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(72) Inventeur/Inventor:
LIM, BOON LEONG, CN

(73) Propriétaire/Owner:
VERSITECH LIMITED, CN

(74) Agent: SMART & BIGGAR LLP

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(54) Title: METHOD FOR SPEEDING UP PLANT GROWTH AND IMPROVING YIELD BY ALTERING EXPRESSION LEVELS OF KINASES

(57) Abrégé/Abstract:
The invention discloses transgenic plants having increased growth rate and increased yield and methods for making the same. In one embodiment, the method comprises: transforming a plant or plant cell with a nucleic acid molecule comprising a plant kinase and/or phosphatase gene selected from NG6, NG21, NG24, NG28, and NG32, and over-expressing said kinase and/or phosphatase gene in the plant or plant cell.

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(71) Applicant *(for all designated States except US)*: **THE UNIVERISTY OF HONG KONG** [CN/CN]; Pokfulam Road, Hong Kong (CN).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **LIM, Boon Leong** [CN/CN]; Flat C, Floor 31, Block 9, Phase 4, Bel-Air on the Peak, 68 Bel-Air Peak Avenue, Island South, Hong Kong (CN).

(74) Agent: **CHINA PATENT AGENT (H K) LTD.**; 22/F., Great Eagle Center, 23 Harbour Road, Wanchai district, Hong Kong (CN).

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(54) **Title**: METHOD FOR SPEEDING UP PLANT GROWTH AND IMPROVING YIELD BY ALTERING EXPRESSION LEVELS OF KINASES AND PHOSPHATASES

(57) **Abstract**: The invention discloses transgenic plants having increased growth rate and increased yield and methods for making the same. In one embodiment, the method comprises: transforming a plant or plant cell with a nucleic acid molecule comprising a plant kinase and/or phosphatase gene selected from NG6, NG21, NG24, NG28, and NG32, and over-expressing said kinase and/or phosphatase gene in the plant or plant cell.

METHOD FOR SPEEDING UP PLANT GROWTH AND IMPROVING YIELD BY ALTERING EXPRESSION LEVELS OF KINASES

1. INTRODUCTION

Described herein are methods for speeding up plant growth and/or elevating plant yields by altering the expression levels of plant kinases and phosphatases. Also described therein are the use of plant kinases and phosphatases, and their respective protein products, as well as fragments, derivatives, homologues, and variants thereof.

2. BACKGROUND OF THE INVENTION

Purple acid phosphatases (PAPs) catalyze the hydrolysis of a wide range of activated phosphoric acid mono- and di-esters and anhydrides (Klabunde et al., 1996). The PAP proteins are characterized by seven conserved amino acid residues (shown in bold face) in the five conserved motifs XDXX, XDXXY, GNH(D/E), XXXH, XHXH, which are involved in the coordination of the dimetal nuclear center (Fe^{3+}-Me^{2+}) in the active site (Li et al., 2002), where Me is a transition metal; Me^{2+} is mostly found to be Fe^{2+} in mammals, and Zn^{2+}, or Mn^{2+} in plants (Klabunde and Krebs, 1997; Schenk *et al.*, 1999).

Multiple PAP-like sequences are present in plant genomes. In the Arabidopsis genome, twenty-nine potential PAP genes have been identified based on sequence comparison. Most of the functions of characterized plant PAPs are related to phosphorus metabolism. None of the plant PAPs that had been functionally or biochemically characterized carry any transmembrane motif. In addition, no AtPAPs or any other plant PAPs had been discovered to affect sugar signalling and carbon metabolism in plants. Overexpression of AtPAP2 in Arabidopsis, a PAP with a C-terminal motif, can significantly speed up plant growth, increase sugar content in plants and improve seed yield (U.S. Patent Application Publication No. 2010/0159065).

3. SUMMARY

In one aspect, provided herein are methods that speed up or increase the rate of plant growth and elevate plant yields by altering the expression levels of plant kinases and phosphatases. Kinases and phosphatases, and their respectively encoded protein products, as well as fragments, derivatives, homologues, and variants thereof, are disclosed. Methods for introducing these genes into plants to speed up or increase the growth rate of plants, and to increase yield of plants, are provided. The kinases and phosphatases of the present invention are selected from the results of a microarray study. Surprisingly, it is discovered that phosphatases (such as NG6) and kinases (such as NG21, NG24, NG28, and NG32) have growth-promoting effects.

Provided herein, a microarray study was carried out to compare the gene expression profiles of the AtPAP2 overexpression lines, AtPAP2 T-DNA (mutant) line, and the wild-type plants. The results showed that expression levels of a number of genes are significantly altered (upregulated or downregulated) in AtPAP2 overexpression lines, when compared to the wild-type. Among these genes, a number of phosphatases and kinases were selected and analyzed using transgenic studies in Arabidopsis.

At least in part, the present inventors discover that altering the expression levels of plant phosphatases (such as NG6) and kinases (such as NG21, NG24, NG28 and NG32) in plants resulted in rapid plant growth and higher yield. In one aspect, provided herein are methods of producing plants with enhanced growth and/or yield. In one embodiment, the method comprises: transforming a plant or plant cell with a nucleic acid molecule comprising a plant kinase and/or phosphatase gene selected from SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 93, 95, 97, 99, or 101, and over-expressing said kinase and/or phosphatase gene in the plant or plant cell. In one embodiment, provided herein are methods of regenerating, from said transformed plant or plant cell, a plant having enhanced growth and/or yield.

In one embodiment, the method comprises: transforming a plant or plant cell with a nucleic acid molecule comprising a plant kinase and/or phosphatase having at least 65%, 70%, 75%, 80%, 85%, 90%, 93%, 95%, 96%, 97%, 98%, or 99% identity with SEQ ID NO: 1, 3, 5, 7,

9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 93, 95, 97, 99, or 101, and over-expressing said kinase and/or phosphatase gene in the plant or plant cell.

In certain embodiments, the method comprises transforming a plant or plant cell with a nucleic acid molecule comprising a plant kinase and/or phosphatase having a nucleic acid fragment from SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 93, 95, 97, 99 or 101. In certain embodiments, the nucleic acid fragment encode a peptide that has the same activity as a peptide encoded by SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 93, 95, 97, 99 or 101.

In certain embodiments, the activity is a kinase and/or phosphatase activity. In certain, embodiments, the method comprises transforming a plant or plant cell with a nucleic and molecule comprising a plant kinase and/or phosphatase having a variant from SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 93, 95, 97, 99 or 101.

In certain embodiments, the variant has 1-5, 6-10, 11-20, 21-30, 31-40, 41-50, 50-70, 71-80, 81-100 nucleic acid deletion, substitution or insertion in the sequence as compared to SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 93, 95, 97, 99, or 101. In certain embodiments, the variants encode a peptide that has the same activity as a peptide encoded by SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 93, 95, 97, 99, or 101. In certain embodiments, the activity is a kinase and/or phosphatase activity.

More specifically, in an embodiment, provided herein is a method to make a transgenic plant having increased rate of plant growth and/or elevated plant yields when compared to a wild-type plant of the same species cultivated under the same conditions, said method comprising: a) transforming a nucleic acid molecule encoding a kinase into a plant or plant cell, wherein the nucleic acid molecule comprises: i) a sequence having at least 95% identity over the full length of SEQ ID NO: 43; and/or ii) a sequence encoding a polypeptide having at least 95% identity over the full length of SEQ ID NO: 44; wherein the nucleic acid molecule is operatively linked to upstream and downstream regulatory components comprising a heterologous promoter; and b) over-expressing the nucleic acid molecule in the plant or plant cell, wherein the promoter drives the overexpression of the nucleic acid molecule.

Provided herein are transgenic plants with enhanced growth and/or yield. In certain embodiments, the transgenic plant comprises a nucleic acid molecule selected from SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 93, 95, 97, 99, or 101, wherein said nucleic acid molecule is overexpressed in the transgenic plant when compared to a wild-type plant of the same species cultivated under the same conditions.

In certain embodiments, the transgenic plant comprises a nucleic acid molecule having at least 65%, 70%, 75%, 80%, 85%, 90%, 93%, 95%, 96%, 97%, 98% or 99% identity with SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 93, 95, 97, 99, or 101, wherein said nucleic acid molecule is overexpressed in the transgenic

plant when compared to a wild-type plant of the same species cultivated under the same conditions.

In certain embodiments, the transgenic plant comprises a nucleic acid fragment from SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 93, 95, 97, 99, or 101. In certain embodiments, the nucleic acid fragment encodes a peptide that has the same activity as a peptide encoded by SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 93, 95, 97, 99, or 101. In certain embodiments, the activity is a kinase and/or phosphatase activity.

In certain embodiments, the transgenic plant comprises a plant kinase and/or phosphatase homologue, derivative, or variant having a nucleic acid sequence of the SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 93, 95, 97, 99, or 101. In certain embodiments, the homologue, derivative or variant has 1-5, 6-10, 11-20, 21-30, 31-40, 41-50, 50-70, 71-80, 81-100 nucleic acid deletion, substitution or insertion in the sequence as compared to SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 93, 95, 97, 99, or 101. In certain embodiments, the variants encode a peptide that has the same activity as a peptide encoded by SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 93, 95, 97, 99 or 101. In certain embodiments, the activity is a kinase and/or phosphatase activity.

More specifically, in an embodiment, provided herein is a transgenic plant cell, comprising: i) the nucleic acid molecule of SEQ ID NO: 43, wherein the nucleic acid molecule is operatively linked to upstream and downstream regulatory components comprising a heterologous promoter, and wherein said nucleic acid molecule is over-expressed in the transgenic plant cell when compared to plant cells of the same type in a wild-type plant of the same species and wherein the promoter drives the overexpression of the nucleic acid molecule; and/or ii) a nucleic acid molecule that encodes the protein of SEQ ID NO: 44, wherein the nucleic acid molecule is operatively linked to upstream and downstream regulatory components comprising a heterologous promoter, and wherein said nucleic acid molecule is over-expressed in the transgenic plant cell when compared to plant cells of the same type in a wild-type plant of the same species and wherein the promoter drives the overexpression of the nucleic acid molecule.

In certain embodiments, the method comprises transforming a plant or plant cell with a nucleic acid molecule that expresses a plant kinase and/or phosphatase peptide, fragment, derivative or variant from a peptide having an amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 94, 96, 98, 100, or 102. In certain embodiments, the peptide, fragment, derivative or variant is overexpressed. In certain embodiments, provided herein are methods of regenerating, from said transformed plant or plant cell, a plant having enhanced growth and/or yield.

In certain embodiments, the transgenic plants express a plant kinase and/or phosphatase peptide, fragment, derivative or variant from a protein having an amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 94, 96, 98, 100, or 102. In certain embodiments, the peptide fragment, derivative or variant is

overexpressed. In certain embodiments, provided herein are regenerated transformed plant having enhanced growth and/or yield.

4. BRIEF DESCRIPTION OF THE FIGURES

The patent application file contains at least one drawing executed in color. Copies of this patent application with color drawings will be provided by the Office upon request and payment of the necessary fee.

FIG. 1 shows a heat map of the microarray analysis of gene expression profile of Arabidopsis shoots, using three biological replicates for wild-type (WT), 2 biological replicates for AtPAP2 T-DNA line (P2), and 3 biological replicates for two independent AtPAP2 overexpression lines (OE7 and OE21).

FIG. 2 shows scatter plots of the microarray analysis of gene expression profile of Arabidopsis shoots. The results showed that the expression profiles of the two independent AtPAP2 overexpression lines (OE7 and OE21) were significantly different from that of the wild-type (WT), whereas the expression profile of the AtPAP2 T-DNA (mutant) lines resembled closely that of the WT.

FIG. 3 shows a schematic diagram of the expression vector pCXSN. (a). The cDNAs of the NG genes were cloned into the pCXSN vector at the XcmI sites to create the overexpression vectors. (b) shows an exemplified overexpression vector pCXSN-NG6.

FIG. 4 shows the mRNA expression levels of NG genes in the respective overexpression lines. The mRNA expression levels in 10-day-old T3 homologous seedlings were determined by quantitative RT-PCR using gene-specific primers. The fold-changes represent the relative expression levels of mRNAs compared to that of the wild-type (WT = 1.0). The results of two trials were obtained from two batches of plant growth studies.

FIG. 5 shows the growth performance of the wild-type and NG6 over-expression lines in soil. The five columns of plants from left to right were AtPAP2 overexpression lines, WT, T3 homologous NG6 overexpression lines NG6-1, NG6-2, and NG6-3. (a) 22-day-old and (b) 25-day-old plants.

FIG. 6 shows the growth performance of the wild-type and T3 homologous NG21, NG24, NG28 and NG32 overexpression lines in soil. The five columns of plants from left to right were WT, NG21, NG24, NG28 and NG32 overexpression lines. (a) 30-day-old plants and (b) 34-day-old plants grown in black tray. (c) 22-day-old plants, (d) 25-day-old plants, (e) 28-day-old plants, and (f) 36-day-old plants grown in white cups.

4.1. BRIEF DESCRIPTION OF THE SEQUENCES

SEQ ID NO:1 is a nucleic acid sequence of Arabidopsis phosphatase NG6 gene.

SEQ ID NO:2 is an amino acid sequence of Arabidopsis phosphatase NG6.

SEQ ID NO:3 is a nucleic acid sequence of maize phosphatase NG6 gene.

SEQ ID NO:4 is an amino acid sequence of maize phosphatase NG6.

SEQ ID NO:5 is a nucleic acid sequence of soybean phosphatase NG6 gene.

SEQ ID NO:6 is an amino acid sequence of soybean phosphatase NG6.

SEQ ID NO:7 is a nucleic acid sequence of rice phosphatase NG6 gene.

SEQ ID NO:8 is an amino acid sequence of rice phosphatase NG6.

SEQ ID NO:9 is a nucleic acid sequence of cotton phosphatase NG6 gene.

SEQ ID NO:10 is an amino acid sequence of cotton phosphatase NG6.

SEQ ID NO:11 is a nucleic acid sequence of Arabidopsis kinase NG21 gene.

SEQ ID NO:12 is an amino acid sequence of Arabidopsis kinase NG21.

SEQ ID NO:13 is a nucleic acid sequence of maize kinase NG21 gene.

SEQ ID NO:14 is an amino acid sequence of maize kinase NG21.

SEQ ID NO:15 is a nucleic acid sequence of soybean kinase NG21 gene.

SEQ ID NO:16 is an amino acid sequence of soybean kinase NG21.

SEQ ID NO:17 is a nucleic acid sequence of rice kinase NG21 gene.

SEQ ID NO:18 is an amino acid sequence of rice kinase NG21.

SEQ ID NO:19 is a nucleic acid sequence of cotton kinase NG21 gene.

SEQ ID NO:20 is an amino acid sequence of cotton kinase NG21.

SEQ ID NO:21 is a nucleic acid sequence of Arabidopsis kinase NG24 gene.

SEQ ID NO:22 is an amino acid sequence of Arabidopsis kinase NG24.

SEQ ID NO:23 is a nucleic acid sequence of maize kinase NG24 gene.

SEQ ID NO:24 is an amino acid sequence of maize kinase NG24.
SEQ ID NO:25 is a nucleic acid sequence of soybean kinase NG24 gene.
SEQ ID NO:26 is an amino acid sequence of soybean kinase NG24.
SEQ ID NO:27 is a nucleic acid sequence of rice kinase NG24 gene.
SEQ ID NO:28 is an amino acid sequence of rice kinase NG24.
SEQ ID NO:29 is a nucleic acid sequence of cotton kinase NG24 gene.
SEQ ID NO:30 is an amino acid sequence of cotton kinase NG24.
SEQ ID NO:31 is a nucleic acid sequence of Arabidopsis kinase NG28 gene.
SEQ ID NO:32 is an amino acid sequence of Arabidopsis kinase NG28.
SEQ ID NO:33 is a nucleic acid sequence of maize kinase NG28 gene.
SEQ ID NO:34 is an amino acid sequence of maize kinase NG28.
SEQ ID NO:35 is a nucleic acid sequence of soybean kinase NG28 gene.
SEQ ID NO:36 is an amino acid sequence of soybean kinase NG28.
SEQ ID NO:37 is a nucleic acid sequence of rice kinase NG28 gene.
SEQ ID NO:38 is an amino acid sequence of rice kinase NG28.
SEQ ID NO:39 is a nucleic acid sequence of cotton kinase NG28 gene.
SEQ ID NO:40 is an amino acid sequence of cotton kinase NG28.
SEQ ID NO:41 is a nucleic acid sequence of Arabidopsis kinase NG32 gene.
SEQ ID NO:42 is an amino acid sequence of Arabidopsis kinase NG32.
SEQ ID NO:43 is a nucleic acid sequence of maize kinase NG32 gene.
SEQ ID NO:44 is an amino acid sequence of maize kinase NG32.
SEQ ID NO:45 is a nucleic acid sequence of soybean kinase NG32 gene.
SEQ ID NO:46 is an amino acid sequence of soybean kinase NG32.
SEQ ID NO:47 is a nucleic acid sequence of rice kinase NG32 gene.
SEQ ID NO:48 is an amino acid sequence of rice kinase NG32.
SEQ ID NO:49 is a nucleic acid sequence of cotton kinase NG32 gene.
SEQ ID NO:50 is an amino acid sequence of cotton kinase NG32.
SEQ ID NO:51 is a primer sequence useful according to the present invention.
SEQ ID NO:52 is a primer sequence useful according to the present invention.
SEQ ID NO:53 is a primer sequence useful according to the present invention.

SEQ ID NO:54 is a primer sequence useful according to the present invention.
SEQ ID NO:55 is a primer sequence useful according to the present invention.
SEQ ID NO:56 is a primer sequence useful according to the present invention.
SEQ ID NO:57 is a primer sequence useful according to the present invention.
SEQ ID NO:58 is a primer sequence useful according to the present invention.
SEQ ID NO:59 is a primer sequence useful according to the present invention.
SEQ ID NO:60 is a primer sequence useful according to the present invention.
SEQ ID NO:61 is a primer sequence useful according to the present invention.
SEQ ID NO:62 is a primer sequence useful according to the present invention.
SEQ ID NO:63 is a primer sequence useful according to the present invention.
SEQ ID NO:64 is a primer sequence useful according to the present invention.
SEQ ID NO:65 is a primer sequence useful according to the present invention.
SEQ ID NO:66 is a primer sequence useful according to the present invention.
SEQ ID NO:67 is a primer sequence useful according to the present invention.
SEQ ID NO:68 is a primer sequence useful according to the present invention.
SEQ ID NO:69 is a primer sequence useful according to the present invention.
SEQ ID NO:70 is a primer sequence useful according to the present invention.
SEQ ID NO:71 is a primer sequence useful according to the present invention.
SEQ ID NO:72 is a primer sequence useful according to the present invention.
SEQ ID NO:73 is a nucleic acid sequence of Arabidopsis AtPAP2 phosphatase gene.
SEQ ID NO:74 is an amino acid sequence of Arabidopsis AtPAP2 phosphatase.
SEQ ID NO:75 is an amino acid sequence of a conserved motif of an NG6 protein.
SEQ ID NO:76 is an amino acid sequence of a conserved motif of an NG6 protein.
SEQ ID NO:77 is an amino acid sequence of a conserved motif of an NG6 protein.
SEQ ID NO:78 is an amino acid sequence of a conserved motif of an NG6 protein.
SEQ ID NO:79 is an amino acid sequence of a conserved motif of an NG21 protein.
SEQ ID NO:80 is an amino acid sequence of a conserved motif of an NG21 protein.
SEQ ID NO:81 is an amino acid sequence of a conserved motif of an NG21 protein.
SEQ ID NO:82 is an amino acid sequence of a conserved motif of an NG21 protein.
SEQ ID NO:83 is an amino acid sequence of a conserved motif of an NG24 protein.

SEQ ID NO:84 is an amino acid sequence of a conserved motif of an NG24 protein.
SEQ ID NO:85 is an amino acid sequence of a conserved motif of an NG24 protein.
SEQ ID NO:86 is an amino acid sequence of a conserved motif of an NG28 protein.
SEQ ID NO:87 is an amino acid sequence of a conserved motif of an NG28 protein.
SEQ ID NO:88 is an amino acid sequence of a conserved motif of an NG28 protein.
SEQ ID NO:89 is an amino acid sequence of a conserved motif of an NG32 protein.
SEQ ID NO:90 is an amino acid sequence of a conserved motif of an NG32 protein.
SEQ ID NO:91 is an amino acid sequence of a conserved motif of an NG32 protein.
SEQ ID NO:92 is an amino acid sequence of a conserved motif of an NG32 protein.
SEQ ID NO:93 is a nucleic acid sequence of rapeseed kinase NG6 gene.
SEQ ID NO:94 is an amino acid sequence of rapeseed kinase NG6.
SEQ ID NO:95 is a nucleic acid sequence of rapeseed kinase NG21 gene.
SEQ ID NO:96 is an amino acid sequence of rapeseed kinase NG21.
SEQ ID NO:97 is a nucleic acid sequence of rapeseed kinase NG24 gene.
SEQ ID NO:98 is an amino acid sequence of rapeseed kinase NG24.
SEQ ID NO:99 is a nucleic acid sequence of rapeseed kinase NG28 gene.
SEQ ID NO:100 is an amino acid sequence of rapeseed kinase NG28.
SEQ ID NO:101 is a nucleic acid sequence of rapeseed kinase NG32 gene.
SEQ ID NO:102 is an amino acid sequence of rapeseed kinase NG32.

5. DETAILED DESCRIPTION

Provided herein are methods of producing plants with enhanced growth and/or yield. In one embodiment, the method comprises: transforming a plant or plant cell with a nucleic acid molecule comprising a plant kinase and/or phosphatase gene selected from NG6, NG21, NG24, NG28, and NG32, and over-expressing said kinase and/or phosphatase gene in the plant or plant cell. In one embodiment, the method further comprises: regenerating, from said transformed plant or plant cell, a plant having enhanced growth and/or yield. Also provided are transgenic plants with enhanced growth and/or yield, comprising a plant kinase and/or phosphatase gene selected from NG6, NG21, NG24, NG28, and NG32, wherein the kinase and/or phosphatase is overexpressed in the plant or plant cell.

The inventors discover that altering the expression levels of one or more phosphatases (such as NG6) and kinases (such as NG21, NG24, NG28, and NG32) results in rapid plant growth and higher yield. The gene expression profiles of the AtPAP2 overexpression lines, AtPAP2 T-DNA (mutant) line, and the wild-type plants are analyzed using microarray. The microarray data show that the expression levels of a range of genes are significantly altered (upregulated or downregulated) in the AtPAP2 overexpression lines, when compared to the wild-type.

The introduction of a representative gene of phosphatases (AT1G05000 (NG6)) and kinases (AT1G13350 (NG21), AT1G28390 (NG24), AT3G24660 (NG28) and AT5G03320 (NG32)), into the genome of Arabidopsis by transgenic technology produced transgenic Arabidopsis that grew faster than the wild-type plants (Table 4, Fig. 5, Fig. 6), and the yield of seeds were elevated by 23 - 70% (Table 5).

While any plant species can be modified using the methods described herein, preferably included without limitation are species from the following genera with representative species in parentheses:

Monocots: genera Asparagus (asparagus), Bromus (cheatgrass), Hemerocallis (daylily), Hordeum (barley), Lolium (ryegrass), Oryza (rice), Panicum (Switchgrass), Pennisetum (fountaingrass), Saccharum (Sugar cane), Sorghum, Trigonella (fenu grass), Triticum (wheat), and Zea (corn); and

Dicots: genera Antirrhinum (flower sp.), Arabidopsis (thaliana), Arachis (peanut), Atropa (deadly nightshade), Brassica (rapeseed), Browallia, Capsicum (pepper), Carthamus (safflower), Cichorium (chicory), Citrus (orange, lemon), Chrysanthemum, Cucumis (cucumber), Datura (thorn apple), Daucus (carrot), Digitalis (foxglove), Fragaria (strawberry), Geranium (flower sp.), Glycine (soybean), Helianthus (sunflower), Hyscyamus, Ipomoea (morning glory), Latuca (lettuce), Linum (linseed), Lotus (flower sp.), Lycopersicon (tomato), Majorana, Malva (cotton), Manihot, Medicago (alfalfa), Nemesia, Nicotiana (tobacco), Onobrychis, Pelargonium (citrosa), Petunia (flower sp.), Ranunculus (flower sp.), Raphanus (radishes), Salpiglossis, Senecio (flower sp.), Sinapis (albae semen), Solanum (potato), Trifolium (clovers), Vigna (mungbean, fava bean), and Vitis (grape).

In certain embodiments, plant species transgenically modified according to the present invention are selected from soybean, maize, potato, rice, sugar canes, switchgrass, cotton, sorghum, alfalfas, rapeseed, canola, rye, sorghum, sunflower, wheat, tobacco, millet, peanuts sweet potato cassava, coffee, coconut, cocoa, tea, banana, citrus, apple, pineapple, avocado, fig, guava, mango, olive, barley ornamentals, and conifers. In preferred embodiments, plant species transgenically modified according to the present invention are selected from soybean, maize, potato, rice, sugar canes, switchgrass, cotton, sorghum, alfalfas, rapeseed, and canola.

In certain embodiment, plant parts, plant tissue, and plant cells including, but not limited to, shoots, stems, seeds, and roots, can be transgenically modified in accordance with the present invention.

4.2 Definitions

The term "protein or peptide homologue," as used herein, refers to one or more of the following proteins or peptides: (i) a protein or polypeptide with at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 98% sequence identity with a protein or polypeptide of the invention; (ii) a protein or polypeptide encoded by a nucleotide sequence that is at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 98% identical to a nucleic acid sequence of the invention; (iii) a protein or polypeptide encoded by a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of the invention; (iv) a protein or polypeptide that is derived from conservative substitution of amino acids of a protein or polypeptide of the invention, or that is derived from conservative substitution of amino acids of a protein or polypeptide of (i) (iii); (v) a fragment of a protein or polypeptide of the invention or a fragment of a protein or polypeptide of (i) through (iv); and (vii) a protein or polypeptide recognized by an antibody that immunospecifically binds to a sequence selected from SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 94, 96, 98, 100 or 102.

The term "an antibody or an antibody fragment that immunospecifically binds to a polypeptide selected from SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 94, 96, 98, 100 or 102" or "an antibody or an antibody fragment that immunospecifically binds to a polypeptide, peptide, or protein of the invention," as

used herein, refers to an antibody or a fragment thereof that immunospecifically binds to a polypeptide selected from SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 94, 96, 98, 100 or 102, or a fragment of these polypeptide, wherein the antibody or the antibody fragment does not non-specifically bind to other peptides, polypeptides, or proteins.

An antibody or a fragment thereof that immunospecifically binds to a polypeptide selected from SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 94, 96, 98, 100 or 102, or a fragment of these polypeptide, may cross-react with other antigens. In a preferred embodiment, an antibody or a fragment thereof that immunospecifically binds to a polypeptide selected from SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 94, 96, 98, 100 or 102, or a fragment of these polypeptides, does not cross-react with other antigens. An antibody or a fragment thereof that immunospecifically binds to a polypeptide selected from SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 94, 96, 98, 100 or 102, or a fragment of these polypeptide, can be identified by, for example, immunoassays or other techniques known to those skilled in the art. An antibody or an antibody fragment that immunospecifically binds a polypeptide selected from SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 94, 96, 98, 100 or 102 may be interchangeably referred to as "anti-PAP antibody".

The term "peptide or protein derivative," as used herein, refers to a given peptide or protein that is modified, *e.g.*, by covalent attachment of another molecule, to the peptide or protein, including the incorporation of non-naturally occurring amino acids. The peptide or protein derivative retains one or more biological activities of the peptide or protein.

The term "nucleic acid fragment," as used herein, refers to a fragment of a nucleic acid molecule of the invention, wherein the fragment comprises at least about 400, at least about 450, at least about 500, at least about 550, at least about 600, at least about 650, at least about 700, at least about 750, at least about 800, at least about 850, at least about 900, at least about 950, at least about 1000, at least about 1050, at least about 1100, at least about 1150, at least about 1200, at least about 1250, at least about 1300, or at least about 1350 contiguous nucleic acid bases of the nucleic acid molecule.

The term "protein or peptide fragment," as used herein, refers to a fragment of a protein or peptide of the invention, wherein the fragment comprises at least about 160, at least about 180, at least about 200, at least about 220, at least about 240, at least about 260, at least about 280, at least about 300, at least about 320, at least about 340, or at least about 360 contiguous amino acid residues of the protein or peptide.

The term "protein or peptide variant," as used herein, includes 1) a naturally occurring allelic variation of a given protein or peptide, and 2) a recombinantly prepared variation of a given protein or peptide, in which one or more amino acid residues have been modified by amino acid substitution, addition, and/or deletion.

An "isolated" nucleic acid molecule has been removed from any environment in which it may exist in nature. For instance, an "isolated" nucleic acid molecule, such as a cDNA molecule, is substantially free of other cellular materials, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. In a preferred embodiment, nucleic acid molecules encoding the polypeptides/proteins of the present invention are isolated or purified.

The term "under stringent conditions" refers to hybridization and washing conditions under which nucleotide sequences having homology to each other remain hybridized to each other. Such hybridization conditions are described in, for example, but not limited to, *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6.; *Basic Methods in Molecular Biology*, Elsevier Science Publishing Co., Inc., N.Y. (1986), pp. 75-78, and 84-87; and *Molecular Cloning*, Cold Spring Harbor Laboratory, N.Y. (1982), pp. 387-389, and are well known to those skilled in the art. A preferred example of stringent hybridization conditions is hybridization in 6X sodium chloride/sodium citrate (SSC), 0.5% SDS at about 68°C followed by one or more washes in 2X SSC, 0.5% SDS at room temperature. Another preferred, example of stringent hybridization conditions is hybridization in 6X SSC at about 45°C followed by one or more washes in 0.2X SSC, 0.1% SDS at about 50-65°C.

To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino acid or nucleic acid sequence). The amino acid residues or nucleotides at

corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % identity = number of identical overlapping positions/total number of positions x 100%). In one embodiment, the two sequences are the same length.

The determination of percent identity between two sequences can also be accomplished using a mathematical algorithm. A preferred, non limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, 1990, Proc. Natl. Acad. Sci. U.S.A. 87:2264 2268, modified as in Karlin and Altschul, 1993, Proc. Natl. Acad. Sci. U.S.A. 90:5873 5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.*, 1990, J. Mol. Biol. 215:403. BLAST nucleotide searches can be performed with the NBLAST nucleotide program parameters set, *e.g.*, for score=100, wordlength=12 to obtain nucleotide sequences homologous to a nucleic acid molecules of the present invention. BLAST protein searches can be performed with the XBLAST program parameters set, *e.g.*, to score 50, wordlength = 3 to obtain amino acid sequences homologous to a protein molecule of the present invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, 1997, Nucleic Acids Res. 25:3389 3402. Alternatively, PSI BLAST can be used to perform an iterated search which detects distant relationships between molecules (Id.). When utilizing BLAST, Gapped BLAST, and PSI Blast programs, the default parameters of the respective programs (*e.g.*, of XBLAST and NBLAST) can be used (see, *e.g.*, the NCBI website). Another preferred, non limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, 1988, CABIOS 4:11 17. Such an algorithm is incorporated in the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically only exact matches are counted.

As used herein, the term "derivative" (*e.g.*, proteins, polypeptides, peptides, and antibodies) refers to an agent that comprises an amino acid sequence which has been altered by the introduction of amino acid residue substitutions, deletions, and/or additions. The term "derivative" as used herein also refers to an agent which has been modified, *i.e.*, by the covalent attachment of any type of molecule to the agent. For example, but not by way of limitation, an antibody may be modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. A derivative of an agent may be produced by chemical modifications using techniques known to those of skill in the art, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Further, a derivative of an agent may contain one or more non-classical amino acids. A derivative of an agent possesses a similar or identical function as the agent from which it was derived.

The term "enhance or promote plant growth and/or yield" refers to for example, increased plant weight, increased leaf number and/or weight, increased number of inflorescence, increased seed production (such as weight/seed and total weight of seeds), increased carbon metabolism, increased carbohydrate (*e.g.*, starch, sugars, cellulose), amino acid, and/or lipid production, early bolting, and also can include combinations of the foregoing, when compared to a wild-type plant of the same species cultivated under the same conditions.

5.1 Growth-promoting Phosphatases and Kinases

Provided herein are phosphatases and kinases that promote plant growth and/or yield. In one embodiment, the growth-promoting phosphatase is NG6, and the growth-promoting kinases are selected from NG6, NG21, NG24, NG28, and NG32. In certain specific embodiments, the growth-promoting phosphatases and kinases are derived from plant species including, but not limited to, Arabidopsis, rice, soybean, maize, and cotton.

In certain embodiments, the phosphatase gene that promotes plant growth and/or yield is an NG6 gene comprising a nucleic acid sequence selected from SEQ ID NO: 1, 3, 5, 7, 9 or 93. In certain embodiments, the phosphatase gene that promotes plant growth and/or yield comprises a nucleic acid sequence having at least 80%, 85%, 90%, 93%, 95%, 96%, 97%, 98% or 99%

identity with SEQ ID NO: 1, 3, 5, 7, 9 or 93. In certain embodiments, the phosphatase gene that promotes plant growth and/or yield is a homologue, derivative, or variant of a nucleic acid molecule derives from a nucleic acid molecule having nucleic acid sequence comprising SEQ ID NO: 1, 3, 5, 7, 9 or 93.

In certain embodiments, the phosphatase gene that promotes plant growth and/or yield comprises a nucleic acid sequence having at least 80%, 85%, 90%, 93%, 95%, 96%, 97%, 98% or 99% identity with SEQ ID NO: 1, 3, 5, 7, 9 or 93. In certain embodiments, the phosphatase gene that promotes plant growth and/or yield comprises the nucleic acid sequence that encodes a protein that comprises one or more of the following conserved motifs: GIFRSGFP (SEQ ID NO:75), YLCPEPYP (SEQ ID NO:76), KEPFVXIP (SEQ ID NO:77), and HCXRGKHRTG (SEQ ID NO:78).

In certain embodiments, the phosphatase gene that promotes plant growth and/or yield is a homologue, derivative, or variant of a nucleic acid molecule that derives from nucleic acid molecule having nucleic acid sequences comprising SEQ ID NO: 1, 3, 5, 7, 9 or 93. In certain embodiments, the phosphatase gene that promotes plant growth and/or yield comprises one or more of the following conserved motifs: GIFRSGFP (SEQ ID NO:75), YLCPEPYP (SEQ ID NO:76), KEPFVXIP (SEQ ID NO:77), and HCXRGKHRTG (SEQ ID NO:78).

In certain embodiments, the kinase gene that promotes plant growth and/or yield is an NG21 gene comprising a nucleic acid sequence selected from SEQ ID NO: 11, 13, 15, 17, 19 or 95. In certain embodiments, the kinase gene that promotes plant growth and/or yield comprises a nucleic acid sequence having at least 65%, 70%, 75%, 80%, 85%, 90%, 93%, 95%, 96%, 97%, 98% or 99% identity with SEQ ID NO: 11, 13, 15, 17, 19 or 95. In certain embodiments, the kinase gene that promotes plant growth and/or yield is a homologue, derivative, or variant of a nucleic acid molecule that derives from nucleic acid molecule having nucleic acid sequence comprising SEQ ID NO: 11, 13, 15, 17, 19 or 95.

In certain embodiments, the kinase gene that promotes plant growth and/or yield comprises a nucleic acid sequence having at least 80%, 85%, 90%, 93%, 95%, 96%, 97%, 98% or 99% identity with SEQ ID NO: 11, 13, 15, 17, or 19 or 95. In certain embodiments, the kinase gene that promotes plant growth and/or yield comprises the nucleic acid sequence that encodes a protein that comprises one or more of the following conserved motifs:

DNWDDA(D/E)GYY (SEQ ID NO:79), YRNHLCLVFESL (SEQ ID NO:80), VLHCDIKPDNMLVNE (SEQ ID NO:81), and TPYLVSRFYRXPEI (SEQ ID NO:82).

In certain embodiments, the kinase gene that promotes plant growth and/or yield is a homologue, derivative, or variant of a nucleic acid molecule that derives from nucleic acid molecule having nucleic acid sequences comprising SEQ ID NO: 11, 13, 15, 17, 19 or 95. In ceratin embodiments, the kinase gene that promotes plant growth and/or yield comprises one or more of the following conserved motifs: DNWDDA(D/E)GYY (SEQ ID NO:79), YRNHLCLVFESL(SEQ ID NO:80), VLHCDIKPDNMLVNE (SEQ ID NO:81), and TPYLVSRFYRXPEI (SEQ ID NO:82). In certain embodiments, the kinase gene that promotes plant growth and/or yield is an NG24 gene comprising a nucleic acid sequence selected from SEQ ID NO: 21, 23, 25, 27, 29 or 97. In certain embodiments, the kinase gene that promotes plant growth and/or yield comprises a nucleic acid sequence having at least 65%, 70%, 75%, 80%, 85%, 90%, 93%, 95%, 96%, 97%, 98% or 99% identity with SEQ ID NO: 21, 23, 25, 27, 29 or 97. In certain embodiments, the kinase gene that promotes plant growth and/or yield is a homologue, derivative, or variant of a nucleic acid molecule that derives from nucleic acid molecule having nucleic acid sequence comprising SEQ ID NO: 21, 23, 25, 27, 29 or 97.

In certain embodiments, the kinase gene that promotes plant growth and/or yield comprises a nucleic acid sequence having at least 80%, 85%, 90%, 93%, 95%, 96%, 97%, 98% or 99% identity with SEQ ID NO: 21, 23, 25, 27, 29 or 97. In certain embodiments, the kinase gene that promotes plant growth and/or yield comprises one or more of the following conserved motifs: VRHRDXKS (SEQ ID NO:83), GTLXGYLDP (SEQ ID NO:84), and DV(F/Y)S(F/Y)G(I/V)LLLEI (SEQ ID NO:85).

In certain embodiments, the kinase gene that promotes plant growth and/or yield is a homologue, derivative, or variant of a nucleic acid molecule that derives from nucleic acid molecule having nucleic acid sequence comprising SEQ ID NO: 21, 23, 25, 27, 29 or 97. In certain embodiments, the kinase gene that promotes plant growth and/or yield comprises one or more of the following conserved motifs: VRHRDXKS (SEQ ID NO:83), GTLXGYLDP (SEQ ID NO:84), and DV(F/Y)S(F/Y)G(I/V)LLLEI (SEQ ID NO:85). In certain embodiments, the kinase gene that promotes plant growth and/or yield is an NG28 gene comprising a nucleic acid sequence selected from SEQ ID NO: 31, 33, 35, 37, 39 or 99. In certain embodiments, the NG24

kinase gene that promotes plant growth and/or yield comprises a nucleic acid sequence having at least 65%, 70%, 75%, 80%, 85%, 90%, 93%, 95%, 96%, 97%, 98% or 99% identity with SEQ ID NO: 31, 33, 35, 37, 39 or 99. In certain embodiments, the NG24 kinase gene that promotes plant growth and/or yield is a homologue, derivative, or variant of a nucleic acid molecule that derives from nucleic acid molecule having nucleic acid sequence comprising SEQ ID NO: 31, 33, 35, 37, 39 or 99.

In certain embodiments, the kinase gene that promotes plant growth and/or yield comprises a nucleic acid sequence having at least 80%, 85%, 90%, 93%, 95%, 96%, 97%, 98% or 99% identity with SEQ ID NO: 31, 33, 35, 37, 39 or 99. In certain embodiments, the kinase gene that promotes plant growth and/or yield comprises one or more of the following conserved motifs: RRHKIALG (SEQ ID NO:86), Y(K/R)APEL (SEQ ID NO:87), and DVYAFGILLLE (SEQ ID NO:88).

In certain embodiments, the kinase gene that promotes plant growth and/or yield is a homologue, derivative, or variant of a nucleic acid molecule that derives from nucleic acid molecule having nucleic acid sequence comprising SEQ ID NO: 31, 33, 35, 37, 39 or 99. In certain embodiments, the kinase gene that promotes plant growth and/or yield comprises one or more of the following conserved motifs: RRHKIALG (SEQ ID NO:86), Y(K/R)APEL (SEQ ID NO:87), and DVYAFGILLLE (SEQ ID NO:88).

In certain embodiments, the kinase gene that promotes plant growth and/or yield is an NG32 gene comprising a nucleic acid sequence selected from SEQ ID NO: 41, 43, 45, 47, 49 or 101. In certain embodiments, the kinase gene that promotes plant growth and/or yield comprises a nucleic acid sequence having at least 65%, 70%, 75%, 80%, 85%, 90%, 93%, 95%, 96%, 97%, 98% or 99% identity with SEQ ID NO: 41, 43, 45, 47, 49 or 101. In certain embodiments, the kinase gene that promotes plant growth and/or yield is a homologue, derivative, or variant of a nucleic acid molecule that derives from nucleic acid molecule having nucleic acid sequence comprising SEQ ID NO: 41, 43, 45, 47, 49 or 101.

In certain embodiments, the kinase gene that promotes plant growth and/or yield comprises a nucleic acid sequence having at least 80%, 85%, 90%, 93%, 95%, 96%, 97%, 98% or 99% identity with SEQ ID NO: 41, 43, 45, 47, 49 or 101. In certain embodiments, the kinase gene that promotes plant growth and/or yield comprises one or more of the following conserved

motifs: CAXDDERG (SEQ ID NO:89), AKLSDFGLAR (SEQ ID NO:90), YELITGR(R/K) (SEQ ID NO:91), and RPKMSEV (SEQ ID NO:92).

In certain embodiments, the kinase gene that promotes plant growth and/or yield is a homologue, derivative, or variant of a nucleic acid molecule that derives from nucleic acid molecule having nucleic acid sequence comprising SEQ ID NO: 41, 43, 45, 47, 49 or 101. In certain embodiments, the kinase gene that promotes plant growth and/or yield comprises one or more of the following conserved motifs: CAXDDERG (SEQ ID NO:89), AKLSDFGLAR (SEQ ID NO:90), YELITGR(R/K) (SEQ ID NO:91), and RPKMSEV (SEQ ID NO:92). In certain embodiments, the phosphatase or kinase gene that promotes plant growth and/or yield encodes a protein selected from SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 94, 96, 98, 100 or 102. In certain embodiments, the phosphatase or kinase gene that promotes plant growth and/or yield encodes a protein having at least 65%, 70%, 75%, 80%, 85%, 90%, 93%, 95%, 96%, 97%, 98% or 99% identity with SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, , 50, 94, 96, 98, 100 or 102. In certain embodiments, the phosphatase or kinase gene that promotes plant growth and/or yield encodes a protein that is a homologue, derivative, or variant of a protein derived from the amino acid molecule having the amino acid sequence selected from SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 94, 96, 98, 100 or 102.

In certain embodiments, the phosphatase gene that promotes plant growth and/or yield encodes an NG6 protein having at least 80%, 85%, 90%, 93%, 95%, 96%, 97%, 98% or 99% identity with SEQ ID NO: 2, 4, 6, 8, 10 or 94. In certain embodiments, the phosphatase gene that promotes plant growth and/or yield one or more of the following conserved motifs: GIFRSGFP (SEQ ID NO:75), YLCPEPYP (SEQ ID NO:76), KEPFVXIP (SEQ ID NO:77), and HCXRGKHRTG (SEQ ID NO:78).

In certain embodiments, the phosphatase gene that promotes plant growth and/or yield encodes an NG6 protein that is a homologue, derivative, or variant of a protein derived from the amino acid molecule having the amino acid sequence comprising SEQ ID NO: 2, 4, 6, 8, 10 or 94. In certain embodiments, the phosphatase gene that promotes plant growth and/or yield comprises one or more of the following conserved motifs: GIFRSGFP (SEQ ID NO:75),

YLCPEPYP (SEQ ID NO:76), KEPFVXIP (SEQ ID NO:77), and HCXRGKHRTG (SEQ ID NO:78).

In certain embodiments, the kinase gene that promotes plant growth and/or yield encodes an NG 21 protein having at least 80%, 85%, 90%, 93%, 95%, 96%, 97%, 98% or 99% identity with SEQ ID NO: 12, 14, 16, 18, 20 or 96. In certain embodiments, the kinase gene that promotes plant growth and/or yield comprises one or more of the following conserved motifs: DNWDDA(D/E)GYY (SEQ ID NO:79), YRNHLCLVFESL (SEQ ID NO:80), VLHCDIKPDNMLVNE (SEQ ID NO:81), and TPYLVSRFYRXPEI (SEQ ID NO:82).

In certain embodiments, the kinase gene that promotes plant growth and/or yield encodes an NG21 protein that is a homologue, derivative, or variant of a protein derived from the amino acid molecule having the amino acid sequence selected from SEQ ID NO: 12, 14, 16, 18, 20 or 96. In certain embodiments, the kinase gene that promotes plant growth and/or yield comprises one or more of the following conserved motifs: DNWDDA(D/E)GYY (SEQ ID NO:79), YRNHLCLVFESL (SEQ ID NO:80), VLHCDIKPDNMLVNE (SEQ ID NO:81), and TPYLVSRFYRXPEI (SEQ ID NO:82).

In certain embodiments, the kinase gene that promotes plant growth and/or yield encodes an NG24 protein having at least 80%, 85%, 90%, 93%, 95%, 96%, 97%, 98% or 99% identity with SEQ ID NO: 22, 24, 26, 28, 30 or 98. In certain embodiments, the kinase gene that promotes plant growth and/or yield comprises one or more of the following conserved motifs: VRHRDXKS (SEQ ID NO:83), GTLXGYLDP (SEQ ID NO:84), and DV(F/Y)S(F/Y)G(I/V)LLLEI (SEQ ID NO:85).

In certain embodiments, the kinase gene that promotes plant growth and/or yield encodes an NG24 protein that is a homologue, derivative, or variant of a protein derived from the amino acid molecule having the amino acid sequence selected from SEQ ID NO: 22, 24, 26, 28, 30 or 98. In certain embodiments, the kinase gene that promotes plant growth and/or yield comprises one or more of the following conserved motifs: VRHRDXKS (SEQ ID NO:83), GTLXGYLDP (SEQ ID NO:84), and DV(F/Y)S(F/Y)G(I/V)LLLEI (SEQ ID NO:85).

In certain embodiments, the kinase gene that promotes plant growth and/or yield encodes an NG28 protein having at least 80%, 85%, 90%, 93%, 95%, 96%, 97%, 98% or 99% identity with SEQ ID NO: 32, 34, 36, 38, 40 or 100. In certain embodiments, the kinase gene that

promotes plant growth and/or yield comprises one or more of the following conserved motifs: RRHKIALG (SEQ ID NO:86), Y(K/R)APEL (SEQ ID NO:87), and DVYAFGILLLE (SEQ ID NO:88).

In certain embodiments, the kinase gene that promotes plant growth and/or yield encodes an NG28 protein that is a homologue, derivative, or variant of a protein derived from the amino acid molecule having the amino acid sequence selected from SEQ ID NO: 32, 34, 36, 38, 40 or 100, wherein the protein comprises one or more of the following conserved motifs: RRHKIALG (SEQ ID NO:86), Y(K/R)APEL (SEQ ID NO:87), and DVYAFGILLLE (SEQ ID NO:88).

In certain embodiments, the kinase gene that promotes plant growth and/or yield encodes an NG32 protein having at least 80%, 85%, 90%, 93%, 95%, 96%, 97%, 98% or 99% identity with SEQ ID NO: 42, 44, 46, 48, 50 or 102. In certain embodiments, the kinase gene that promotes plant growth and/or yield comprises one or more of the following conserved motifs: CAXDDERG (SEQ ID NO:89), AKLSDFGLAR (SEQ ID NO:90), YELITGR(R/K) (SEQ ID NO:91), and RPKMSEV (SEQ ID NO:92).

In certain embodiments, the kinase gene that promotes plant growth and/or yield encodes an NG32 protein that is a homologue, derivative, or variant of a protein derived from the amino acid molecule having the amino acid sequence selected from SEQ ID NO: 42, 44, 46, 48, 50 or 102, wherein the protein comprises one or more of the following conserved motifs: CAXDDERG (SEQ ID NO:89), AKLSDFGLAR (SEQ ID NO:90), YELITGR(R/K) (SEQ ID NO:91), and RPKMSEV (SEQ ID NO:92).

5.2 Production of Transgenic Plants with Enhanced Growth and/or Yield

Another aspect of the present invention provides methods of producing plants with enhanced growth and/or yield. In one embodiment, the method comprises: transforming a plant or plant cell with a nucleic acid molecule comprising a plant kinase and/or phosphatase gene of the present invention. In one embodiment, the method comprises overexpressing said kinase and/or phosphatase gene in the plant or plant cell. In one embodiment, the present invention further comprises: regenerating, from said transformed plant or plant cell, a plant having enhanced growth and/or yield.

The term "overexpressing," "overexpression," or any of the grammatical variations thereof (*e.g.*, over-expressing, over-expression) refers to an increase in the level of expression of a gene, or the level of a protein product encoded by a gene, wherein such increase is at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, or 200%, when compared to cells of the same type in a wild-type plant of the same species cultivated under the same conditions.

In one embodiment, the method further comprises: transforming a plant or a plant cell with a nucleic acid molecule comprising an AtPAP2 gene. In certain embodiments, the method comprises overexpressing the AtPAP2 gene in the plant or plant cell. In one embodiment, the AtPAP2 gene comprises SEQ ID NO: 73. In certain embodiments, the AtPAP2 gene comprises a nucleic acid molecule having a nucleic acid molecule having sequence having at least 65%, 70%, 75%, 80%, 85%, 90%, 93%, 95%, 96%, 97%, 98%, or 99% identity with SEQ ID NO: 73.

In one embodiment, the method further comprises: transforming a plant or a plant cell with a nucleic acid molecule encoding AtPAP2 phosphatase. In certain embodiment, the method comprises overexpressing the nucleic acid molecule encoding AtPAP2 phosphatase in the plant or plant cell. In one embodiment, AtPAP2 phosphatase comprises SEQ ID NO: 74. In certain embodiments, AtPAP2 phosphatase comprises an amino acid molecule having an amino acid nucleic acid sequence having at least 65%, 70%, 75%, 80%, 85%, 90%, 93%, 95%, 96%, 97%, 98%, or 99% identity with SEQ ID NO: 74.

Provided herein are transgenic plants with enhanced growth and/or yield. In certain embodiments, the transgenic plant comprises a nucleic acid molecule having a nucleic acid sequence selected from SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 93, 95, 97, 99 or 101. In certain embodiments, the nucleic acid molecule is overexpressed in the transgenic plant when compared to a wild-type plant of the same species cultivated under the same conditions. In certain embodiments, the transgenic plant comprises a nucleic acid molecule having a nucleic acid sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 93%, 95%, 96%, 97%, 98% or 99% identity with SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 93, 95, 97, 99 or 101. In certain embodiments, the nucleic acid molecule is overexpressed in the transgenic plant when compared to a wild-type plant of the same species cultivated under the same conditions. In

certain embodiments, the transgenic plant comprises a nucleic acid molecule that is a homologue, derivative, or variant of a nucleic acid molecule derived from the nucleic acid molecule having a nucleic acid sequence selected from SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 93, 95, 97, 99 or 101. In certain embodiments, the nucleic acid molecule is overexpressed in the transgenic plant when compared to a wild-type plant of the same species cultivated under the same conditions.

In certain embodiments, the transgenic plant comprises a nucleic acid that encodes a protein having an amino acid sequence selected from SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 94, 96, 98, 100 or 102. In certain embodiments, the nucleic acid molecule is overexpressed in the transgenic plant when compared to a wild-type plant of the same species cultivated under the same conditions. In certain embodiments, the transgenic plant comprises a nucleic acid that encodes a protein having an amino acid sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 93%, 95%, 96%, 97%, 98% or 99% identity with SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 94, 96, 98, 100 or 102. In certain embodiments, the nucleic acid molecule is overexpressed in the transgenic plant when compared to a wild-type plant of the same species cultivated under the same conditions. In certain embodiments, the transgenic plant comprises a nucleic acid that encodes a protein that is a homologue, derivative, or variant of a protein derived from the peptide having an amino acid sequence selected from SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 94, 96, 98, 100 or 102. In certain embodiments, the nucleic acid molecule is overexpressed in the transgenic plant when compared to a wild-type plant of the same species cultivated under the same conditions.

In certain embodiments, the transgenic plant comprises a protein having an amino acid sequence selected from SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 94, 96, 98, 100 or 102. In certain embodiments, the level of the protein in the transgenic plant is higher than that of a wild-type plant of the same species cultivated under the same conditions. In certain embodiments, the transgenic plant comprises a protein having an amino acid sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 93%, 95%, 96%, 97%, 98% or 99% identity with SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24,

26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 94, 96, 98, 100 or 102. In certain embodiments, the level of the protein in the transgenic plant is higher than that of a wild-type plant of the same species cultivated under the same conditions. In certain embodiments, the transgenic plant comprises a protein that is a homologue, derivative, or variant of a protein derived from the peptide having an amino acid and sequence selected from SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 94, 96, 98, 100 or 102. In certain embodiments, the level of the protein in the transgenic plant is higher than that of a wild-type plant of the same species cultivated under the same conditions.

In addition, the present invention provides transgenic plant cells transformed with a nucleic acid molecule of the present invention. In one embodiment, the invention provides transgenic plant cells comprising a kinase or phosphatase nucleic acid molecule of the invention. In certain embodiments, the nucleic acid molecule is overexpressed in the transgenic plant cells when compared to plant cells of the same type in a wild-type plant of the same species cultivated under the same conditions. In another embodiment, the invention provides transgenic plant cells comprising a kinase or phosphatase protein of the invention, wherein the level of said protein in the transgenic plant cells is higher (*e.g.*, at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, or 200% higher) than that of plant cells of the same type in a wild-type plant of the same species cultivated under the same conditions.

In certain embodiments, the transgenic plant comprises a nucleic acid molecule encoding a phosphatase having an amino acid sequence selected from SEQ ID NO: 2, 4, 6, 8, 10, or 94 and/or a kinase selected from SEQ ID NO: 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 96, 98, 100 or 102. In another embodiment, the transgenic plant comprises a nucleic acid molecule encoding a phosphatase having an amino acid sequence selected from SEQ ID NO: 2, 4, 6, 8, 10, or 94 and/or a kinase selected from SEQ ID NO: 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 96, 98, 100 or 102. In certain embodiment, all or a portion, particularly an N-terminal portion, of amino acid residues 1 to 80, preferably all or a portion of amino acid residues 1 to 30, are replaced by a heterologous plant signal peptide by genetic engineering. In such a transgenic plant, the phosphatases or kinases are directed to various organelles/compartments of the cells.

In certain embodiments, the present invention provides chimeric gene constructs for genetic modification of plants to increase growth rate and to improve yield. In a specific embodiment, the chimeric gene constructs comprise a nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 93, 95, 97, 99 or 101. In another specific embodiment, the chimeric gene constructs comprise a sequence that hybridizes under stringent conditions to a nucleic acid molecule comprising a nucleic acid sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 93, 95, 97, 99 or 101, or a complement thereof, wherein the nucleic acid sequence encodes a protein or a polypeptide that exhibits at least one structural and/or functional feature of the polypeptides and enhances plant growth and/or yield.

The phosphatase or kinase- coding sequence is operatively linked to upstream and downstream regulatory components, preferably heterologous to the phosphatase or kinase sequence, such as for example, CMV 35S promoter, which acts to cause expression of the gene (production of the enzyme) in plant cells (*see* Fig. 3). Preferably, when a construct comprising a gene encoding a phosphatase or kinase of the present invention is introduced into plant cells by a conventional transformation method, such as microparticle bombardment, Agrobacterium infection, or microinjection, the gene is expressed in the cells under the control of the regulatory sequences. The expressed phosphatase interacts with the biosynthetic machinery that is naturally present in the plant cells to alter the carbon metabolism. By altering the carbon metabolism, the method of the present invention promotes the growth rate of the plant, resulting in faster growth rate and higher yield. As a result, the time required for the maturation of the plant and the time required for flowering is shortened. Also provided are methods for increasing growth rate and yield of plants, comprising the step of inserting into such plant cells, or cells of such whole plants, a chimeric gene construct.

In one specific embodiment, Arabidopsis is genetically modified by introducing an overexpression construct comprising nucleic acid molecules encoding a growth-promoting phosphatase or kinase of the present invention.

In an embodiment, the growth-promoting phosphatase and kinase genes are derived from Arabidopsis. As shown in the examples, transgenic Arabidopsis plants with over-expression of

NG6, NG21, NG24, NG28, and/or NG32 have enhanced growth and/or yield, when compared to wild-type Arabidopsis plants (*see* Table 5, and Figs. 5 and 6).

In one embodiment, a transgenic plant overexpressing a nucleic acid comprises the sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 93, 95, 97, 99 or 101 or homologues thereof, wherein the nucleic acid molecule encodes polypeptides or proteins of the invention.

5.3 Homologues, Derivatives, and Variants of Kinases and Phosphatases

The present invention also provides homologues, derivatives, and variants of kinases and phosphatases of the present invention; nucleic acid molecules encoding the polypeptides and homologues, derivatives, and variants; vectors, plant cells and transgenic plants comprising these nucleic acid molecules; and uses thereof for promoting plant growth and/or yield. The homologues, derivatives and variants of kinases and phosphatases are derived from the wild-type kinases and phosphatases, respectively. The methods of deriving the homologues, derivatives and variants are well known in the art which include routine conventional techniques of chemical modifications of amino acid residues or using molecular biology and recombinant DNA manipulation and production. Such techniques are available to the skilled artisan in laboratory manuals such as Sambrook and Russell, Molecular cloning: A Laboratory Manual, 3rd edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (2001).

In one embodiment, a homologue of the nucleic acid or polypeptide molecule of the present invention includes: (i) a polypeptide with at least about 65%, at least about 70%, at least about 80%, at least about 90%, or at least about 98% sequence identity of the polypeptide of the invention; (ii) a polypeptide encoded by a nucleotide sequence that is at least about 65%, at least about 70%, at least about 80%, at least about 90%, or at least about 98% identical to one or more of the nucleotide sequences encoding a polypeptide of the invention, or a fragment thereof; (iii) a polypeptide encoded by a nucleotide sequence that hybridizes, under stringent conditions, to a nucleotide sequence of the present invention; (iv) a polypeptide having an amino acid sequence that is at least about 65%, at least about 70%, at least about 80%, at least about 90%, or at least about 98% identical to a polypeptide of the present invention, and wherein the polypeptide of the invention is conservatively substituted; (v) a nucleic acid sequence encoding an amino acid

sequence that is at least about 70%, at least about 80%, at least about 90%, or at least about 98% identical to a polypeptide of the present invention and wherein the polypeptide of the invention is conservatively substituted; and (vi) a fragment of a polypeptide described in (i) through (iv), wherein the polypeptide fragment has at least 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 450, 500, 550, 600, 650, 700, or 750 contiguous amino acid residues of a polypeptide of the invention.

In one embodiment, a homologue polypeptide has an amino acid sequence that is at least about 65%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or at least about 98% identical to a kinase or phosphatase of the present invention. In one embodiment, the homologue polypeptide is obtained by conservative substitution.

In one aspect, the homologues derivatives and variants are derived from the wild type kinase and phosphatase by substitution, deletion, insertion of one or more nucleic acid in a nucleic acid molecule or one or more amino acid residues in an amino acid molecule. The term "derived" as used herein includes the modifications of a wild type nucleic acid molecule or amino acid molecule as described below. For example, non-natural amino acids can be substituted for the amino acids of the kinases and phosphatases so long as the kinases and phosphatases having the substituted amino acids retain substantially the same functional activity as the kinases and phosphatases in which amino acids have not been substituted. Those having skill in the art will recognize that mutations can be made to polynucleotides encoding protein and peptides, or complementary thereto, and that such mutations do not cause structural changes that affect functionality.

Conservative substitutions whereby a modified protein or polypeptide of the present invention having an amino acid of one class is replaced with another amino acid of the same class fall within the scope of the subject invention so long as the modified protein or polypeptide having the substitution still retains substantially the same functional activity as the protein or polypeptide that does not have the substitution. For instance, amino acid residue of any of the following 11 groups may be conservatively substituted with another amino acid of the same group: (1) acidic (negatively charged) amino acids, such as aspartic acid and glutamic acid; (2) basic (positively charged) amino acids, such as arginine, histidine, and lysine; (3) neutral polar amino acids, such as glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; (4)

neutral nonpolar (hydrophobic) amino acids, such as alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; (5) amino acids having aliphatic side chains, such as glycine, alanine, valine, leucine, and isoleucine; (6) amino acids having aliphatic-hydroxyl side chains, such as serine and threonine; (7) amino acids having amide-containing side chains, such as asparagine and glutamine; (8) amino acids having aromatic side chains, such as phenylalanine, tyrosine, and tryptophan; (9) amino acids having basic side chains, such as lysine, arginine, and histidine; (10) amino acids having sulfur-containing side chains, such as cysteine and methionine; and (11) amino acids having similar geometry and hydrogen bonding patterns, such as aspartic acid, asparagine, glutamic acid and glutamine.

Examples of non-natural amino acids include, but are not limited to, ornithine, citrulline, hydroxyproline, homoserine, phenylglycine, taurine, iodotyrosine, 2,4-diaminobutyric acid, α-amino isobutyric acid, 4-aminobutyric acid, 2-amino butyric acid, γ-amino butyric acid, ε-amino hexanoic acid, 6-amino hexanoic acid, 2-amino isobutyric acid, 3-amino propionic acid, norleucine, norvaline, sarcosine, homocitrulline, cysteic acid, τ-butylglycine, τ-butylalanine, phenylglycine, cyclohexylalanine, β-alanine, fluoro-amino acids, designer amino acids such as β-methyl amino acids, C-methyl amino acids, N-methyl amino acids, and amino acid analogues in general. Non-natural amino acids also include amino acids having derivatized side groups. Furthermore, any of the amino acids in the protein can be of the D (dextrorotary) form or L (levorotary) form.

The structure of a polypeptide can be determined by methods known to those skilled in the art, including but not limited to, X-ray crystallography, nuclear magnetic resonance, and crystallographic electron microscopy. A sequence having sequence homology can be made using standard molecular biology techniques, including site-directed mutagenesis and by insertion or deletion of sequences.

In one aspect, the homologues, derivatives and variants are derived from the wild type kinase and phosphatase. In certain embodiments, provided herein are derivatives of the disclosed polypeptides. For example, but not by way of limitation, derivatives may include peptides or proteins that have been modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical

modifications may be carried out by known techniques including, but not limited to, specific chemical cleavage, acetylation, formylation, etc. Additionally, the derivative may contain one or more non-classical amino acids. The subject invention also concerns variants of the polynucleotides of the present invention. Variant sequences include those sequences wherein one or more nucleotides of the sequence have been substituted, deleted, and/or inserted.

The nucleotides that can be substituted for natural nucleotides of DNA have a base moiety that can include, but is not limited to, inosine, 5-fluorouracil, 5-bromouracil, hypoxanthine, 1-methylguanine, 5-methylcytosine, and tritylated bases. The sugar moiety of the nucleotide in a sequence can also be modified and includes, but is not limited to, arabinose, xylulose, and hexose. In addition, the adenine, cytosine, guanine, thymine, and uracil bases of the nucleotides can be modified with acetyl, methyl, and/or thio groups. Sequences containing nucleotide substitutions, deletions, and/or insertions can be prepared and tested using standard techniques known in the art.

Unless otherwise specified, as used herein percent sequence identity and/or similarity of two sequences can be determined using the algorithm of Karlin and Altschul (1990), modified as in Karlin and Altschul (1993). Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.* (1990). BLAST searches can be performed with the NBLAST program, score = 100, wordlength = 12, to obtain sequences with the desired percent sequence identity. To obtain gapped alignments for comparison purposes, Gapped BLAST can be used as described in Altschul *et al.* (1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (NBLAST and XBLAST) can be used. *See* NCBI/NIH website.

The subject invention also contemplates those polynucleotide molecules having sequences which are sufficiently homologous with the polynucleotide sequences exemplified herein so as to permit hybridization with that sequence under standard stringent conditions and standard methods (Maniatis *et al.*, 1982).

In one embodiment, the present invention further provides isolated nucleic acid molecules that comprise, or consist of, at least about 550, at least about 600, at least about 650, at least about 700, at least about 750, at least about 800, at least about 850, at least about 900, at least about 950, at least about 1000, at least about 1050, at least about 1100, at least about 1150,

at least about 1200, at least about 1250, at least about 1300, or at least about 1350 contiguous nucleotides of a nucleic acid molecule of the present invention.

In another embodiment, an isolated nucleic acid molecule encodes a variant of a polypeptide whose amino acid sequence has been modified by genetic engineering so that biological activities of the polypeptides are either enhanced or reduced, or the local structures thereof are changed without significantly altering the biological activities. Amino acid modifications can be made by methods known in the art.

In one embodiment, the present invention embodies isolated nucleic acid molecules that hybridize, under stringent conditions, to nucleic acid molecules having the nucleic acid sequence comprising SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 93, 95, 97, 99 or 101, or homologues thereof. In certain embodiments, the nucleic acid molecules encode proteins or polypeptides that exhibit at least one structural and/or functional feature of the polypeptides of the invention (e.g. enhance plant growth and/or yield).

A further embodiment includes methods for preparing a polypeptide as provided herein by recombinant DNA technology. In one embodiment, the preparation method comprises culturing host cells containing a recombinant expression vector encoding a polypeptide as provided herein, or a nucleotide sequence encoding a polypeptide as provided herein operably linked to a heterologous promoter, and producing the polypeptide as provided herein.

5.4 Vectors and Expression Constructs

Another embodiment includes nucleic acid molecules suitable for use as primers or hybridization probes for the detection of nucleic acids encoding a phosphatase or kinase polypeptide as provided herein or other sequences.

Yet another embodiment includes vectors, *e.g.*, recombinant expression vectors, comprising a nucleic acid molecule as provided herein. Furthermore, host cells containing such a vector or engineered to contain and/or express a nucleic acid molecule as provided herein and host cells containing a nucleotide sequence as provided herein operably linked to a heterologous promoter are disclosed.

As used herein, the term "expression construct" refers to a combination of nucleic acid sequences that provides for transcription of an operably linked nucleic acid sequence. In general, operably linked components are in contiguous relation.

Expression constructs of the invention will also generally include regulatory elements that are functional in the intended host cell in which the expression construct is to be expressed. Regulatory elements include promoters, transcription termination sequences, translation termination sequences, enhancers, and polyadenylation elements.

An expression construct as provided herein can comprise a promoter sequence operably linked to a polynucleotide sequence encoding a peptide. Promoters can be incorporated into a polynucleotide using standard techniques known in the art. Multiple copies of promoters or multiple promoters can be used in an expression construct. In a preferred embodiment, a promoter can be positioned about the same distance from the transcription start site as it is from the transcription start site in its natural genetic environment. Some variation in this distance is permitted without substantial decrease in promoter activity. A transcription start site is typically included in the expression construct.

Unique restriction enzyme sites can be included at the 5' and 3' ends of the expression construct to allow for insertion into a polynucleotide vector. As used herein, the term "vector" refers to any genetic element, including for example, plasmids, cosmids, chromosomes, phage, virus, and the like, which is capable of replication when associated with proper control elements and which can transfer polynucleotide sequences between cells. Vectors contain a nucleotide sequence that permits the vector to replicate in a selected host cell.

The term "operably linked," as used herein, refers to when transcription under the control of the "operably linked" promoter produces a functional messenger RNA, translation of which results in the production of the polypeptide encoded by the DNA operably linked to the promoter.

5.5 Fusion Proteins

Also provided herein are fusion proteins. In one embodiment, the polypeptides as provided herein, or fragments thereof, are recombinantly fused or chemically conjugated (*e.g.*, covalent and non-covalent conjugations) to heterologous polypeptides (*i.e.*, an unrelated polypeptide or portion thereof, preferably at least 10, at least 20, at least 30, at least 40, at least

50, at least 60, at least 70, at least 80, at least 90 or at least 100 amino acids of the polypeptide) to generate fusion proteins. The fusion can be direct, or may occur through linker sequences.

In one embodiment, the fusion protein comprises a polypeptide fused to a heterologous signal sequence at its N-terminus. For example, the signal sequence naturally found in the polypeptide can be replaced by a signal sequence that is derived from a heterologous origin. Various signal sequences are commercially available.

In another embodiment, a polypeptide can be fused to tag sequences, *e.g.*, a hexa-histidine peptide, among others, many of which are commercially available. As described in Gentz *et al.*, 1989, *Proc. Natl. Acad. Sci. USA*, 86:821-824, for instance, hexa-histidine provides for convenient purification of the fusion protein. Other examples of peptide tag include the hemagglutinin "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson *et al.*, 1984, *Cell*, 37:767), and the "flag" tag (Knappik *et al.*, 1994, *Biotechniques*, 17(4):754-761). These tags are useful for purification of recombinantly produced polypeptides.

Fusion proteins can be produced by standard recombinant DNA techniques or by protein synthetic techniques, *e.g.*, by use of a DNA synthesizer. For example, a nucleic acid molecule encoding a fusion protein can be synthesized by conventional techniques including, for example, automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers, which give rise to complementary overhangs between two consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, *e.g.*, *Current Protocols in Molecular Biology*, Ausubel *et al.*, eds., John Wiley & Sons, 1992).

The nucleotide sequence encoding a fusion protein can be inserted into an appropriate expression vector, *i.e.*, a vector that contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. In a specific embodiment, the expression of a fusion protein is regulated by an inducible promoter.

In another embodiment, the present invention provides methods for detecting the presence, activity or expression of a polypeptide of the invention or similar polypeptide in a biological material, such as cells, or culture media. The increased or decreased activity or expression of the polypeptide in a sample relative to a control sample can be determined by

contacting the biological material with an agent that can detect directly or indirectly the presence, activity or expression of the polypeptide. In a particular embodiment, such an agent is an antibody or a fragment thereof which immunospecifically binds to one of the disclosed polypeptides.

In a still another embodiment, a fusion protein comprising a bioactive molecule and one or more domains of a disclosed polypeptide or fragment thereof is provided. In particular, fusion proteins comprising a bioactive molecule recombinantly fused or chemically conjugated (including both covalent and non-covalent conjugations) to one or more domains of a disclosed polypeptide or fragments thereof.

5.6 Preparation of Transgenic Plants

Genetic engineering of plants can be achieved in several ways. The most common method is *Agrobacterium*-mediated transformation. In this method, *A. tumefaciens*, which naturally infects plants by inserting tumor-causing genes into a plant's genome, is genetically altered. Selected genes can be engineered into the T-DNA of the bacterial Ti (tumor-inducing) plasmid of *A. tumefaciens* in laboratory conditions so that they become integrated into the plant chromosomes when the T-DNA is transferred to the plant by the bacteria's own internal transfer mechanisms.

The only essential parts of the T-DNA are its two small (25 base pair) border repeats, at least one of which is needed for plant transformation. The bacterial genes encoding for plant hormones that promote tumor growth are excised from the T-DNA and replaced with a sequence of DNA that typically contains: a selectable marker (*e.g.* an antibiotic-resistance gene; usually kanamycin resistance), a restriction site - a site with a specific sequence of nucleotides where a restriction enzyme will cut the DNA, and the desired genes to be incorporated into the plant (B. Tinland, 1996. The integration of T-DNA into plant genomes. Trends in Plant Science 1,178-184; D. Grierson (ed.) 1991. Plant Genetic Engineering. Blackie, Glasgow).

Agrobacterium can be added to plant protoplasts (plant cells with cell walls removed) in culture; the plant protoplasts then regenerate cell walls at which point non-transformed plants are killed with antibiotics for which the transformed plants have been given resistance genes.

Plantlets are then regenerated from the surviving transformed cells using standard plant tissue culture techniques.

In an alternative technique, sterile disks or fragments of vegetative portions of plants are placed in liquid culture medium with Agrobacterium, and then hormones are used to induce rooting, thereby regenerating plantlets grown on selection media. Another technique for delivering genes is possible for some plants such as Arabidopsis, where the Agrobacterium or even "naked" DNA can be infused through the seed coat to cause transformation (Clough SJ and Bent AF, 1998. Floral dip: a simplified method for Agrobacterium-mediated transformation of *Arabidopsis thaliana*. Plant J 16:735-43).

The biolistic method for genetic engineering of plants was developed more recently and is becoming more widely employed. In this method, very small particles (microprojectiles) of tungsten or gold coated with biologically active DNA are propelled at high-velocities into plant cells using an electrostatic pulse, air pressure, or gunpowder percussion. As the particles pass through the cell, the DNA dissolves and can then integrate into the genome of that cell and its progeny. This method can produce stable transformants (Christou, P., *et al.*, 1988. Stable transformation of soybean callus by DNA-coated gold particles, *Plant Physiology* 87:671-674). The method can be practiced on whole plants and is particularly effective on meristematic tissue. It is also capable of delivering DNA either to the nucleus or into mitochondria (Johnston, S.A., *et al.*, 1988. Mitochondrial transformation in yeast by bombardment with microprojectiles (Science 240,1538-41) and chloroplasts (Svab, Z., *et al.*, 1990, Stable transformation of plastids in higher plants, *Proc Natl Acad Sci.* USA 87, 8526-8530).

The electroporation method of plant genetic engineering has met with less success. In this technique, protoplasts in culture take up pure DNA when treated with certain membrane-active agents or with electroporation - a rapid pulse of high-voltage direct current. Once the DNA enters the protoplast, it can be integrated into the cells genome. Standard tissue culture techniques are then used to regenerate transgenic plants.

The microinjection method of plant genetic engineering is perhaps the most difficult. In this method, DNA is microinjected into target plant cells using very thin glass needles in a method similar to that used with animals. The technique is laborious, ineffective, and impractical for generating large numbers of transgenic plants.

It is within the ability of a skilled artisan to select known methods for producing genetically engineering plants, taking into account various factors such as the targeted plant species and which methods have been proven effective therein.

5.7 Preparation of Antibodies

In one aspect, provided herein are antibodies against the kinase and phosphatase. Antibodies which specifically recognize one of the described phosphatase polypeptides or fragments thereof can be used for detecting, screening, and isolating the polypeptide that is provided herein or fragments thereof, or similar sequences that encode similar enzymes from other organisms. For example, an antibody which immunospecifically binds a protein or protein fragments thereof can be used for various *in vitro* detection assays, including enzyme-linked immunosorbent assays (ELISA), radioimmunoassays, Western blot, etc., for the detection of the polypeptide that is provided herein or fragments, derivatives, homologues, or variants thereof, or similar molecules having the similar enzymatic activities as the phosphatase and/or kinase polypeptides.

Embodiments further provide antibodies that immunospecifically bind a polypeptide that is provided herein. Such antibodies include, but are not limited to, antibodies from various animals, humanized, chimeric, polyclonal, monoclonal, bi-specific, multi-specific, single chain antibodies, Fab fragments, $F(ab')_2$ fragments, disulfide-linked Fvs, fragments containing a VL or VH domain or a complementary determining region (CDR), wherein the antibody or antibody fragment immunospecifically binds to a polypeptide that is provided herein.

Antibodies specific for the described phosphatase polypeptides can be generated by any suitable method known in the art. Once an antibody molecule has been produced, it may then be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (*e.g.*, ion exchange, affinity, particularly by affinity for the specific antigen after Protein A or Protein G purification, and sizing column chromatography), centrifugation, differential solubility, or by any other standard techniques for the purification of proteins. Further, the antibodies or fragments thereof may be fused to heterologous polypeptide sequences described herein or otherwise known in the art to facilitate purification.

Antibodies fused or conjugated to heterologous polypeptides may be used in *in vitro* immunoassays and in purification methods (*e.g.*, affinity chromatography) well known in the art. See *e.g.*, PCT publication Number WO 93/21232; EP 439,095; Naramura *et al.*, 1994, *Immunol. Lett.* 39:91-99; U.S. Patent 5,474,981; Gillies *et al.*, 1992, PNAS 89:1428-1432; and Fell *et al.*, 1991, *J. Immunol.* 146:2446-2452.

Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the described polypeptides or fragments, derivatives, homologues, or variants thereof, or similar molecules having the similar enzymatic activities as the polypeptide of the invention. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, and polystyrene.

5.8 Detection Assays

An exemplary method for detecting the presence or absence of an over-expressed phosphatase/ kinase polypeptide or an inserted phosphatase/kinase-encoding nucleic acid in a biological sample involves obtaining a biological sample from various sources and contacting the sample with a compound or an agent capable of detecting a polypeptide or nucleic acid (*e.g.*, mRNA, genomic DNA) such that the presence of a heterologous polypeptide or nucleic acid is detected in the sample.

An exemplary agent for detecting mRNA or genomic DNA encoding an inserted phosphatase polypeptide is a labeled nucleic acid probe capable of hybridizing to mRNA or genomic DNA encoding any of the described phosphatase and kinase polypeptides. The nucleic acid probe can be, for example, a full-length cDNA, such as the nucleic acid of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 93, 95, 97, 99, 101 or a portion thereof, such as an oligonucleotide of at least one of at least about 15, at least about 20, at least about 25, at least about 30, at least about 50, at least about 100, at least about 250, at least about 500, or more nucleotides in length and sufficient to specifically hybridize under stringent conditions to a mRNA or genomic DNA encoding a polypeptide of the invention.

An exemplary agent for detecting an over-expressed phosphatase / kinase polypeptide is an antibody capable of binding to a phosphatase / kinase polypeptide product of an inserted gene,

preferably an antibody with a detectable label. Antibodies can be polyclonal and monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or $F(ab')_2$) can be used.

The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin.

The detection method can be used to detect mRNA, protein, or genomic DNA in a sample *in vitro* as well as *in vivo*. For example, in vitro techniques for detection of mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of a heterologous polypeptide include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of a heterologous polypeptide include introducing into a subject organism a labeled antibody directed against the polypeptide. For example, the antibody can be labeled with a radioactive marker whose presence and location in the subject organism can be detected by standard imaging techniques, including autoradiography.

In a specific embodiment, the methods further involve: 1) obtaining a control sample from a control subject, 2) contacting the control sample with a compound or agent capable of detecting an over-expressed polypeptide product, or the mRNA transcription product, or genomic DNA encoding an inserted phospatase gene, such that the presence of the polypeptide or mRNA or genomic DNA encoding the phosphatase polypeptide is detected in the sample, and 3) comparing the level of the phosphatase / kinase polypeptide or mRNA or genomic DNA encoding the polypeptide in a control sample with the level of the polypeptide or mRNA or genomic DNA encoding endogenous phosphatase polypeptides in the test sample.

5.9 Applications of Transgenic Plants

The transgenic plants generated can have many useful applications, including in food, feed, biomass, biofuels (starch, cellulose, seed lipids) and wood pulp industry. The enhanced

growth rate of the transgenic plants can provide additional carbon dioxide fixation per hectare of land per year, and, thus is useful for generating carbon credits.

6.0 EXAMPLES

Following are examples that illustrate embodiments for practicing the invention. These examples should not be construed as limiting. Unless otherwise noted, all percentages are by weight, all solvent mixture proportions are by volume, all temperatures are in Centigrade, and all pressure is at or near atmospheric pressure.

6.1 SCREENING OF GROW-PROMOTING NG GENES

Two independent AtPAP2 overexpression lines (OE7 and OE21, homozygous T3 plants), an AtPAP2 T-DNA mutant line that cannot express the full length AtPAP2, and the wild-type Arabidopsis (Col-0) were employed for microarray analysis. The AtPAP2 overexpression lines (OE7 and OE21, homozygous T3 plants), the AtPAP2 T-DNA mutant line, and the wild-type Arabidopsis (Col-0) line have been disclosed by the present inventor in U.S. Patent Application Serial No. 12/640,674 (U.S. Patent Application Publication No. 2010/0159065).

Briefly, seeds were germinated on MS medium supplemented with 2% (w/v) sucrose, grew in a growth room under 12 hour-light/12 hour-dark cycle at 22°C for 10 days, and were then transferred to soil and grew in a growth chamber under a 16-hour light (22°C) and 8-hour dark (18°C) cycle. Shoots of 20-day-old Arabidopsis (WT, T-DNA, OE7 and OE21) prior to bolting were collected in the middle of day (4 plants/line/tube, 3 biological triplicates/line, 3 tubes/line) and ground in liquid nitrogen. RNA extraction was performed with on-column DNase digestion according to the manufacturer's instruction (RNeasy Plant Mini Kit, Cat.No.74904, Qiagen). Total RNA was dissolved in DEPC water and quantified by the Bioanalyzer 2100 (Agilent Technologies, Boblingen, Germany). Double strand DNA synthesis and Cy 3 labeling from three biological replicates were performed by NimbleGen Systems, Inc. (Madison, WI). Statistical analyses of normalized microarray data (RMA algorithm, quantile normalization) and drawing of scatter plots, heatmaps were performed using ArrayStar 3.0 (DNASTAR, Madison, WI). Identification of GO and classification were carried out using software available from TAIR

database and KEGG pathway database. In all three replications, genes were considered to be significantly regulated if their fold change values were positively or negatively beyond 1.3 ($p< 0.05$).

20-day-old plants did not show any differences in appearance so that any differences in gene expression between the lines were not due to difference in developmental stage or additional tissues (e.g. inflorescence). The transcripts levels of 30360 genes in shoots were determined using the Arabidopsis Genome NimbleGen chips. The average hybridization signals detected in each line were normalized from the log2 average signal and compared with the signal strengths in the wild-type Arabidopsis.

An overview of the expression data of OE7, OE21 and T-DNA plants versus wild-type control is presented as a heat map (Fig. 1) and scatter plots (Fig. 2) that show a linear bias in the graphs. Gene expression patterns in transgenic shoots are different comparative to their wild-type controls.

The data show that AtPAP2 overexpression altered expression levels of other genes, nearly half of which have not been characterized yet. AtPAP2 overexpression lines exhibit more dramatic changes in gene expression than the AtPAP2 T-DNA line.

Differentially expressed genes are identified using *P*-value <0.05 and fold change >1.3 as the cutoff, and the results show that the expression of about 6312, 7831, and 672 genes in the shoots of OE7, OE21 and T-DNA lines are significantly altered. An overall view of the altered genes in the heat map (Fig. 1) revealed that most genes were down-regulated in the fold change >=2.0. In addition, the fold change in expression levels is smaller in up-regulated genes than in down-regulated genes.

Based on the microarray data, 33 putative phosphatase and kinase genes were selected, and were introduced into Arabidopsis to produce overexpression lines. The results show that the overexpression of NG6, NG21, NG24, NG28 and NG32 in Arabidopsis promotes the growth of Arabidopsis and increases seed yield (see Example 2). The expression level of the five growth-promoting NG genes in the AtPAP2 OE lines and T-DNA lines are shown in Table 1.

Table 1. Microarray data of the 5 growth-promoting genes in AtPAP2 overexpression lines (OE7, OE21), T-DNA line and wild type (WT) Arabidopsis.

NG No.	AGI code	WT-Mean	T-DNA Mean	OE7 Mean	OE21 Mean	OE7/WT Fold	OE21/WT Fold	T-DNA/WT Fold	Gene Description
NG 6	AT1G05000	637	533	976	1131	1.52*	1.78**	0.83	Protein phosphatase
NG21	AT1G13350	2406	2151	3543	3441	1.47*	1.43*	0.89	Protein kinase
NG24	AT1G28390	778	710	1853	1915	2.37**	2.42**	0.91	Protein kinase
NG28	AT3G24660	2514	1839	3313	4422	1.32**	1.74**	0.73	Protein kinase
NG32	AT5G03320	1325	1063	1884	2053	1.43*	1.56**	0.80	Protein kinase

6.2 PRODUCTION OF NG OVEREXPRESSION LINES IN ARABIDOPSIS

To create transgenic NG gene overexpressing lines, the full length coding region of each NG gene's cDNA was amplified by PCR using the following primers (Table 2). The PCR products were inserted into the pCXSN vector with classical TA cloning method (Fig. 3).

Table 2. Primers used for to amplify the full CDS of the aimed NG genes

Gene name		Sequence(5'---3')
NG6	Forward Primer	5'-TCGAGCTAGCATGAAGCTTGTGGAGAAGAC-3' (SEQ ID NO: 51)
	Reverse Primer	5'-CGACGAGCTCTTACCTGATGGAACAAGAG-3 (SEQ ID NO: 52)
NG21	Forward Primer	5'-ATGGTGAGTGACAAGCATGTAG-3' (SEQ ID NO: 53)
	Reverse Primer	5'-TCACTTGCCCGTGATGAATG-3' (SEQ ID NO: 54)
NG24	Forward Primer	5'-ATGGGTTATCTCTCTTGCAAC-3' (SEQ ID NO: 55)
	Reverse Primer	5'-TCAGTATCTCTTCCGCGACG-3' (SEQ ID NO: 56)
NG28	Forward Primer	5'-ATGGGCATGGAAGCTTTGAG-3' (SEQ ID NO: 57)
	Reverse Primer	5'-TCAAAATGGAGTTTCGGCGT-3' (SEQ ID NO: 58)
NG32	Forward Primer	5'-ATGAAATGCTTCTTATTCCC-3' (SEQ ID NO: 59)
	Reverse Primer	5'-TCAACAAGCTCTCACATTCT-3' (SEQ ID NO: 60)

The vector was introduced into *Agrobacterium tumefaciens* strain GV3101 and then transformed by the floral dip method (Clough and Bent, 1998) into wild-type Col-0 to generate NG-overexpressing lines. Through two generations of selection on MS agar plate with 30 mg/l hygromycin, homologous NG transgenic lines were obtained. The resistant plants were transferred to soil to grow to maturity, and their transgenic status was confirmed by qRT-PCR analysis.

6.3 CONFIRMATION OF OVEREXPRESSION OF NG GENES IN TRANSGENIC PLANTS

The transcription levels of the NG genes in the hygromycin resistant, homologous T3 overexpression lines were confirmed by quantitative Real Time-PCR. Total RNA was extracted from 10-day-old seedlings grown on Murashige and Skoog (MS) with 3% (w/v) sucrose using the TRIzol RNA isolation method with DNase I treatment. cDNAs were generated using Superscript III reverse transcriptase (Invitrogen, Carlsbad, CA, USA) using an oligo15 dT primer. Two gene-specific primers were used to amplify the 80-150bp coding region of each NG gene. The ACTIN primers were used for control experiment. As shown in Figure 4, the transcript levels of each overexpression line were consistently higher than their respective expression levels in the wild-type.

Table 3. Primers used in the quantitative RT-PCR

NG6	Forward Primer	5'-TGTGCCCGGAGCCCTACC-3' (SEQ ID NO: 61)
	Reverse Primer	5'-CTTTCAGTGCCATGCGGATTTT-3 (SEQ ID NO: 62)
NG21	Forward Primer	5'-GGCACAAGTCCCGTCATCACC-3' (SEQ ID NO: 63)
	Reverse Primer	5'-TCCCCAATCCCTTCTTTTCCTA-3' (SEQ ID NO: 64)
NG24	Forward Primer	5'-GCCGCCGTCAAGAGAACAAC-3' (SEQ ID NO: 65)
	Reverse Primer	5'-CTCCGGTGGTCAACGCAGTAA-3' (SEQ ID NO: 66)
NG28	Forward Primer	5'-TGTTGTTGTGGCCTCGTTGTTA-3' (SEQ ID NO: 67)
	Reverse Primer	5'-CTTTCCTTCACCGCCTTCTTTC-3' (SEQ ID NO: 68)
NG32	Forward Primer	5'-AAGCTTTCGGATTTCGGTTTG-3' (SEQ ID NO: 69)

	Reverse Primer	5'-TGGCCTTCTTCCTGTAATGAGC-3' (SEQ ID NO: 70)
ACTIN	Forward Primer	5'- CCCGCTATGTATGTCGC -3' (SEQ ID NO: 71)
	Reverse Primer	5'- AAGGTCAAGACGGAGGAT -3' (SEQ ID NO: 72)

6.4 GROWTH PHENOTYPES OF NG GENE OVER-EXPRESSION LINES

Arabidopsis seeds were soaked in water at 4°C for 3 days. The seeds were surface sterilized and sown on MS medium supplemented with 3% (w/v) sucrose for 10 days. Seedlings with 2 rosette leaves of the same size were transferred to soil under Long Day condition (16h light at 22°C/ 8h dark at18°C) in a plant growth chamber. Bolting time was measured when the primary inflorescence reached 1 cm above the rosette leaves. (Liu *et al.*, 2008; Wu *et al.*, 2008).

The inflorescences of NG gene OE lines emerged earlier (4-5 days) than the WT at Long Day conditions (Table 4, Fig. 5 and Fig. 6). This phenotype observation was repeated at least 3 times and the results of two of the experiments are shown here.

Table 4A. Earlier bolting time of NG OE lines (Trial 1)

	WT	NG6	NG21	NG24	NG28	NG32
Average bolting time (Day)	24.4	21.2	20.1	21.4	20.8	19.8
SD	1.4	1.0	1.4	0.9	1.0	1.3
N	12	12	12	9	9	9

Table 4B. Earlier bolting time of NG OE lines (Trial 2)

	WT	NG6	NG21	NG24	NG28	NG32
Average bolting time (Day)	24.3	19.2	19	19	18.3	19
SD	0.8	0.8	1.1	1	1.0	0.9
N	12	6	6	9	6	6

At maturity (Long Day), the number of inflorescence and the total weight of seeds harvested from each line were recorded. The results of two separate experimental trials are shown in Tables 5A and B. The results show that the overexpression of each of the five NG

genes (NG6, NG21, NG24, NG28, and NG32) resulted in increased number of inflorescences and seed yield. Compared to that of the wild-type, the seed yield of each NG over-expression line increased 30-50% (Table 5).

Table 5A.OE lines produced more seeds (Trial 1).

Lines	Weight of seeds (mg)/plant	SD
WT(Col-0)	80.4	4.9
NG6	113.6	12.2
NG21	127.8	26.9
NG24	99.6	17.3
NG28	130.6	26.7
NG32	135.9	23.5

The plants were grown in small black trays (N = 6 – 9).

Table 5B. OE lines produced more seeds (Trial 2).

Lines	Weight of seeds (mg)/plant	SD
WT(Col-0)	142.0	14.6
NG6	190.3	15.7
NG21	180.4	26.3
NG24	203.8	20.0
NG28	186.0	39.5
NG32	241.8	23.8

The plants were grown in large white cups (N = 6 – 9).

The results show that, when compared to the wild-type, Arabidopsis plants transformed with NG6, NG21, NG24, NG28 and/or NG32 have the following advantageous phenotypes: (1) faster growth rate; (2) higher seed yield.

6.5 SEQUENCE ALIGNMENT AND PHYLOGENETIC ANALYSIS

All the CDS of 5 NG genes in the Arabidopsis Col-0 ecotype were obtained from the TAIR website. Sequence alignment of each NG gene was retrieved by tblastn program from Plant GDB database and NCBI database using the amino acid sequence of each Arabidopsis NG gene as the bait sequences. Partial sequences recovered were aligned and compared to produce a full length coding sequence if feasible. Sequence alignment and phylogenetic tree were conducted using MEGA4 (Kumar *et al.*, 2004) and *ClustalW* program. Amino acid sequence comparisons were performed using CLC Sequence Viewer 5.1.1.

Those skilled in the art will recognize, or be able to ascertain many equivalents to the specific embodiments of the invention described herein using no more than routine experimentation. Such equivalents are intended to be encompassed by the following claims.

Citation or discussion of a reference herein shall not be construed as an admission that such is prior art to the present invention.

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United States Patent Application Publication No. 2010/0159065

SEQUENCE LISTING IN ELECTRONIC FORM

In accordance with Section 111(1) of the Patent Rules, this description contains a sequence listing in electronic form in ASCII text format (file: 73140-38 Seq 15-OCT-13 v1.txt).

A copy of the sequence listing in electronic form is available from the Canadian Intellectual Property Office.

CLAIMS:

1. A method to make a transgenic plant having increased rate of plant growth and/or increased plant yields when compared to a wild-type plant of the same species cultivated under the same conditions, said method comprising:

a) transforming at least one nucleic acid molecule encoding a kinase into a plant or plant cell, wherein the at least one nucleic acid molecule comprises:

i) a sequence having at least 95% identity over the full length of SEQ ID NO: 43; or

ii) a sequence encoding a polypeptide having at least 95% identity over the full length of SEQ ID NO: 44;

wherein the at least one nucleic acid molecule is operatively linked to upstream and downstream regulatory components comprising a heterologous promoter; and

b) over-expressing the nucleic acid molecule in the plant or plant cell, wherein the promoter drives the overexpression of the nucleic acid molecule.

2. The method of claim 1, wherein the at least one nucleic acid molecule comprises:

i) the sequence of SEQ ID NO: 43; or

ii) a sequence encoding the polypeptide of SEQ ID NO: 44.

3. The method of claim 1 or 2, further comprising regenerating, from said transformed plant cell, a plant having increased growth and/or yield.

4. The method of claim 1 or 2, wherein plant growth rate and plant yield are increased.

5. The method of any one of claims 1 to 4, wherein the plant is of a genus selected from the group consisting of: Asparagus, Bromus, Hemerocalli, Hordeum, Loliu, Panicum,

Pennisetum, Saccharum, Sorghum, Trigonell, Triticum, Zea, Antirrhinum, Arabidopsis, Arachis, Atropa, Brassica, Browallia, Capsicum, Carthamus, Cichorium, Citrus, Chrysanthemum, Cucumis, Datura, Daucus, Digitalis, Fragaria, Geranium, Glycine, Helianthus, Hyscyamus, Ipomoea, Latuca, Linum, Lotus, Lycopersicon, Majorana, Malva, Gossypium, Manihot, Medicago, Nemesia, Nicotiana, Onobrychis, Pelargonium, Petunia, Ranunculus, Raphanus, Salpiglossis, Senecio, Sinapis, Solanum, Trifolium, Vigna, and Vitis.

6. The method of any one of claims 1 to 4, wherein the plant is a species of Brassica.

7. The method of any one of claims 1 to 4, wherein the plant is selected from the group consisting of soybean, maize, potato, rice, sugar cane, switchgrass, cotton, sorghum, alfalfa, rapeseed, and canola.

8. The method of any one of claims 1 to 7, wherein the plant cell is a seed, stem, shoot, or root cell.

9. The method of any one of claims 1 to 8, wherein total number of inflorescence and seed yield per plant are increased, and bolting time is earlier when compared to a wild-type plant of the same species cultivated under the same conditions.

10. A transgenic plant cell, comprising at least one nucleic acid molecule selected from the group consisting of:

i) the nucleic acid molecule of SEQ ID NO: 43, wherein the nucleic acid molecule is operatively linked to upstream and downstream regulatory components comprising a heterologous promoter, and wherein said nucleic acid molecule is over-expressed in the transgenic plant cell when compared to plant cells of the same type in a wild-type plant of the same species and wherein the promoter drives the overexpression of the nucleic acid molecule; and

ii) a nucleic acid molecule that encodes the protein of SEQ ID NO: 44, wherein the nucleic acid molecule is operatively linked to upstream and downstream regulatory components comprising a heterologous promoter, and wherein said nucleic acid molecule is

over-expressed in the transgenic plant cell when compared to plant cells of the same type in a wild-type plant of the same species and wherein the promoter drives the overexpression of the nucleic acid molecule.

11. The transgenic plant cell of claim 10, wherein said plant cell is of a monocotyledonous species or a dicotyledonous species.

12. The transgenic plant cell of claim 10, wherein the plant cell is of a genus selected from the group consisting of: Asparagus, Bromus, Hemerocalli, Hordeum, Loliu, Panicum, Pennisetum, Saccharum, Sorghum, Trigonell, Triticum, Zea, Antirrhinum, Arabidopsis, Arachis, Atropa, Brassica, Browallia, Capsicum, Carthamus, Cichorium, Citrus, Chrysanthemum, Cucumis, Datura, Daucus, Digitalis, Fragaria, Geranium, Glycine, Helianthus, Hyscyamus, Ipomoea, Latuca, Linum, Lotus, Lycopersicon, Majorana, Malva, Gossypium, Manihot, Medicago, Nemesia, Nicotiana, Onobrychis, Pelargonium, Petunia, Ranunculus, Raphanus, Salpiglossis, Senecio, Sinapis, Solanum, Trifolium, Vigna, and Vitis.

13. The transgenic plant cell of claim 10, wherein the plant cell is a Brassica cell.

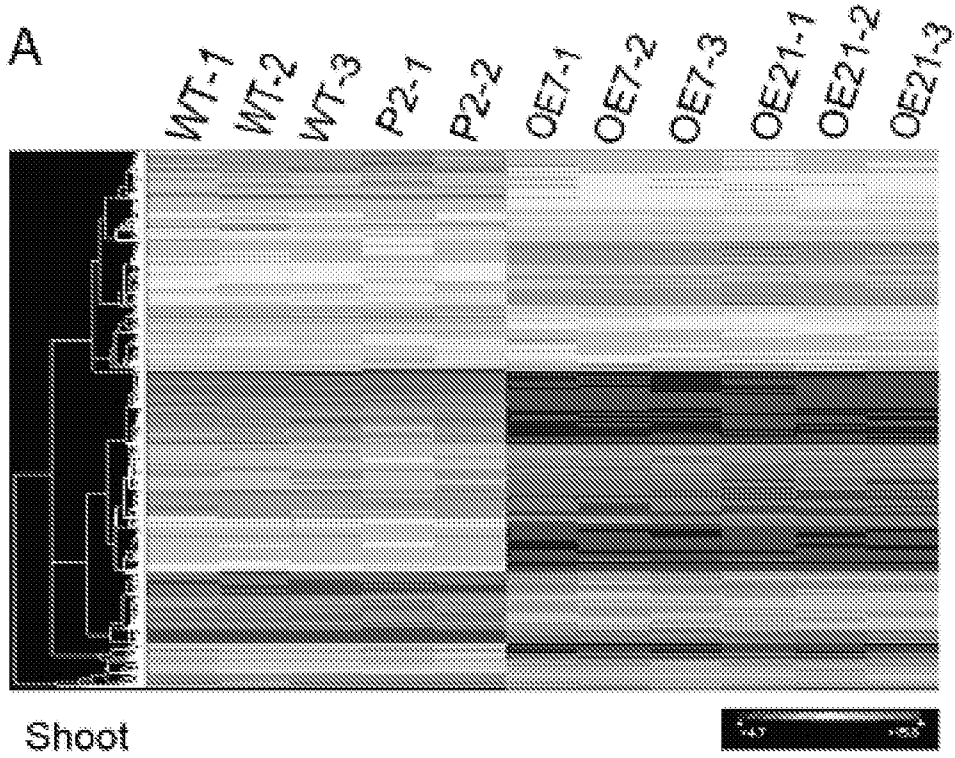


FIG. 1

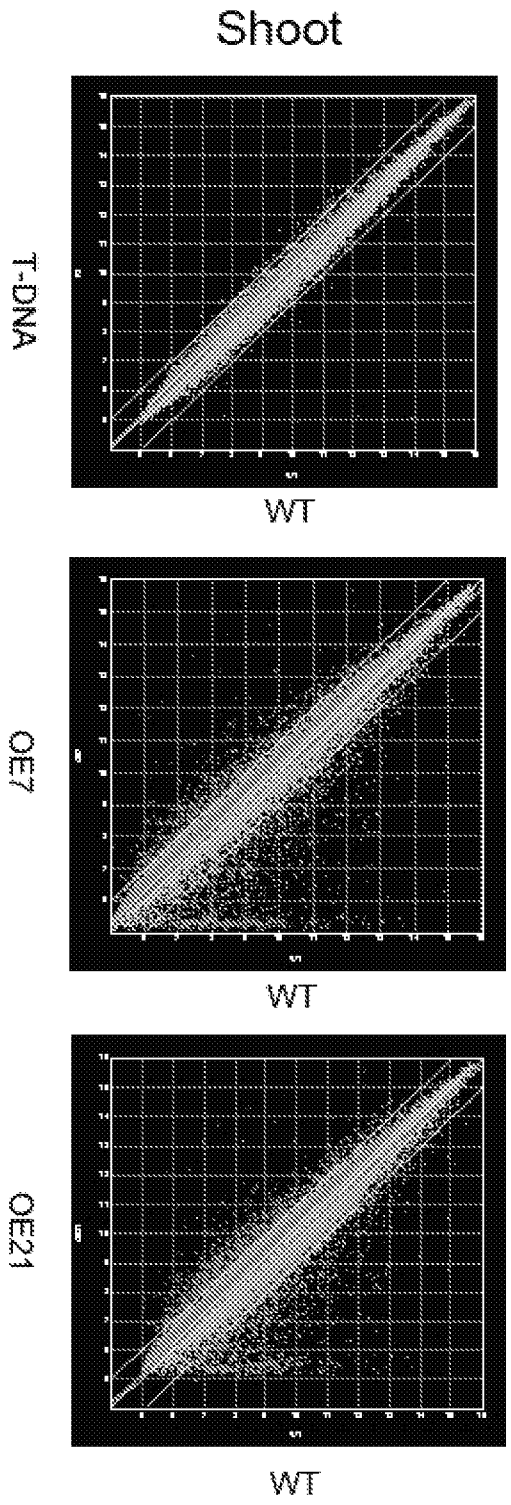
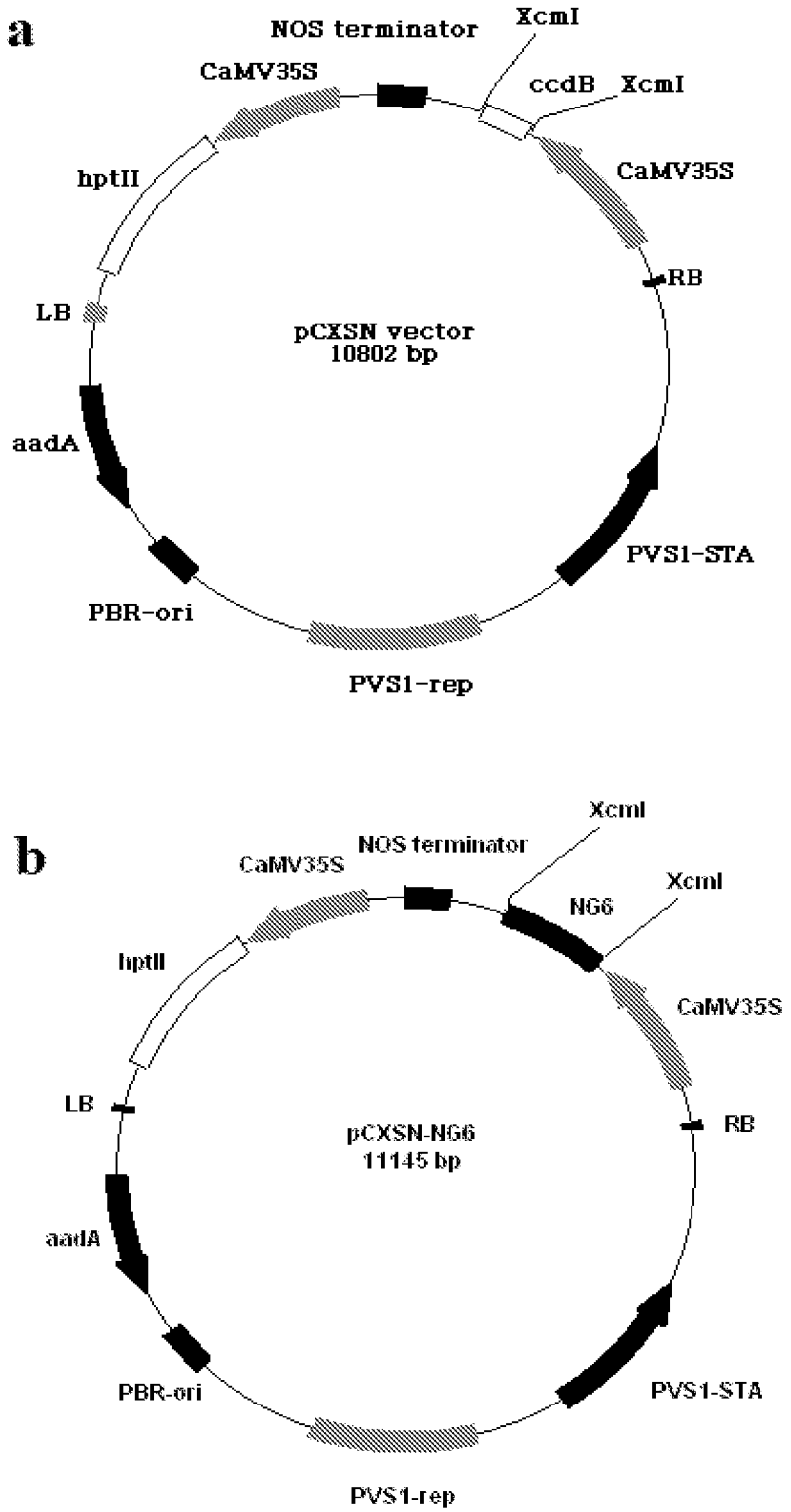


FIG. 2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PBR-ori
PVS1-rep
b
XcmI
NOS terminator
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FIG. 3

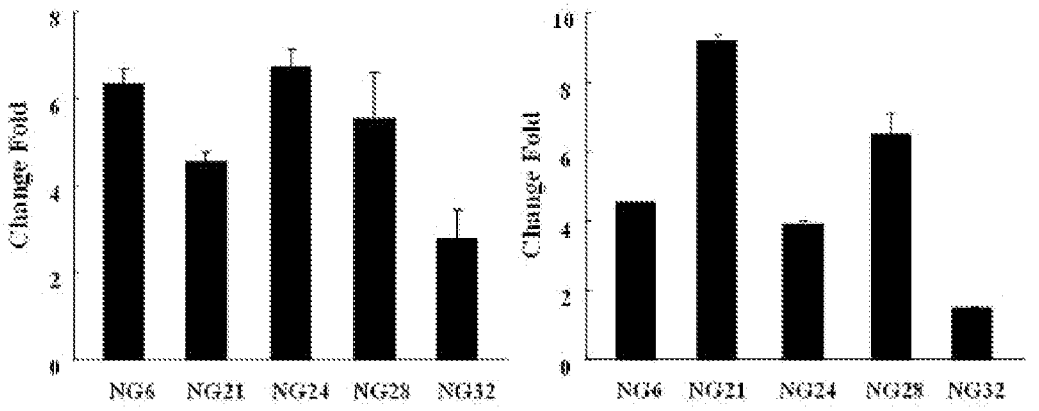

Fig4A. qRT-PCR of NG gene OE lines (Trial 1) Fig4B. qRT-PCR of NG gene OE lines (Trial 2)

FIG. 4

FIG. 5

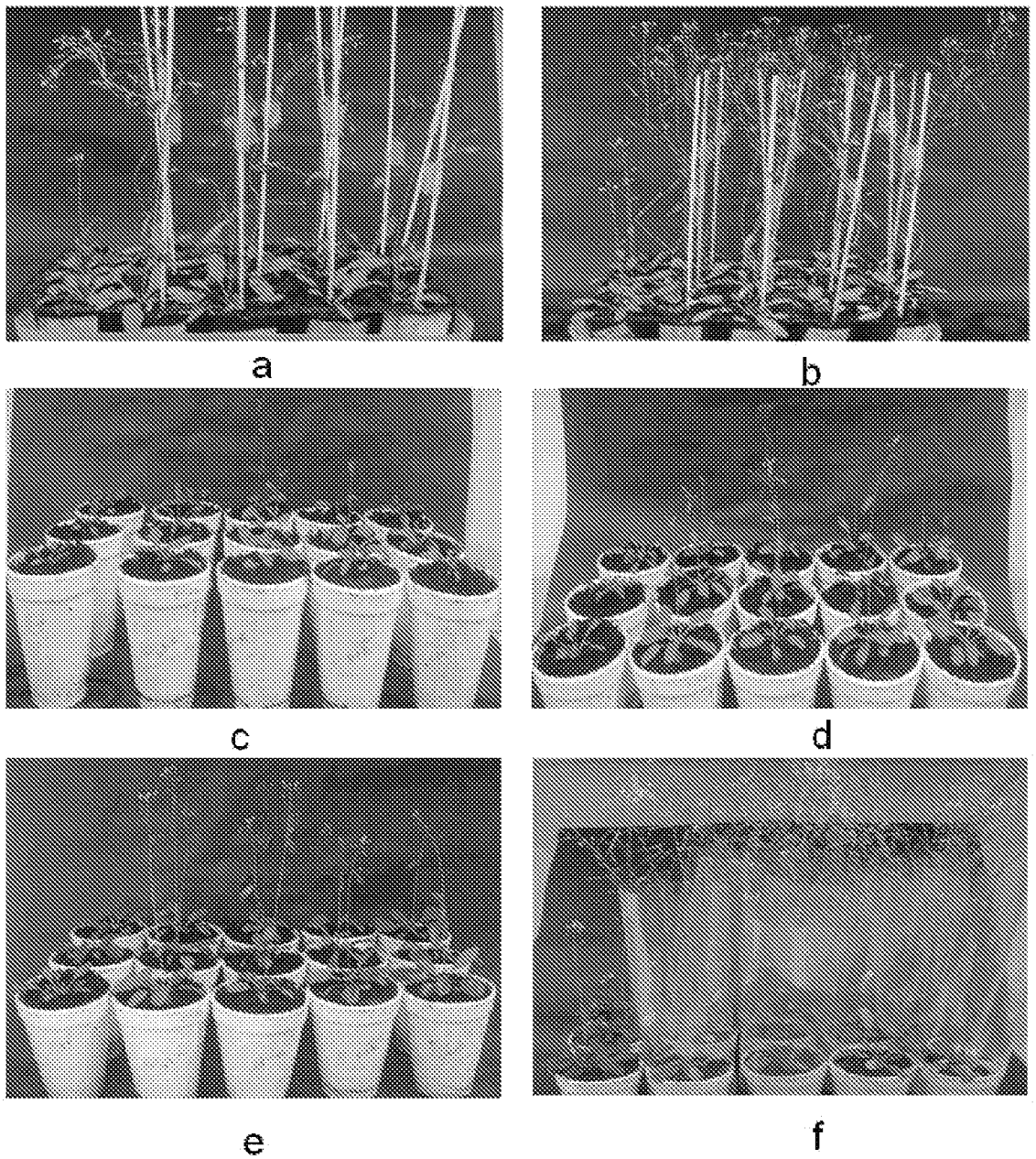

FIG. 6