogenetic analysis was performed using Clustal Omega (e.g., via the website ebi.ac.uk/Tools/msa/clustalo/) and BoxShade software (e.g., via the website embnet.vital-it.ch/software/BOX_form.html) to identify and visualize conserved sequences. The resulting phylogenetic tree was generated using the Neighbor-joining method (Clustal Omega) and modified using FigTree v1.4.3 (FIG. 6A). A list of the protein sequences used as input can be found in Table 6.

Table 6. List of amino acid sequences used for phylogenetic analysis.

Name	UniProt	Amino acid sequence
Human DP1	Q14186	MAKDAGLIEANGELKVFIDQNLSPGKGVVSLVAVHPSTVNPLGK QLLPKTFGQSNVNIAQQVVIGTPQRPAASNTLVVGSPTHPSTHFA SQNQPSDSSPWSAGKRNRKGEKNGKGLRHFSMKVCEKVQRKGT TSYNEVADELVAEFSAADNHILPNESAYDQKNIRRRVYDALNVL MAMNIISKEKKEIKWIGLPTNSAQECQNLEVERQRRLERIKQKQS QLQELILQQIAFKNLVQRNRHAEQQASRPPPPNSVIHLPFIIIVNTSK KTVIDCSISNDKFEYLFNFDNTFEIHDDIEVLKRMGMACGLES GS

		CSAEDLKMARSLVPKALEPYVTEMAQGTVGGVFITTAGSTSNGT RFSASDLTNGADGMLATSSNGSQYSGSRVETPVSYVGEDDEEDD DFNENEDDD
Human DP2	Q14188	MIISTPQRLTSSGSVLIGSPYTPAPAMVTQTHIAEATGWVPGDRK RARKFIDSDFSESKRSKKGDKNGKGLRHFSMKVCEKVQRKGTTTS YNEVADELVSEFTNSNNHLAADSAYDQKNIRRRVYDALNVLMA MNIISKEKKEIKWIGLPTNSAQECQNLEIEKQRRIERIKQKRAQLQ ELLLQQIAFKNLVQRNRQNEQQNQGPALNSTIQLPFIINTSRKT VIDCSISSDKFEYLFNFDNTFEIHDDIEVLKRMGMSFGLES GKCSL EDLKLAKSLVPKALEGYITDISTGPSWLNQGLLLNSTQSVSNLDL TTGATLPQSSVNQGLCLDAEVALATGQFLAPNSHQSSSAASHCSE SRGETPCSFNDEDEDEDEEDSSSPE
Wheat DP	Q9FET1	MAPPRGGAATAALDLTGWHILEASSVPPLPERGGNAVQRKG AVDPDKDRKKEKAAAPRITGWGLREYSKIVCEKVEAKGRTTYN EVADEIYSELKSMAHIGQGFDEKNIRRRVYDAFNVLIALRVIAKE KKEIRWMGLSNRYRYEKIKKLEEVRELKVNKIRNKKALLQEIEKQ FDDLQNIKLNRNQTLESSAENVNGIRLPFVLVKTSRKARVEIEISDD SKFAHFEFNGAPFTLHDDL SILEGVRGNSIGKAGRATLH
At5g03415 DPB	Q9FNY2	MTTGSNSNHNHESNNNNNNPSTRSWGTA VSGQSVSTSGSMG SPSSRSEQTITVVTSTSDTTTFQRLNNLDIQGDDAGSQGASGVKKK KRGQRAAGPDKTGRGLRQFSMKVCEKVESKGRTTYNEVADELV AEFALPNNDGTSPDQQQYDEKNIRRRVYDALNVLMAMDIISKDK KEIQWRGLPRTSLSDIEELKNERLSLRNRIEKKTAYSQELEEYV GLQNLQIRNEHLYSSGNAPSGGVALPFILVQTRPHATVEVEISED MQLVHFDFNSTPFELHDDNFVLKTMKFCQPPQPPNGRNNSQL VCHNFTPENPNKGPSTGTPQLDMYETHLQSQQHQQHSQQLIIPM PETNNVTSSADTAPVKSPSLPGIMNSSMKPEN
At5g02470 DPA	Q9FNY3	MSMEMELFVTPEKQRQHPSVSVEKTPVRRKLIVDDDSEIGSEKK GQSRTSGGGLRQFSVMVCQKLEAKKITTYKEVADEIISDFATIKQ NAEKPLNENEYNEKNIRRRVYDALNVFMALDIIARDKKEIRWKG LPITCKKDVEEVKMDRNKVMSSVQKKA AFLKELREKVSSLES LM SRNQEMVVKTQGAEGFTLPFILLETNPHAVVEIEISEDMQLVHL DFNSTPFSVHDDAYILKLMQEQKQEQNRVSSSSSTHHQSQHSSA HSSSSSCIASGTSGPVCWNSGSIDTR
At5g22220 E2F1	Q9FV71	MSEEVQQFPSSKRQLHPSLSMKPPLVAPGEYHRFDAEETRGG GAVADQVVS DAIVIKSTLKRKTDLVNQIVEVNELNTGVLQTPVS GKGKAKKTSRSKSNKSGTLASGSNAGSPGNFQAAGTCRYD SSLGLLTKKFINLIKQAE DGILDLNKAADTLEVQKRRIYDITNVLE GIGLIEKTLKNRIQWKGLDVSKPGETIESIANLQDEVQNLAAEEA RLDDQIRESQERLTSLSEDENNKRLLFVTENDIKNLPCFQNKTLIA VKAPHGTTLEVDPDEAGGYQRRYRIILRSTMGPIDVYLVSQFEE SFEDIPQADEPSNVPDEPSNVPDVPSNLPSTSGLPENHDVSMPMK EESTERNMETQEVD DTDQRVYSDIESHDFVDGIMKIVPPDLDMGV DYWFRSEVGEVSITDMWPDES GPDWNQMITFDQDHAGPSDNKI LEQPQTPSSPTPEESTATRSPTGS
At1g47870 E2F2	Q9FV70	MAATNSGEDPTLSYHHRSPFRFELLQSISSSDPRYSSLTPSST NRPFVSQSLPNSQLSPLISPHWDDSYSQITQKVQKSRKNHRI QLGSIANMSGGESIDIAKVIVKQESSPQNVKRVYNKSKGGTKL LKAGKRMANGEVQNGGLNGASINCRYDSSLGLLTKKFVKLI QEAEDGTLDLNYCAVVLEVQKRRIYDITNVLEGIGLIEKTTK NHIRWKGADNLGQKDLGDQISRLKSEVESMQSEESRLDDLIR ERQEALRSLEEDDYCRRYMFMTTEEDITS LPRFQNTLLAIKA PTASYIEVPDPDEMSEFPQQYRMVIRSRMGPIDVYLLSKYKGS AETSDKLGNESDQKAPVGVDTPSLKIVTSDTDLKADYWFESD AEVSLTDLWSNFNS
LOC_Os03g05760	Q84VA0	MNLLPNLQIPITAFCHQIRIIPMQQQYDEKNIRRRVYDALNVLMA MEIISKDKKEIQWKGLPRTSINDIEDLQTEL VGLKSRIEKKNTYLQ ELQDQFVGMQKLIQRNEQLYSGGNIPSGGVALPFILVQTRPHATV EVEISEDMQLVHFDFNSTPFELHDDSFVLKAMSSCGEEQIDGIHD LISNGGESSMPNIYRQQVQQPARSTNGTARLPSSPPIPGILKGRV KHEH

LOC_Os01g48700	Q84VF4	MAPPCGDAAAASAAPGLANLLIREGAGLPSRPERYPPFRPCTSD SFAPISREGDDIPPQKKSLSLRSGGGGNAAREEGGANRNGKKEK TGAQRITGWGLREFSKIVSKKVEAKGRTTYNEVADEIFAELKSIT QNGLEFDEKNIRRRVYDAFNVLIAIRVIAKDKKEIKWMGLTNYR YEKIQKLEEVHKELITRIKNKKLLQEIEKQFDDLQNTLRNQASQ RPAESVNGILLPFLIKTSRKARVEIEISED SKFARDFNGAPFTMH DDVSILEAIRRNKGRAGLSIHP
LOC_Os10g30420	Q84VD5	MVSGAAHSASTSGGGGGSEGSPTGRAAPGMQGGGSAATPAASA SASTPASETTVARRLDGLDIQGDDAPSSQPATSKKKKRGPGTRAT GPDKGGRGLRQFSMKVCEKVESKGRTTYNEVADELVAEFADPN NNFASPDNDNPNTQFDEKNIRRRVYDALNVLMMAMDIISKDKKEI QWKGLPRTSMDSVEELKTEIIGLKGRIDKKNAYLQELEDQFVGL QNLAQRNEQLYSGSNAPSGGVALPFILVQTRPHATVEVEISEDM QLVHFDNFSTPFELHDDSFVLKALGFSGKEPDDTQAWVGNGGEC STTPIYHQSPQVARPNGVRLPTSPPIPGILKGRVKHEH

[00199] Possible functions of *PZR1/Os03g05760* were investigated by screening the TAIR Arabidopsis database using the full-length protein sequence of the product of *Os03g05760* (Q84VA0) for homologous genes. The highest scores corresponded to two DP (Q9FNY2 and Q9FNY3) and three E2F (Q9FV71, Q9FV70, and F4ILT1) proteins, all from a family of transcription factors with roles in the cell cycle. Plant DP proteins have been identified in Arabidopsis and *Triticum aestivum* (Magyar et al., 2000; Ramirez-Parra and Gutierrez, 2000), and three possible DP homologs in rice have been found using genome-wide analysis (Guo et al., 2007). Phylogenetic analysis of the PZR1 sequence, along with those of known DP proteins in plants, supported the idea that PZR1 is likely a homolog of Arabidopsis DPb (FIG. 14). The expression pattern of *PZR1* in various tissues from rice seedlings and adult rice plants was investigated (FIG. 6B to FIG. 6E). The expression level of this gene was relatively low in seedlings, with lower expression levels in roots compared to aerial tissues (FIG. 6B and FIG. 6C). Nonetheless, these transcripts were detected in all adult plant tissues examined, with leaf tissues having the highest expression levels. This expression pattern was even more pronounced in the *pzr1-D* mutant (FIG. 6D and FIG. 6E).

EXAMPLE 5

Transcriptome analysis and identification of DEGs between wild type and *pzr1-D* mutant seedlings

[00200] This example describes methods for identifying and analyzing differentially expressed genes (DEGs) in plants and plant lines. Plants and plant lines that have undergone genetic modification (e.g., as described in Example 1) can be evaluated using these methods to determine changes to gene pathway signaling resulting from the genetic modification. The applications for these methods include determining effects of specific genetic modifications on target and off-target signaling pathways. Such methods are especially useful when genetic

modifications directed to poorly understood pathways are employed (e.g., in methods for increasing yield of a plant or plant line).

[00201] Total RNA was extracted from seedlings using Trizol (Sigma-Aldrich) reagent following the manufacturer's instructions. Treatments were performed in triplicate for seedlings of two genotypes, wild type (WT) and *pzr1-D* (MUT), which were grown in the light or total darkness. A total of 12 samples were prepared and subjected to quality inspection. Of these, duplicates of each treatment with the best purity value were used to construct sequencing libraries. Quality inspection, library preparation, and raw data processing were conducted. Quality assessment was performed on an Agilent Bioanalyzer 2100 with RNA integrity number (RIN) >7 (>9 for most samples). Libraries were generated using an UltraTM RNA Library Prep Kit for Illumina and sequenced on an Illumina HiSeq 4000 system. Differentially expressed genes (DEGs) were identified in the samples as wild-type seedlings grown in darkness versus *pzr1-D* seedlings grown in darkness (WT dark vs. *pzr1-D* dark) and wild-type seedlings grown in light versus *pzr1-D* seedlings grown in light (WT light vs. *pzr1-D* light). The expression level of each gene was calculated using HTseq software and normalized. DEGs were identified based on $\log_2$ (fold changes) ≥ 1 and a corrected P-value (Q-value) of ≤ 0.05 . Gene ontology (GO) analysis, Venn diagram construction, and promoter analysis were performed using tools available from the website pantherdb.org/, the website interactivenn.net/, and the website plantpan2.itps.ncku.edu.tw/gene_group.php?#multipromoters, respectively.

[00202] To elucidate the possible molecular basis of the relationship between BR and cell cycle processes through PZR1, genome-wide transcriptome analysis was performed comparing wild-type and mutant *pzr1-D* seedlings. To avoid detecting individual differences among plants, three to four seedlings per treatment (one biological replicate) were sampled, and two biological replicates per analysis were used.

[00203] In total, and considering the consistency of all samples, were identified 1,141 differentially expressed genes (DEGs) among wild-type and mutant seedlings. Of these, 678 genes were differentially expressed only under dark conditions, 229 were differentially expressed in seedlings grown in the light, and 234 were differentially expressed independently of light treatment (FIG. 7A). DEGs that were upregulated or downregulated in the mutant compared to the wild type were identified, and the top 20 most significant DEGs under each condition were listed (FIG. 7B; Table 1 and Table 2).

Table 1. List of the top 20 most significant differentially expressed genes (DEGs) in *pzr1-D* compared with the wild type (WT) in the light.

Gene Accession Number	Description	Value WT	Value pzt1-D	log ₂ (fold change)	q-value
Upregulated genes					
OS11G0549665		0	117.0	infinite	0.004
OS10G0381601		0	84.5	infinite	0.004
OS11G0573100		0	39.0	infinite	0.004
OS11G0549675		0	34.4	infinite	0.004
OS11G0549680		0	27.1	infinite	0.004
OS11G0639300		0	26.6	infinite	0.004
OS01G0146101		0	17.9	infinite	0.004
OS03G0629800		0	13.9	infinite	0.004
OS11G0636050		0	11.3	infinite	0.004
OS07G0159200		0	10.0	infinite	0.004
OS12G0100100	RNA recognition motif family protein	0	10.0	infinite	0.004
OS11G0606400	NB-ARC domain containing protein	0.1	7.6	7.01	0.046
OS03G0223301		0.9	68.3	6.27	0.004
OS07G0486000		0.3	13.8	5.77	0.004
OS02G0129000		0.1	5.9	5.69	0.004
OS11G0540600		0.5	17.3	5.08	0.004
OS07G0531900		0.3	10.0	4.95	0.004
OS12G0431300		2.5	68.2	4.74	0.004
OS03G0576200	Potassium transporter 21	0.2	3.7	4.60	0.031
OS08G0203350		5.4	131.0	4.60	0.004
OS11G0562100		0.4	8.9	4.47	0.004
Downregulated genes					
OS11G0701700		79.4	0	--infinite	0.004
OS12G0222650		75.6	0	--infinite	0.004
OS12G0172150		72.7	0	--infinite	0.004
OS12G0257450		36.3	0	--infinite	0.004
OS12G0247700	Mannose-specific jacalin-related lectin	26.9	0	--infinite	0.004
OS12G0222300		24.5	0	--infinite	0.004
OS12G0255200	Staphylococcal nuclease family protein	21.7	0	--infinite	0.004
OS12G0250900		19.7	0	--infinite	0.004
OS12G0254400		16.3	0	--infinite	0.004
OS10G0175500		12.7	0	--infinite	0.004
OS05G0202800	Metallothionein-like protein 3B	123	2.2	-5.78	0.004
OS11G0518900		13.3	0.3	--5.63	0.004
OS08G0255500		8.2	0.2	--5.62	0.004

OS11G0689800		25.2	0.5	--5.59	0.004
OS07G0543500	Putative BLE2 protein	5.2	0.2	--4.86	0.004
OS12G0209800		24.8	1.3	--4.31	0.004
OS11G0255300	Cysteine endopeptidase; Cysteine proteinase-rice; Thiol protease SEN102, putative	22.7	1.2	--4.22	0.004
OS12G0211500		6.9	0.4	--4.08	0.004
OS06G0254300		163.0	9.6	--4.08	0.004
OS11G0134300	Similar to Serine/threonine kinase	16.9	1.0	--4.04	0.004

Table 2. List of the top 20 most significant differentially expressed genes (DEGs) in *pzr1-D* compared with the wild type (WT) in darkness.

Gene Accession Number	Description	Value WT	Value <i>pzr1-D</i>	log ₂ (fold change)	q-value
Upregulated genes					
OS09G0467700		0	32.6	infinite	0.003
OS01G0146101		0	20.9	infinite	0.003
OS01G0148100		0	13.8	infinite	0.003
OS07G0187001		0	12.0	infinite	0.003
OS11G0640300	Leucine Rich Repeat family protein, expressed	0	8.5	infinite	0.003
OS12G0257400		0	7.5	infinite	0.003
OS07G0159200		0	7.4	infinite	0.003
OS01G0845950		0	7.0	infinite	0.003
OS11G0691100		0	5.9	infinite	0.003
OS07G0153150		0.2	68.4	8.16	0.003
OS11G0605100	NB-ARC domain containing protein	0.1	11.8	7.18	0.006
OS03G0223301		0.5	68.2	7.08	0.003
OS08G0367300		0.1	7.1	6.67	0.019
OS11G0618700		0.1	9.6	6.44	0.005
OS07G0162450		0.5	44.8	6.37	0.003
OS02G0129000		0.1	8.9	6.30	0.003
OS03G0299700		1.6	123	6.25	0.003
OS11G0549680		0.4	26.1	6.00	0.003
OS07G0486000		0.2	15.8	5.97	0.003
OS11G0569800	Receptor kinase, putative, expressed	0.2	11.2	5.83	0.003
OS09G0467700	-	0	32.6	infinite	0.003
Downregulated genes					
OS12G0250900	-	20.9	0.5	--5.33	0.003
OS12G0406000	-	6.4	0.2	--5.14	0.003
OS11G0696600	-	26.1	0.8	--5.09	0.003

OS11G0532600	-	5.5	0.2	---5.01	0.010
OS01G0520180	-	81.5	2.6	---4.99	0.003
OS07G0297400	-	93.3	2.9	---4.97	0.003
OS07G0535200	-	6.74	0.2	---4.78	0.003
OS12G0425800	-	56.2	2.1	---4.74	0.003
OS11G0685200	-	7.5	0.3	---4.62	0.003
OS12G0204600	NB-ARC domain containing protein	6.5	0.3	---4.62	0.003
OS12G0239300	-	281.0	12.1	---4.53	0.003
OS07G0677100	Peroxidase	40.3	1.8	---4.50	0.003
OS07G0103000	-	15.1	0.7	---4.49	0.010
OS05G0414400	-	6.2	0.3	---4.32	0.003
OS11G0693800	-	18.2	0.9	---4.25	0.003
OS05G0369900	-	8.1	0.5	---4.09	0.003
OS11G0687100	Von Willebrand factor type A domain containing protein, expressed	41.0	2.5	---4.04	0.003
OS12G0425500	-	3.7	0.2	---4.04	0.003
OS08G0255500	-	6.6	0.4	---3.99	0.003
OS11G0689800	-	16.1	1.2	---3.77	0.003

[00204] To identify characteristics shared by these genes, gene ontology (GO) analysis was performed. Among the most abundant GO terms was cellular processes (GO:0009987), containing 202 DEGs. In the cellular processes category, GO terms such as cell cycle (GO:0007049), cellular component movement (GO:0006928), chromosome segregation (GO:0007059), and cytokinesis (GO:0000910) were enriched. Other enriched terms include cellular component organization (GO:0016043) and cellular component organization or biogenesis (GO:0071840), which are primarily involved in the cell cycle process and regulation (FIG. 7C to FIG. 7E).

[00205] In fact, several known rice cell cycle genes were differentially expressed in *pzr1-D* compared to the wild type (Table 3). Several other GO terms were enriched as well, such as catalytic activities (GO:0003824), kinase activity (GO:0016301), protein serine/threonine kinase activity (GO:004674), nucleic acid binding (GO:0003676), nucleotide binding (GO:000166), and protein binding (GO:0005515).

Table 3. List of differentially expressed rice cell cycle genes in *pzr1-D* compared with the wild type (WT).

Common name	Description	Sample	Value in WT	Value in <i>pzr1-D</i>	log ₂ (fold change)
OsRAA1	Flowering-promoting factor 1-	WT vs <i>pzr1-D</i> in the	6.625	19.519	1.559

	like protein 1	dark			
		WT vs pzl1-D in the light	5.698	16.287	1.515
CycB1;1	Cyclin-B1-1	WT vs pzl1-D in the dark	65.441	39.659	-0.723
		WT vs pzl1-D in the light	21.389	22.576	0.078
Psd1	Similar to Lipid transfer protein.	WT vs pzl1-D in the dark	649.943	336.993	-0.948
		WT vs pzl1-D in the light	192.136	278.211	0.534
CDKB;1	Similar to Cyclin-dependent kinase B1-1	WT vs pzl1-D in the dark	25.206	15.539	-0.698
		WT vs pzl1-D in the light	13.253	11.259	-0.235
CycT1;3	Cyclin-dependent kinase A-2	WT vs pzl1-D in the dark	18.959	12.393	-0.613
		WT vs pzl1-D in the light	9.255	8.796	-0.073
CDKG;1	Cyclin-dependent kinase G-1	WT vs pzl1-D in the dark	18.775	32.877	0.808
		WT vs pzl1-D in the light	39.671	40.669	0.036
PCNA	Proliferating cell nuclear antigen	WT vs pzl1-D in the dark	129.495	68.486	-0.919
		WT vs pzl1-D in the light	37.115	45.642	0.298
CKS1	Cyclin-dependent kinases regulatory subunit 1	WT vs pzl1-D in the dark	49.949	31.799	-0.651
		WT vs pzl1-D in the light	36.218	35.047	-0.047
CDKG;2	Cyclin-dependent kinase G-2	WT vs pzl1-D in the dark	17.678	24.627	0.478
		WT vs pzl1-D in the light	28.401	24.079	-0.238
CycB2;1	Cyclin-B2-1	WT vs pzl1-D in the dark	24.121	15.741	-0.616
		WT vs pzl1-D in the light	8.909	8.037	-0.149
CycU1;1	Cyclin-P1-1	WT vs pzl1-D in the dark	0.497	1.333	1.424
		WT vs pzl1-D in the light	4.273	8.237	0.947
CycD2;2	Similar to Cyclin-D3-1	WT vs pzl1-D in the dark	10.231	6.013	-0.767
		WT vs pzl1-D in the light	5.588	5.224	-0.097
CycB2;2	Cyclin-B2-2	WT vs pzl1-D in the dark	46.600	27.177	-0.778
		WT vs pzl1-D in the light	13.102	14.476	0.144
CycD6;1	Cyclin-D6-1	WT vs pzl1-D in the dark	1.046	0.290	-1.849
		WT vs pzl1-D in the light	0.257	0.373	0.537
CycD2;1	Cyclin-D2-2	WT vs pzl1-D in the dark	17.749	9.653	-0.879
		WT vs pzl1-D in the light	6.290	6.813	0.115

CDKB2; 1	Cyclin-dependent kinase B2-1	WT vs <i>pzr1-D</i> in the dark	58.090	34.057	-0.770
		WT vs <i>pzr1-D</i> in the light	24.909	26.196	0.073
CDKB2; 2	Cyclin-dependent kinase B2-1	WT vs <i>pzr1-D</i> in the dark	58.090	34.057	-0.770
		WT vs <i>pzr1-D</i> in the light	24.909	26.196	0.073
CycA3; 2	Cyclin-A3-2	WT vs <i>pzr1-D</i> in the dark	9.151	5.852	-0.645
		WT vs <i>pzr1-D</i> in the light	3.704	4.441	0.262

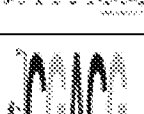
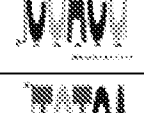
[00206] The promoter sequences of the top DEGs listed in Table 1 and Table 2 were examined, and it was found that 77 out of 80 contained E2F and E2F10SPCNA consensus promoter binding sequences, which match the consensus sequence defined as TTTC[CG]CGC (Vandepoele et al., 2005) (Table 4). RNA sequencing revealed that the promoters of differentially expressed genes are enriched with cognate sequences for both BZR1 and EF-DPb transcription factors, suggesting that PZR1 functions in BR-mediated cell division in rice. Together, these results suggest that the *pzr1-D* mutation affects the expression of the genes involved in cell division.

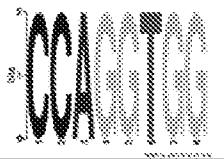
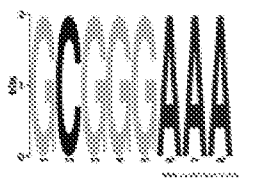
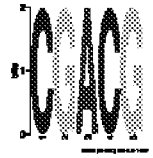
[00207] The relative lack of information about BR processes in monocot plants has stimulated the development of new genetic tools and studies. To date, several genes controlling rice architecture and yield were found to be related to BR responses, and many mutants have been identified with potential uses for agronomic improvement (Sakamoto et al., 2006; Wu et al., 2008; Yang and Hwa, 2008; Wu et al., 2016). Like other BR-related mutants in rice, the *pzr1-D* mutant displays semi-dwarfism and an increased number of tillers, two traits that are usually related; dwarf plants generally have more tillers than the wild type. However, unlike other BR mutants, the dwarfism in *pzr1-D* was not accompanied by a significant reduction in seed size. On the contrary, seed size is almost unaffected in this mutant, while an increased tiller number results in an increased number of panicles in the plant, all contributing to improved yield per plant.

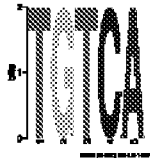
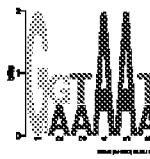
[00208] It was determined that the mutant phenotype of *pzr1-D* resulted from the activation of *PZR1*, a homolog of an Arabidopsis DP transcription factor gene (FIG. 5 and FIG. 6). DP is a dimerization partner of the transcription factor E2F that activates the transcription of genes involved in progression to the S phase. It was found that overexpression of *PZR1* in rice recapitulated the phenotypes and yield increases to those of the T-DNA activation-tagging lines (FIG. 2 and FIG. 5). The function of the E2F/DP heterodimer is well conserved in animals and plants, and phylogenetic analysis showed that the heterodimerization domain is well conserved in PZR1, suggesting that PZR1 is likely involved in cell cycle regulation in rice along with E2F, like their Arabidopsis homologs (FIG. 6) (De Veylder et al., 2002; del Pozo et al., 2006). In addition to

the results of phylogenetic analysis, other results of this study indicate that PZR1 functions in cell cycle regulation. First, the number cells in roots and leaves were increased in the mutant compared to the wild type, whereas the size of the cells were decreased possibly due to accelerated cell division before cell growth (FIG. 4A to FIG. 4D and FIG. 11B and FIG. 11C). Second, overall shape of the calli derived from *pzr1-D* seeds were smaller and the calli accompanied root-like structures, indicating that the balance between cell division and cell differentiation was clearly altered in the mutant background (FIG. 4E and FIG. 4F). Additionally, genome-wide transcriptome analysis revealed an association of PZR1 with a number of genes involved in cell cycle regulation (Table 4). Many GO categories related to cellular processes were enriched in the DEGs between wild type and *pzr1-D* (FIG. 7). Interestingly, most DEGs have consensus E2F/DP binding sequences in their promoters, again confirming the notion that PZR1 functions like an Arabidopsis DP transcription factor (FIG. 13; Table 4).

Table 4. Transcription Factor Binding Sites (TFBS) in the promoters of the top 20 most significant differentially expressed genes (DEGs) from each condition.

UPREGULATED GENES			
TFBS ID	TFBS Name	Support	Binding Site Sequence
TF_MOTIF_SEQ_0343	ANAERO1CONSENSUS	90.9	
TF_MOTIF_SEQ_0372	ACGTOSGLUB1	90.9	
TF_MOTIF_SEQ_0458	E2FCONSENSUS	93.9	
TF_MOTIF_SEQ_0429	E2F1OSPCNA	97	
TF_MOTIF_SEQ_0259	CGACGOSAMY3	100	
TF_MOTIF_SEQ_0274	BIHD1OS	100	

TF_MOTIF_SEQ_0321	GT1CONSENSUS	100	
TF_MOTIF_SEQ_0425	SITEIOSPCNA	100	
DOWNREGULATED GENES			
TFBS ID	TFBS Name	Support	Binding Site Sequence
TF_MOTIF_SEQ_0389	TATABOXOSPAL	94.4	
TF_MOTIF_SEQ_0343	ANAERO1CONSENSUS	91.7	
TF_MOTIF_SEQ_0425	SITEIOSPCNA	97.2	
TF_MOTIF_SEQ_0458	E2FCONSENSUS	97.2	
TF_MOTIF_SEQ_0344	AACACOREOSGLUB1	97.2	
TF_MOTIF_SEQ_0429	E2F1OSPCNA	100	
TF_MOTIF_SEQ_0259	CGACGOSAMY3	100	

TF_MOTIF_SEQ_0274	BIHD10S	100	
TF_MOTIF_SEQ_0321	GT1CONSENSUS	100	

[00209] The dwarf phenotype observed in BR-deficient or -insensitive mutants is mainly caused by decreased cellular elongation, (Kauschmann et al., 1996; Szekeres et al., 1996), but cell proliferation is altered in this type of mutant as well (Hu et al., 2000; Gonzalez-Garcia et al., 2011; Zhiponova et al., 2013). In Arabidopsis, the overexpression of *E2F* alone produced seedlings with enlarged cotyledons, but the overexpression of *E2F* and its partner *DP* together caused severe dwarfism (De Veylder et al., 2002). Similar to observations in the roots of the *pzr1-D* mutant, cotyledon and roots of *E2F*- and *DP*- overexpressing lines contained more but smaller cells than those of the wild type, and these cells possessed an enlarged nucleus possibly due to enhanced endoreduplication. Extra cells in the *E2F/DP* transgenic plants may result from a prolonged proliferative phase of the cell cycle, which would delay cell differentiation (De Veylder et al., 2002). The characteristic phenotype of *pzr1-D* callus may result from shortening of the cell cycle and precocious differentiation (FIG. 4E). The PZR1 transcription factor likely serves as an important decision maker determining whether cells should divide.

[00210] Interestingly, the overexpression of *DP* alone did not cause any altered phenotype in Arabidopsis seedlings, and overexpression of *E2F* alone resulted in shorter roots (De Veylder et al., 2002; Ramirez-Parra et al., 2004). Overexpression of the rice version of *PZR1*, in Arabidopsis produced seedlings with longer primary roots, increased root hair density, and overall higher root biomass (FIG. 12A to FIG. 12E). The phenotypes produced by overexpression of *PZR1* in rice and Arabidopsis, and the observation that this phenotype is opposite to that described for transgenic plants overexpressing *E2F* suggest that rice PZR1 and Arabidopsis DP might not have completely overlapping functions and that PZR1 might play a different role aside from merely being a dimer partner of E2F.

[00211] The phosphorelay signal transduction pathway plays important roles in BR signaling pathways involving BRI1, BAK1, BSKs, BIN2, and BZR1 (He et al., 2002; Li et al., 2002; Nam and Li, 2002; Wang et al., 2002; Kim and Wang, 2010; Tang et al., 2011; Wang et al., 2011).

Similarly, phosphorylation plays essential roles in cell-cycle control, where cyclin-dependent kinases (CDKs) and cyclins form complexes that phosphorylate targets through progression of cell cycle phases (Inze and De Veylder, 2006). The observation that PZR1 is a cell cycle regulator with BIN2 kinase target motifs in its sequence (FIG. 14) led us to hypothesize that it might be a target of phosphorylation by a component of the BR cascade, suggesting a possible link between both processes. One of the most important components of the BR signaling pathway is the protein kinase BIN2, or OsGSK2 in rice, which acts at different levels and even mediates different pathways (Li et al., 2001; He et al., 2002; Koh et al., 2007; Kim et al., 2012; Tong et al., 2012; Khan et al., 2013). A recent example of BIN2-mediated cell cycle regulation was described in rice, where BIN2 was shown to interact and phosphorylate the U-type cyclin, CYC U4 (Sun et al., 2015). Most known BIN2 and other GSK3 substrates contain a short consensus sequence, S/TxxxS/T, where S/T corresponds to serine or threonine and x represents any other residue (Zhao et al., 2002). Indeed, the sequence of PZR1 harbored many typical motifs (e.g. T³⁵xxxS³⁹ and S⁷⁷T⁷⁸xxS⁸¹), raising the possibility that the regulation of PZR1 involves OsGSK2 (FIG. 14).

[00212] The DP pathway could be manipulated to direct cell division in plants to allow yield and architecture to be adjusted. Since E2F and DP are well-conserved proteins, DP homologs are likely to exist in other cereals of agronomic importance as well. Therefore, similar approaches can be applied to other species through modulating the expression of these homologous genes to boost yield by increasing tiller and panicle number, or to increase drought resistance by amplifying both primary and lateral root production.

EXAMPLE 6

Methods of creating a high-yield crop field

[00213] This example describes methods for producing a high-yield crop field comprising plants generated using methods disclosed herein, including methods for genetic modification (e.g., gene overexpression), and removing a weed from the crop field.

[00214] A *pzr1-D* rice plant produced by methods disclosed herein is grown in a paddy field using transplantation growth methods. A wild type plant is grown in an adjacent plant field. The *pzr1-D* and wild type rice plants can also be grown in a greenhouse either by transplantation or direct seeding if a paddy field is unavailable.

[00215] A comparison of crops produced by the field planted with *pzr1-D* plants and the field planted with wild type plants indicates that the field planted with *pzr1-D* plants shows a yield more than 5% greater than the field planted with wild type plants.

EXAMPLE 7

Methods of clearing a weed from a crop field

[00216] This example describes methods for producing a crop field comprising plants generated using methods disclosed herein, including methods for genetic modification (e.g., gene overexpression), and removing a weed from the crop field.

[00217] A plant produced by methods disclosed herein (e.g., a *pzr1-D* plant) is grown in a paddy field. An adjacent field is planted with wild type rice plants. Each field is also seeded with weeds. Each crop field is treated with 20 μ M propiconazole.

[00218] In fields seeded with *pzr1-D* plants, weeds are cleared and *pzr1-D* plants are able to produce rice for harvest. In fields seeded with wild type rice plants, both the weeds and the wild type rice plants are destroyed.

CLAIMS

WHAT IS CLAIMED IS:

1. A method of increasing yield in a crop field comprising a plant having a mutation affecting a signaling pathway selected from the group consisting of: a cell cycle pathway, a hormone signaling pathway, and a metabolic pathway.
2. The method of claim 1, wherein the mutation affects a hormone signaling pathway.
3. The method of claim 2, wherein the hormone signaling pathway comprises BR signaling.
4. The method of claim 3, wherein the mutation causes increased BR signaling.
5. The method of claim 1, wherein the mutation affects a cell cycle pathway.
6. The method of claim 5, wherein the mutation affects Rb signaling.
7. The method of claim 6, wherein the mutation results in a downregulation of Rb signaling activity.
8. The method of claim 5, wherein the mutation alters the expression of at least one gene implicated in DP-E2F signaling.
9. The method of claim 8, wherein the mutation causes overexpression of at least one gene implicated in DP-E2F signaling.
10. The method of claim 9, wherein the mutation comprises an exogenous nucleic acid sequence.
11. The method of claim 9, wherein the mutation affects the expression of the *PZR1* gene.
12. The method of claim 11, wherein the mutation causes the overexpression of the *PZR1* gene.
13. The method of claim 12, wherein the mutation is a *pzr1-D* mutation.
14. The method of claim 13, wherein the plant is homozygous for the *pzr1-D* mutation.
15. The method of claim 5, wherein the at least one gene is selected from the group consisting of: OS11G0549665, OS10G0381601, OS11G0573100, OS11G0549675, OS11G0549680, OS11G0639300, OS01G0146101, OS03G0629800, OS11G0636050, OS07G0159200, OS12G0100100, OS11G0606400, OS03G0223301, OS07G0486000, OS02G0129000,

OS11G0540600, OS07G0531900, OS12G0431300, OS03G0576200, OS08G0203350, OS11G0562100, OS11G0701700, OS12G0222650, OS12G0172150, OS12G0257450, OS12G0247700, OS12G0222300, OS12G0255200, OS12G0250900, OS12G0254400, OS10G0175500, OS05G0202800, OS11G0518900, OS08G0255500, OS11G0689800, OS07G0543500, OS12G0209800, OS11G0255300, OS12G0211500, OS06G0254300, OS11G0134300, OS09G0467700, OS01G0146101, OS01G0148100, OS07G0187001, OS11G0640300, OS12G0257400, OS07G0159200, OS01G0845950, OS11G0691100, OS07G0153150, OS11G0605100, OS03G0223301, OS08G0367300, OS11G0618700, OS07G0162450, OS02G0129000, OS03G0299700, OS11G0549680, OS07G0486000, OS11G0569800, OS09G0467700, OS12G0250900, OS12G0406000, OS11G0696600, OS11G0532600, OS01G0520180, OS07G0297400, OS07G0535200, OS12G0425800, OS11G0685200, OS12G0204600, OS12G0239300, OS07G0677100, OS07G0103000, OS05G0414400, OS11G0693800, OS05G0369900, OS11G0687100, OS12G0425500, OS08G0255500, OS11G0689800, Flowering-promoting factor 1-like protein 1, Cyclin-B1-1, Similar to Lipid transfer protein, Similar to Cyclin-dependent kinase B1-1, Cyclin-dependent kinase A-2, Cyclin-dependent kinase G-1, Proliferating cell nuclear antigen, Cyclin-dependent kinases regulatory subunit 1, Cyclin-dependent kinase G-2, Cyclin-B2-1, Cyclin-P1-1, Similar to Cyclin-D3-1, Cyclin-B2-2, Cyclin-D6-1, Cyclin-D2-2, Cyclin-dependent kinase B2-1, Cyclin-dependent kinase B2-1, and Cyclin-A3-2.

16. The method of claim 1, wherein at least one aspect of the plant is increased, the aspect being selected from the group consisting of: plant weight, tiller number, panicle number, total length, root length, and coleoptile length.

17. The method of claim 1, wherein the yield of the crop field is at least 5% greater than that of a crop field planted with a reference line and grown under similar conditions as the crop field comprising the plant.

18. The method of claim 17, wherein the mutation is a *pzr1-D* mutation.

19. The method of claim 1, wherein the crop field is treated with a herbicide.

20. The method of claim 19, wherein the crop field comprises a weed, and the herbicide inhibits brassinosteroid synthesis in the weed prior to harvest.

21. The method of claim 20, wherein the herbicide is selected from the group consisting of Brassinazole and propiconazole.

22. The method of claim 21, wherein the mutation affects the expression of the *PZRI* gene.
23. The method of claim 22, wherein the mutation causes overexpression of the *PZRI* gene.
24. The method of claim 23, wherein the mutation is a *pzr1-D* mutation.
25. A method of clearing a weed from a field comprising: planting the field using a plant having a mutation affecting a signaling pathway selected from the group consisting of: a cell cycle pathway, a hormone signaling pathway, and a metabolic pathway; and administering a herbicide that impacts brassinosteroid signaling.
26. The method of claim 25, wherein the mutation affects a hormone signaling pathway.
27. The method of claim 25, wherein the mutation affects BR signaling.
28. The method of claim 25, wherein the mutation affects a cell cycle pathway.
29. The method of claim 28, wherein the mutation affects Rb signaling.
30. The method of claim 28, wherein the mutation alters the expression of at least one gene implicated in DP-E2F signaling.
31. The method of claim 30, wherein the mutation affects the expression of the *PZRI* gene.
32. The method of claim 31, wherein the mutation causes the overexpression of the *PZRI* gene.
33. The method of claim 32, wherein the mutation is a *pzr1-D* mutation.
34. The method of claim 25, wherein the herbicide is selected from the group consisting of Brassinazole and propiconazole.

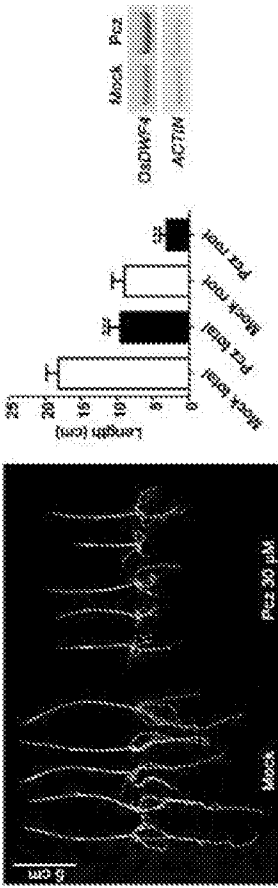


FIG. 1A

FIG. 1B

FIG. 1C

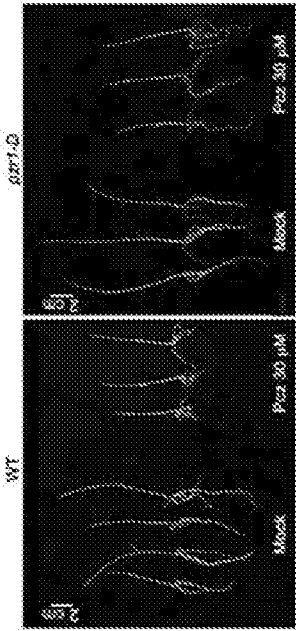


FIG. 1D

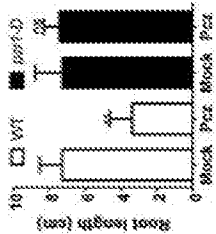


FIG. 1E

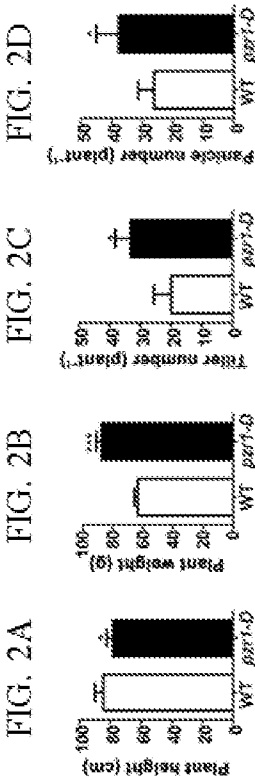


FIG. 2E FIG. 2F



FIG. 2I

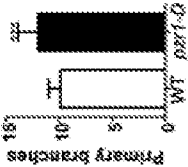


FIG. 2H

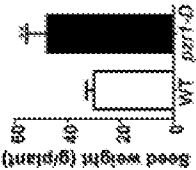


FIG. 2G

FIG. 3A

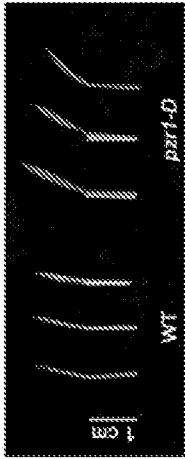


FIG. 3B

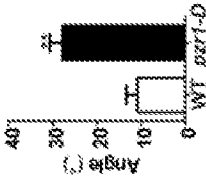


FIG. 3C

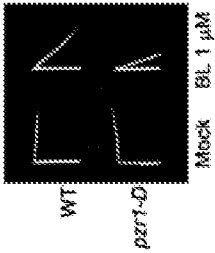


FIG. 3D

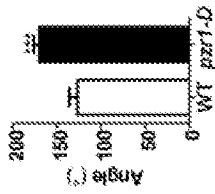
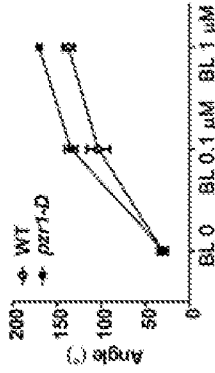


FIG. 3E



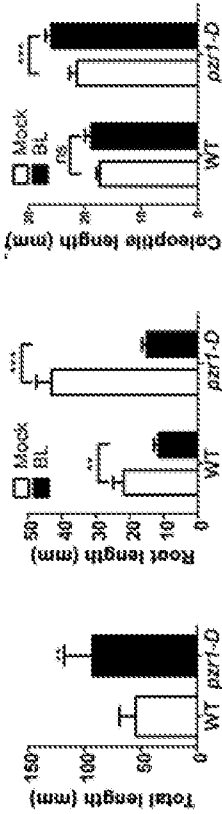


FIG. 3H

FIG. 3G

FIG. 3F

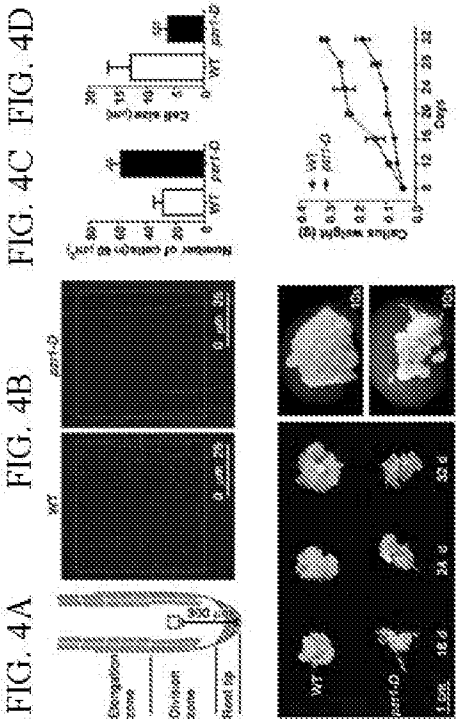


FIG. 4F

FIG. 4E

FIG. 5E

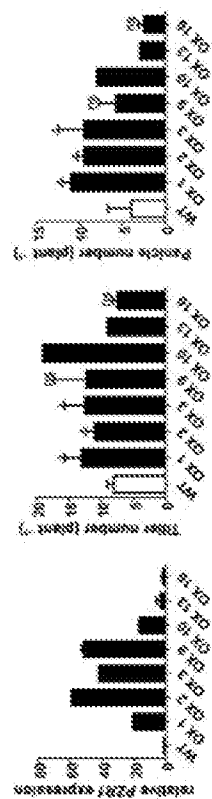
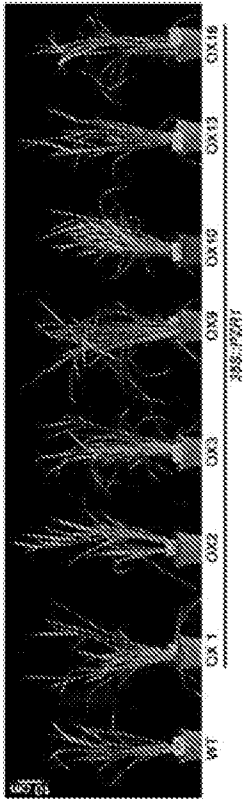


FIG. 5H

FIG. 5G

FIG. 5F

10/21

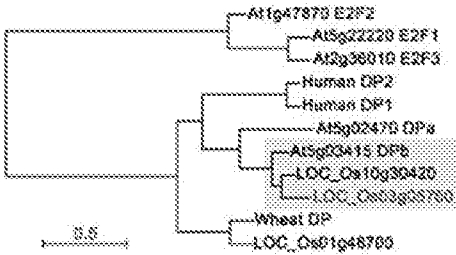


FIG. 6A

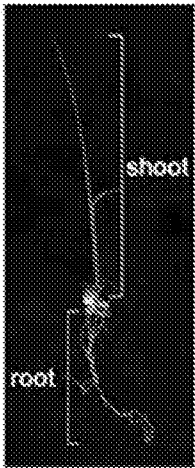


FIG. 6B

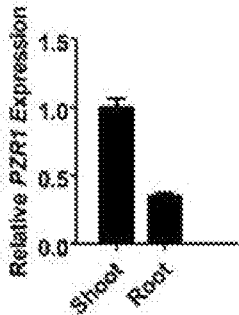


FIG. 6C

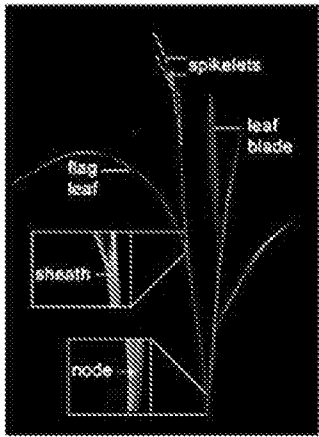


FIG. 6D

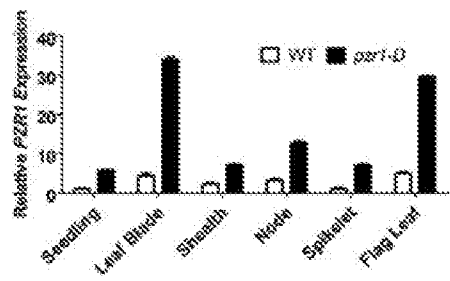


FIG. 6E

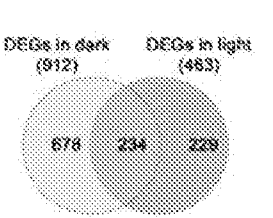


FIG. 7A

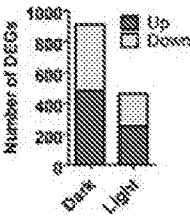


FIG. 7B

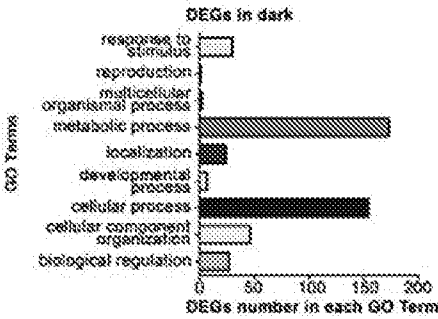


FIG. 7C

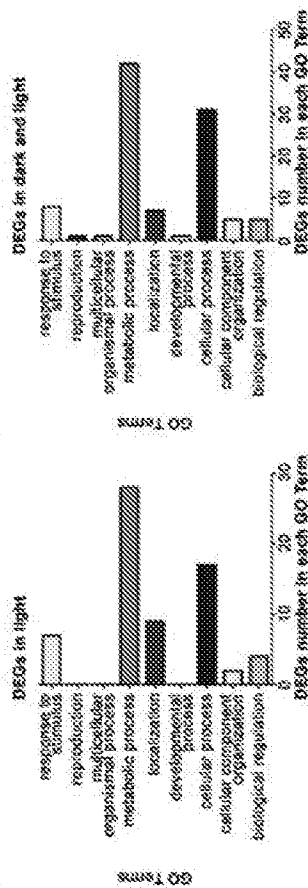


FIG. 7E

FIG. 7D

FIG. 8A

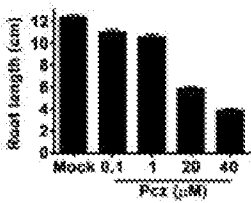
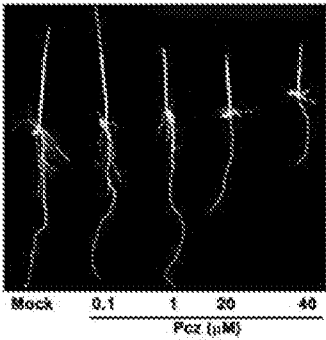


FIG. 8B

FIG. 8C



FIG. 8D

FIG. 9A

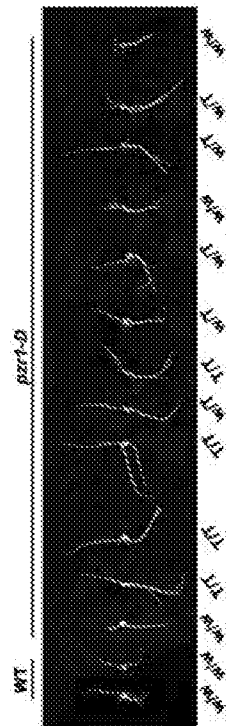
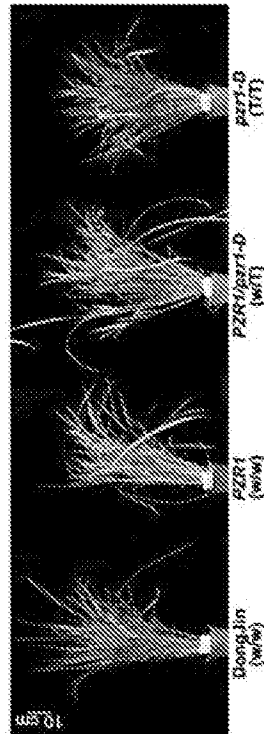


FIG. 9B



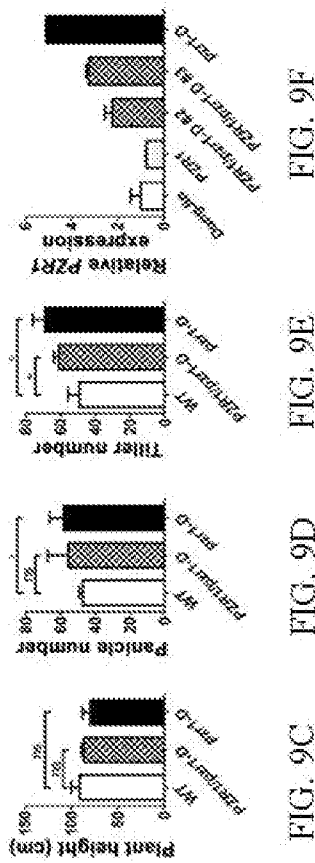


FIG. 10A

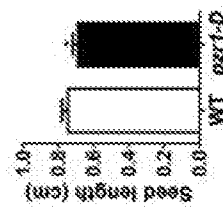
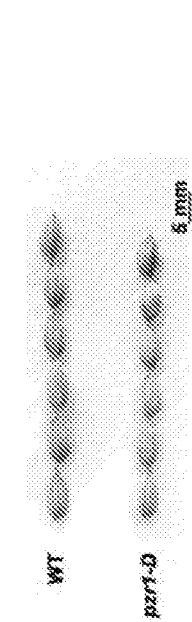


FIG. 10B

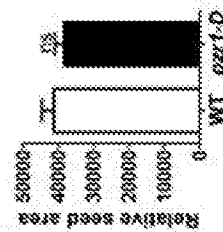


FIG. 10C

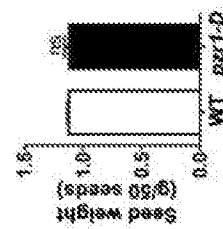


FIG. 10D

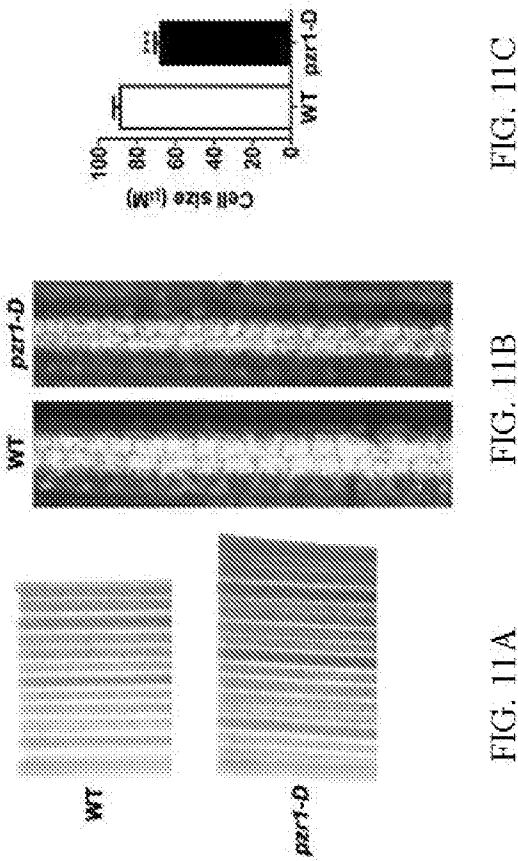


FIG. 12A



FIG. 12B

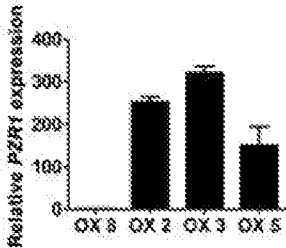


FIG. 12C

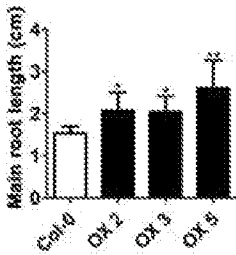


FIG. 12D

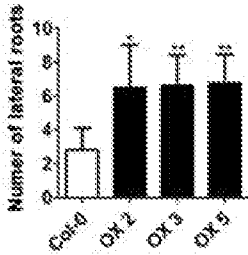


FIG. 12E

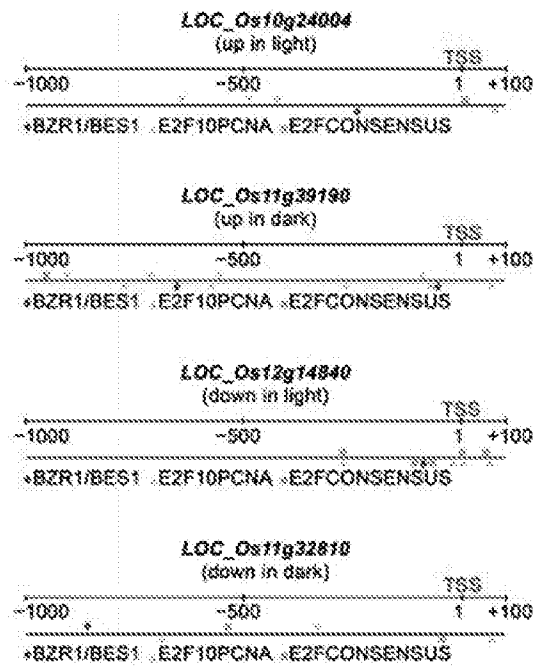


FIG. 13

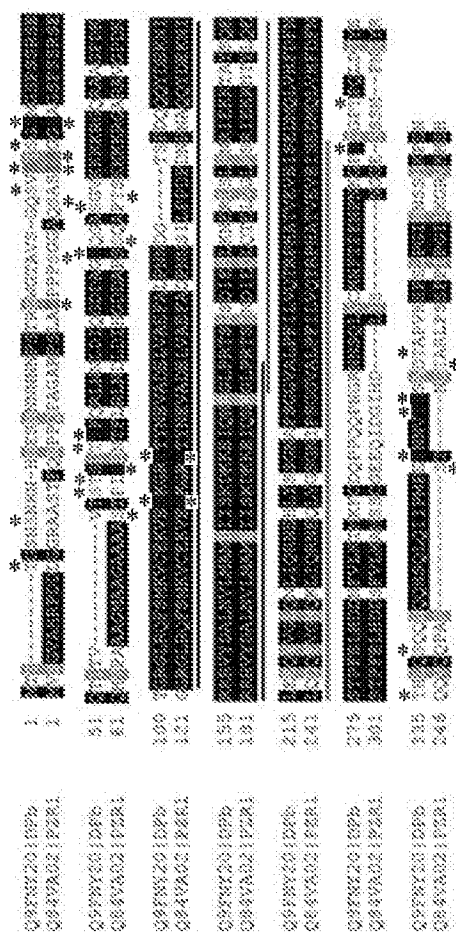


FIG. 14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2019/000161**A. CLASSIFICATION OF SUBJECT MATTER****C12N 15/82(2006.01)i**

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N 15/82; A01H 1/06; A01H 5/00; A01H 5/10; C12N 15/29

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

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

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

eKOMPASS(KIPO internal) & keywords: yield, crop field, plant, mutation, signaling pathway, cell cycle, hormone, metabolic pathway, herbicide

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009-0025100 A1 (CHOE, S. et al.) 22 January 2009 See paragraphs [0081], [0093], [0101], [0145]; claims 41-47.	1-5, 16-17, 19-21, 25-28, 34
Y		6-7, 15, 29
A		8-14, 18, 22-24, 30-33
Y	US 2002-0046416 A1 (GUTIERREZ-ARMENTA, C. et al.) 18 April 2002 See paragraphs [0002], [0032]; claims 1-14.	6-7, 15, 29
X	US 2003-0150027 A1 (GIROUX, M.) 7 August 2003 See abstract; paragraphs [0004]-[0010], [0141]; claims 1, 10, 13, 25, 37, 41, 45.	1, 5, 16, 19-21, 25, 28, 34
X	US 2014-0143908 A1 (BASU, S. et al.) 22 May 2014 See paragraphs [0095], [0146]-[0147], [0252]; claims 1-20.	1, 5, 16, 19-21, 25, 28, 34
A	US 2011-0302673 A1 (MCKERSIE, B. D. et al.) 8 December 2011 See the whole document.	1-34



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

24 July 2019 (24.07.2019)

Date of mailing of the international search report

24 July 2019 (24.07.2019)

Name and mailing address of the ISA/KR

International Application Division

Korean Intellectual Property Office

189 Cheongsa-ro, Seo-gu, Daejeon, 35208, Republic of Korea

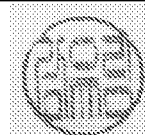


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Telephone No. +82-42-481-3516



INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/IB2019/000161

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/IB2019/000161

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