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(54) METHOD FOR SPEEDING UP PLANT GROWTH AND IMPROVING YIELD BY INTRODUCING PHOSPHATASES IN TRANSGENIC PLANT

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(57) ABSTRACT

Transgenic plants having increased growth rate, increased sugar content, and increase yield are disclosed, and methods for making the same. The transgenic plants have a gene coding for a phosphatase having a C-terminal motif under control of a heterologous promoter incorporated into the genomic DNA of the plant.

FIG. 1

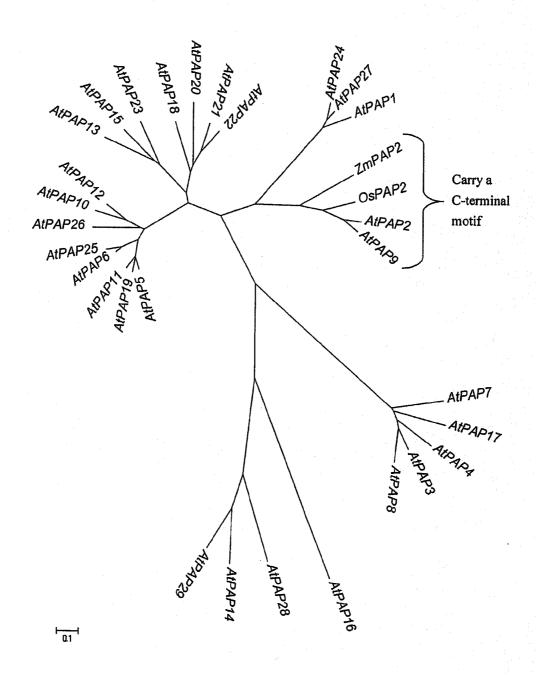


FIG. 2A

Protein Sequences (A) Transmembrane	e motif (Aligned)
AtPAP2_gi 15222978 ref NP_172843	A A LMVV VLLEFIIEF
AtPAP9_g1 20257481 gb AAM15910	L XI BA VMVV VIFEEFV FL
Brassica_rapa_subsppekinensis_clone	
Hordeum_vulgare_PUT-161a-Hordeum_vulg	. INL I BO VMFALML FAL FL
Medicago_AC202582_HTG_Medicago_trunca	
OSPAP2_NM_001065273	EFELDIVE VMFALVL FELDEL
Poplar_trichocarpa_ref[NC 008476.1]	ANVER VLVL AFVENEL MA
Saccharum_officinarum_28138_PlantGDB	LYLICEVLFALLLEFEFFL
Solanum tuberosum PUT-157a-Solanum tu	. SOYV V VLML AFMEVIV FL
Vitis_viniferaAM458569_modified	ANTILVI AFMENVIEV
Zea mays EU975503	LYLIGOVMFALLLGFGFGIL
<u></u>	
Physicomitrella_patens_subsppatens_g Protein Sequences (B) 614th-636-a.a. (
Physicomitrella_patens_subsppatens_g Protein Sequences (B) 614th-636-a.a. (Unaligned)
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Physcomitrella patens_subsppatens_g	LELEIGHEC) LEEL CONTROL CONTR

FIG. 2B

AtDA D2	MIRNES-E-F	LIESTOVEVO		WARRING WAS AN	O VIIV 1 201110 ALC M	
BnPAP2 GmPAP2 ZmPAP2	MINOFE - E - F MINOFE - LEF MYRENEHLEF	LLELXXXXXXX ULGEUSVFUS LESLEUUFEH LLELXXXXXXX	SANNKATLE		SXYIQWSOVD SIEIKWSNVD BYNERWSONE	SPSDLDWLGI 57 SPSDLDGLAI 59
AIPAP 15 Consensus	MIESSS - 9 - F MXXXXX - XXF	LLLLUEGFUS LLXXXXXFXX		GE-HABEES	XXXXXM2XX EREESBEES GUK I UMS GEE	
AtPAP2 BnPAP2	YSPPESPNDH YSPPESPHDH	FIGYEFLNES	ETWEDGE CEN	SLPLT-NLRS	NYTER I FRWS	ESELÖPKHKD 117 GSEL <u>n</u> pkhkd 116
GmPAP2 ZmPAP2	YSPPESTORD	FIGYUFLEDS FUGYUFLNUS	ATWRINGS CNU ASWREGS CEU	SLPLUSTLRE	NYEFRIFEWT EYOFRUFRWE	
ALPAP15 Consensus	HSEPSELDOR YSPPXSXXXX	FXGYXFLXXS	XXWXXGXGXX		登録問題問題問題 X Y X F R X F X W X	NXEIXXXXXD
BnPAP2	HDONPLPGTK HDONPLPGTK	HLLAESEQVE	FGS - GYGMPE FGS AGYGRPE	QIHLMF近M·· QIHLAF頁D··	- MV NEMR VMF - KVNRMR V世 F	VAGDGE 169 VAGDGE 169
GmPAP2 ZmPAP2	HOHNPLPYTE	HRYANSADVE	YGDEN - RPE		· EV DEMR VME	WARDER 172
AtPAP15 Consensus	HDXNPLPXXX	HXXAXSXXXX	FEE · B · · 其PE FXXXXVGXPE	OIELEHES DH	- 区区区医III Y EW	Mightore 92
AtPAP2 BnPAP2	E	REVRYGEEK -	- DELENSAAA - DELENSAAA	RÖMRYEREHM RÖMRYEREHM	CDEPANSTIIG C <u>N</u> APANSTVC	WRDPGWIFDT 218 WRDPGWIFHT 218
GmPAP2 ZmPAP2		MANNYGERE -	- DKLDGUAXA	RYERYEREHM DYSTYEORHM	CDAPANTEVG CDWPANSEVA	WROPGENED 219
Consensus	ARERDETZUM	ZXVQECTURH XXVRYCXXXX	-DXLXXXAXX	XXXXXEXXHW HELLYAROUND	ED透信題N致工程語	WRDPGX I XXX
AIPAP2 BnPAP2	VMKNLNGGVR VMKNLNGGVR	AAABAGSDSK	- CWSEIHSKI	ERDYNSEET!	A FMFGDM A FMFGDM	GEARPYNTFI 274
GmPAP2 ZmPAP2	NKGLE PGES	A A A K A C K D N C	- GWSEIMSFI	SRNEDSDET!	A FUFGDM A FUFGDM	GERNPYNTEL 277
AlPAP 15 Consensus	RINGLEPETT	YYYKGGDESK YYYXVGXDXX	-GWSX1XSFX	TWEVESEES Y	A FXFGDM	GERETANTEE 201
AtPAP2 BnPAP2	RTODES ISTV RTODES ISTV	KWILRDIEAL KWILRDIEAL	GDKPAMISHI GDKPAMSHI	GDISYAR		311
GmPAP2 ZmPAP2 AlPAP15	RTQDES I STM RTQSESESTV	KWILRDYEAL	GDKPAEVSH!	GD ISYAR GD ISYAR		******* 312
Consensus	RECEDENT SHU	KWILROXEAL	GDKPAXXSHI	GDUSYANUNU GDISYAR	INGIESDENE	CLESEMENTE 249
AIPAP2 BnPAP2	GYSWVWDEFF GYSWVWDEFF	AQUEPIASTV	PYHVC I GNHE PYHVC I GNHE	YDESTOPWKP YDEPTQPWKP	DWARSHYGND DWGT - YCHD	GGGECGVPYS 371
GmPAP2 ZmPAP2 AlPAP15	CYSWLWDHFF CYSWVWHFF LYBERWDWWG	AQIEPVASQV EQIEPIAANT	AYHVC I GNHE PYHVC I GNHE	YDWP EQPWKP	WWAT - YOND	GGGECGNPYS 372
Consensus	GYSWXWDXFF	XOXEBXV8XA KEMENDOSKA	SAHAC I GNHE SEMATECHHE	ADXXXOBMK b	XMXX SEES	GOOF COX DAS 503
AIPAP2 BnPAP2 GmPAP2	LKFNMPGNSS	ESTG-MKAPP EETG-MKAPP	TRNLYYSYDM TRNLYYSYDM	GEVHFUYIST GEVHFEYIST	ETNFUKCOSO ETNFUKCORO	YEFEKRDLES 430 YEFEKRDLES 428
ZMPAP2 AlPAP15	LRFNMPGNSS ZRFEMPGNSE ERFEEPENES	ELTGNAAPP	TRNLYYSFDM	CMVHFVYIST CMVHFVYMST	ETNFYEGE KO	HNFLKHOLES 432
Consensus	XXFXMPGNSS	SEEK EEEE	日間近LYYSFNA TRNLYYSXDX	GENHFVMEGA GXVHFVYXST	注測器MDK変数EQ ETNFXXCXXO	YEWLKKOLEK YXFXKXOLEX
AtPAP2 BnPAP2 GmPAP2	VORKKTPFVV VNREKTPFVV VNRSKTPFVV	VQGHRPMYTT VQGHRPMYTT	-SNEVROTMT	ROKMVEHLEP ROKMVEHLEP	LEVENNVTLA	LWGHVHRYER 489 LWGHVHRYER 487
ZmPAP2 AlPAP15	VNRSETPFVV VDRSETPFVV	EOGHRPMYTT EOGHRPMYTS ESWHEPWYES	-SHENROARD	ROCKMUEHLEP	LLV和ENVTLA	LWGHVHRYER 491 LWGHVHRYER 488
Consensus	VXRXXTPFVV	XQGHRPMYTX	*SXEXRDAXX	XXXMXEXFE EEGWREVWEE	LXXXXNVTLA	ENGHVHRYER 402
AlPAP2 OnPAP2 OmPAP2	FCPISNNT	-cekom	RGSPVHL	VIGMEGODWQ VIGMEGODWQ	PEWO	PRPNHPDE 531 PRPNHPEE 529
ZmPAP2 AlPAP15	ENEXXNXEDD ECHEMNED	-cante ess	EGZECH LAHF GDKKGKJAHD	VIGMAGODWO	PWG	PRPDHPDD 540
Consensus	FCFXXNXX	-cgxxx ·xxx	XXXXGXPVHX	A I CWX CODMO	P XWX	KEPEPUTED 449
BnPAP2	PIF PIF	*********	POPEOSMYRM POPEOSMYRM	GEFGYTRLVA GEFGYTRLVA	NKEKLTY-SE	VGNHDG EV 571 VGNHDG EV 569
ZmPAP2	PIF		POPENSTYRG POPENSMYRG	GEFGYTRLVA GEFGYARLVA	TORKITI - HW	VCNHDC. EV 580 VGNHDG. QV 575 MENQDESEV 509
Consensus	P P		POPXXSXYRX	GEFGYXRLVA	XXEKTXX-XX	ACHHOC EA
BnPAP2	HDEVELLASG HDGELLASG	MV 13 GEKEST	RUENCKEALX	SATLEGER	ESDYLWYVKG	AGLMVMGVLL 629
ZmPAP2	HDMVE ESSC EDQUX: VROE	MASSEMERNY		DREGUCES DA	KKNEEDAREN	GEVLUNCKEN 633
Consensus	HDXXEIXXSG	XXXXXXXXX	· · · · XXXXXX	XXXXXGXXXV	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	XXXXXXXXXXXX
BRIPAP2	GFUMGFFTRG	KK-ESSEONR KKOESSEONR RKKSEVEESU	WHPVKNFFTM	653		
ZmPAP2 AtPAP15	CFSECULVER PROBLEMENT	ER-BOSS-SO KKENDES-SO KKENDES-SO	MEGAKNEERS	655 533		
Consensus	GXXXGXXXXX	xxxxxxxxx	MX X A K X E E X X			

FIG. 3

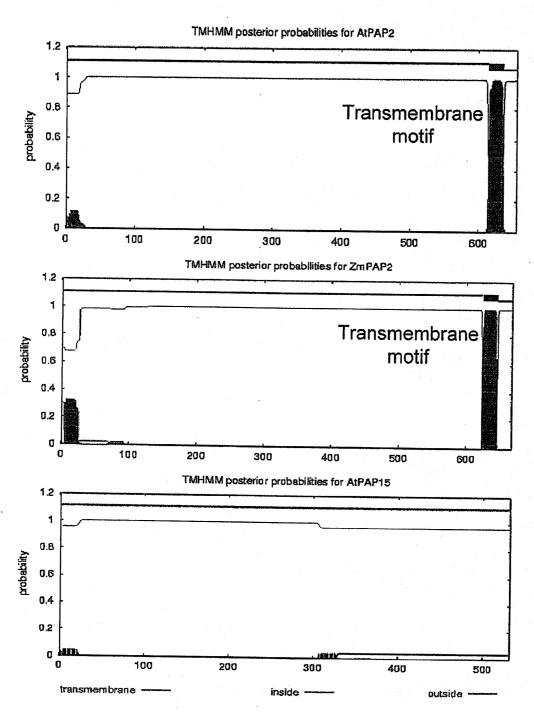
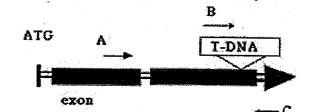


FIG. 4



atpap2-8 structure

(a) Genomic PCR

WTatpap2-8



(b) RT-PCR

WT atpap2-8 AtPAP2 EF

(c) Western blotting

atpap2-8

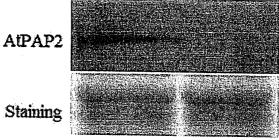


FIG. 5

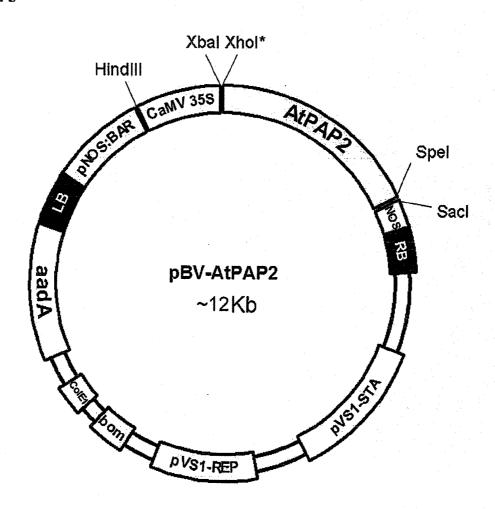
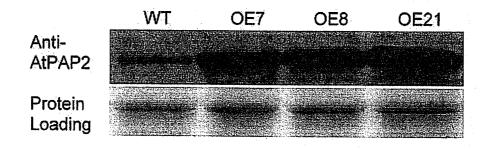
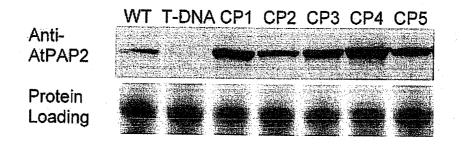


FIG. 6





B

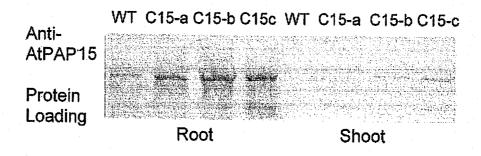
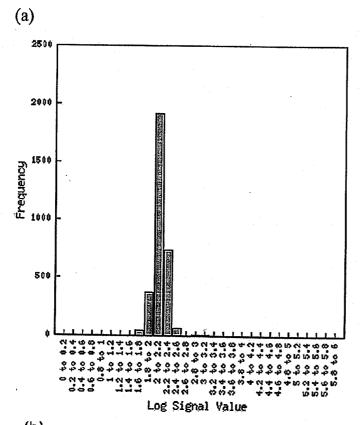
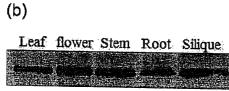
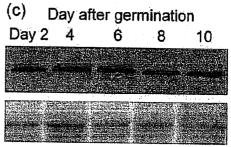


FIG. 7







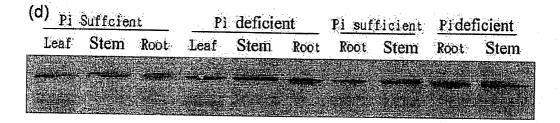


FIG. 8

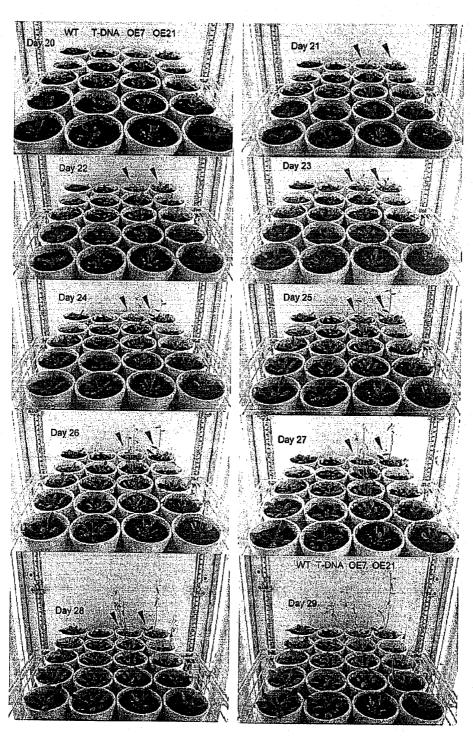
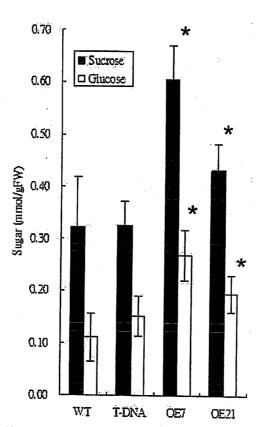


FIG. 9



*Statistically (p<0.001) different from the WT (n = 10).

FIG. 10

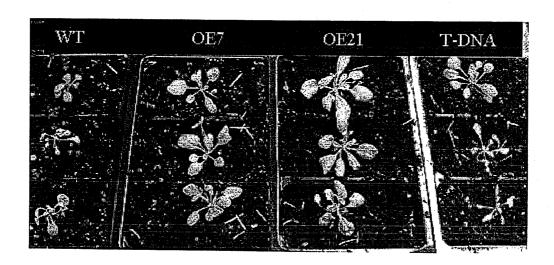


FIG. 11

Cell Nucleus Mito Soluble Membrane Chlorop.

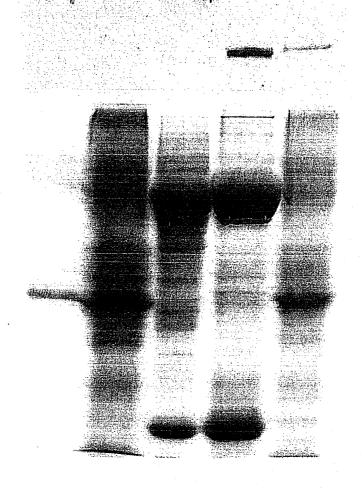


FIG. 12

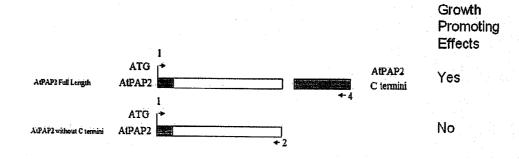
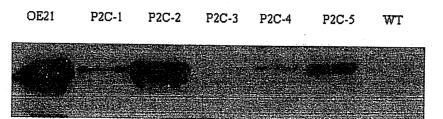


FIG. 13



METHOD FOR SPEEDING UP PLANT GROWTH AND IMPROVING YIELD BY INTRODUCING PHOSPHATASES IN TRANSGENIC PLANT

RELATED APPLICATIONS

[0001] This application claims priority to provisional application Ser. No. 61/138,918, filed on Dec. 18, 2008, which is incorporated herein by reference.

1. TECHNICAL FIELD

[0002] The present disclosure provides methods that speeds up plant growth and elevates plant yields by introducing phosphatases with a C-terminal motif into plants. The present disclosure relates to phosphatases with a C-terminal motif, and their respectively encoded protein products, as well as fragments, derivatives, homologues, and variants thereof. Methods for introducing these genes into plants to (1) speed up the growth rate of plants, (2) to increase the sugar contents of plants, and (3) to increase of yield of plants, are provided.

2. BACKGROUND

[0003] 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 (Fe³⁺-Me²⁺) in the active site (Li et al., 2002), where Me is a transition metal and Me²⁺ is mostly found to be Fe²⁺ in mammalian, and Zn²⁺, or Mn²⁺ in plants (Klabunde and Krebs, 1997; Schenk et al., 1999).

[0004] Purple acid phosphatases are distinguished from the other phosphatases by their characteristic purple color, which is caused by a charge transfer transition at 560 nm from a metal-coordinating tyrosine to the metal ligand Fe³⁺ (Klabunde and Krebs, 1997; Schenk et al., 2000). Different from the other acid phosphatases, PAPs are insensitive to inhibition by tartrate, so they are also known as tartrate-resistant acid phosphatases (TRAPs).

[0005] The biochemical properties of some plant PAPs have been characterized, firstly in red kidney bean, and later in soybean suspension cell, soybean seedlings, rice culture cells, spinach leaves, sweet potato tubers, tomato, yellow lupin seeds, medicago and Arabidopsis, etc. (Schenk et al., 1999). Plant PAPs are generally considered to mediate phosphorus acquisition and redistribution based on their ability to hydrolyze phosphate compounds (Cashikar et al., 1997; Bozzo et al., 2004; Lung et al., 2008). Regulation of some plant PAPs transcripts by external phosphate level in medium or soil, strongly suggest their involving in phosphate acquisition. For example, the transcription level of Medicago MtPAP1 in roots was increased under P stress, implicating a role in P acquisition or internal mobilization (Xiao et al., 2005; Xiao et al., 2006). Some plant PAPs could be secreted from root cells to extracellular environment, then hydrolyze various phosphate esters. Lung et al. purified a secreted PAP phosphatase from tobacco, which could hydrolyze broad substrates and help to alleviate P starvation (Lung et al., 2008). Certain plant PAPs can also hydrolyze phytate, a major storage compound of phosphorus in plants. Hegeman and Grabeu (2001) purified a novel PAPs (GmPhy) from the cotyledon of the germinating soybean seedlings. GmPhy was introduced into soybean tissue culture and was assayed to show phosphatase activity. Most recently, AtPAP15 and 23 in *Arabidopsis* sharing high sequence homology (73-52%) with this soybean PAP, were found to exhibit phytase activity (Zhu et al., 2005; Zhang et al., 2008).

[0006] Besides involvement in P acquisition, plant PAPs may perform some other physiological roles. For example, the PAPs AtACP5 (AtPAP17), SAP 1, and SAP2 (del Pozo et al., 1999; Bozzo et al., 2002) display not only phosphatase but also peroxidase activity, suggesting their involvement in the removal of reactive oxygen compounds in plant organs. A pollen-specific PAP from Ester lily was suggested to function as an iron carrier in mature pollen (Kim and Gynheung, 1996). Other studies indicate that plant PAPs may also be involved in NaCl stress adaption or cell regeneration (Kaida, 2003; Liao et al., 2003).

[0007] In the *Arabidopsis* genome, twenty-nine potential PAP genes were identified based on sequence comparison. Twenty-four of these putative enzymes contain seven conserved amino-acids residues involved in metal binding. One (AtPAP13) lacked four of these seven residues, and the other four (AtPAP14, 16, 28 and 29) lacked either the first, the second, or both motifs of the five conserved motifs. Twenty-eight are actively transcribed in *Arabidopsis* (Zhu et al., 2005).

[0008] To date, relatively little is known about AtPAPs biochemical properties and physiological roles, though several members have been characterized (del Pozo et al., 1999). AtPAP17 (AtACP5) was first known to be induced by phosphorus starvation. The transcription of AtPAP17 was also responsive to ABA, salt stress (NaCl), oxidative stress ($\rm H_2O_2$) and leaves senescence, according to GUS activity assay. No alteration in the expression of AtPAP17 was observed during the nitrogen or potassium starvation, and paraquat or salicylic acid. Like the other type 5 acid phosphatases, AtPAP17 displayed peroxidation activity, which may be involved in the metabolism of reactive oxygen species in stressed or senescent parts of plants.

[0009] Besides AtPAP17, several AtPAPs were found to be involved in phosphorus metabolism in *Arabidopsis*. Root secretion of AtPAP12 was induced by P stress, and its regulation was mainly at transcriptional level (Patel et al., 1998; Coello, 2002/11). AtPAP4, as well as AtPAP10, AtPAP11 and AtPAP12 were involved in phosphorus starvation response since their transcription levels increased during phosphate deprivation (Li et al., 2002; Wu et al., 2003). In contrast, AtPAP20, 21 and 22 were irrespective to P starvation and expressed constitutively in Pi sufficient or deficient condition. Fluorescent signals were detected in the cytoplasm via the baculovirus expression system, indicating that they may function in the cytoplasm (Li and Wang, 2003).

[0010] AtPAP26 was purified and characterized from Pistarved *Arabidopsis* suspension cell culture (Veljanovski et al., 2006). It exists as a homodimer with 55 kDa glycosylated protein, showing wide substrate specificity with the highest activity against phosphoenolpyruvate (PEP) and polypeptide phosphate. AtPAP26 also displayed alkaline peroxidase activity with the probable roles in the metabolism of reactive oxygen species. Proteomic study suggested that it may be localized in vacuole, and involved in recycling Pi from intracellular P metabolites (Shimaoka et al., 2004).

[0011] PAPs can act on a wide range of substrates, but not all of them exhibit phytase activity. An enzyme assay involving the GST-AtPAP23 fusion protein revealed that AtPAP23 exhibits phytase activity. A GUS study showed that AtPAP23 is exclusively expressed in the flower of the *Arabidopsis*, and may play certain roles in flower development (Zhu et al., 2005). In a recent report, a recombinant AtPAP15 expressed and partial purified in *E. coli* and yeast was also found to exhibit phytase activity) (Zhang et al., 2008). It was proposed that AtPAP15 may be involved in ascorbic acid biosynthesis with the end product myo-inositol of phytate hydrolysis as the precursor of ascorbic acid synthesis.

[0012] As stated above, most of the functions of characterized plant PAPs are related to phosphorus metabolism. None of the functionally or biochemically characterized plant PAPs carry transmembrane motif, and none of them were shown to be associated with membrane. Furthermore, to date, no AtPAPs or any plant PAPs, have been showed to affect sugar signalling and carbon metabolism in plant.

[0013] The first report of transgenic expression of plant PAP in plant was reported in 2005 (Xiao et al., 2005). The PAP-phosphatase gene from *Medicago* (MtPHY1) was expressed in transgenic *Arabidopsis*, resulting in increased capacity of P acquisition from phytate in agar culture (Xiao et al., 2005). Nonetheless, the growth performance of the plants was not reported to be different under normal growth.

3. SUMMARY

[0014] The present disclosure provides a method that speeds up plant growth and elevates plant yields by introducing phosphatases with a C-terminal motif into plants, Phosphatases with a C-terminal motif, and their respectively encoded protein products, as well as fragments, derivatives, homologues, and variants thereof are disclosed. Methods for introducing this class of genes into plants to speed up the growth rate of plants, to increase the sugar contents of plants, and to increase of yield of plants, are provided. Without wishing to be bound by any particular theory, the C-terminal motif is believed to function as a transmembrane structural element (transmembrane motif).

[0015] As stated above in the Background section, most of the functions of characterized plant PAPs are related to phosphorus metabolism. None of the functionally or biochemically characterized plant PAPs carry transmembrane motif, and none of them were shown to be associated with membrane. Furthermore, to date, no AtPAPs or any plant PAPs, have been showed to affect sugar signalling and carbon metabolism in plant.

[0016] The first report of transgenic expression of plant PAP in plant was reported in 2005 (Xiao et al., 2005). The PAP-phosphatase gene from *Medicago* (MtPHY1) was expressed in transgenic *Arabidopsis*, resulting in increased capacity of P acquisition from phytate in agar culture. Nonetheless, the growth performance of the plants was not reported to be different under normal growth.

[0017] We also produced transgenic tobacco and *Arabidopsis* that overexpressed AtPAP15, a PAP with phosphatase activity, which does not carry any C-terminal motif equivalent to that of AtPAP2; phosphatase activity was secreted into extracellular growth medium. Significant secretion of phosphatase activity was observed in the transgenic plants and the transgenic plants showed larger biomass than the control plants in agar and soil supplemented with exogenous phytate. Higher P content was also obtained in overexpressed trans-

genic lines in phytate treatment. However, the growth of transgenic plants overexpressing AtPAP15 did not show any difference in growth phenotypes when it was compared with the wild-type, under treatments of K—P or No—P, or in soil. [0018] Here, we have developed a technology to speed up plant growth and improve seed yield by overexpressing a phosphatase with a C-terminal motif in plants. An example is the use of a purple acid phosphatase (PAP). This disclosure is the first report to show that overexpressing a phosphatase with a C-terminal motif in transgenic plant is able to speed up the growth of the plants, to increase the sugar contents of plants, and to increase the yield of plants, by altering the carbon metabolism of the plants.

[0019] The present advances are based, in part, on the characterization of a group of purple acid phosphatases (SEQ ID NOS: 1-8 and 18-47) from plants and the observations that overexpression of a purple acid phosphatase (AtPAP2, SEQ ID NO:1) of this group in plants resulted in rapid plant growth, higher sugar content, and higher yield. Accordingly, nucleotide sequences of a group of purple acid phosphatase genes (SEQ ID NOs:1, 3, 5, 7, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44 and 46), which share a C-terminal motif/domain, from plants and amino acid sequences of their encoded proteins (SEQ ID NOS:2, 4, 6, 8, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45 and 47), as well as fragments, derivatives, homologues, and variants thereof, as defined herein, are disclosed. Furthermore, nucleic acid molecules encoding the polypeptides of interest, and include cDNA, genomic DNA, and RNA, are disclosed.

[0020] As used herein, italicizing the name of a gene shall indicate the gene, in contrast to its encoded protein or polypeptide product which is indicated by the name of the gene in the absence of any italicizing. For example, "Gene" shall mean the Gene gene, whereas "Gene" shall indicate the protein or polypeptide product of the Gene gene.

[0021] In one embodiment, isolated nucleic acid molecules hybridize under stringent conditions, as defined herein, to nucleic acids having the sequence of SEQ ID NOS: 1, 3, 5, 7, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44 or 46, or homologues thereof, wherein the nucleic acid molecules encode proteins or polypeptides which exhibit at least one structural and/or functional feature of the polypeptides of the invention.

[0022] Another embodiment includes, nucleic acid molecules, which are suitable for use as primers or hybridization probes for the detection of nucleic acids encoding one of the disclosed phosphatase polypeptides or other sequences.

[0023] Yet another embodiment includes vectors, e.g., recombinant expression vectors, comprising a nucleic acid molecule of the invention. Furthermore, host cells containing such a vector or engineered to contain and/or express a nucleic acid molecule of the invention and host cells containing a nucleotide sequence of the invention operably linked to a heterologous promoter are disclosed.

[0024] A further embodiment includes methods for preparing a polypeptide of the invention by a recombinant DNA technology in which the host cells containing a recombinant expression vector encoding a polypeptide of the invention or a nucleotide sequence encoding a polypeptide of the invention operably linked to a heterologous promoter, are cultured, and the polypeptide of the invention are produced.

[0025] In still further another embodiment, a transgenic plant contains a nucleic acid molecule which encodes an isolated polypeptides or proteins comprising the five con-

served motifs of purple acid phosphatases, including XDXX, XDXXY, GNH(D/E), XXXH, XHXH, and linked to a C-terminal motif.

[0026] Embodiments further provide antibodies that immunospecifically bind a polypeptide of the invention. 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 either a VL or VH domain or even a complementary determining region (CDR), that immunospecifically binds to a polypeptide of the invention.

[0027] In an additional embodiment, method for detecting the presence, activity or expression of a polypeptide of the invention or similar polypeptide in a biological material, such as cells, culture media, and so forth are provided. 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 of the invention. In a particular embodiment, such an agent is an antibody or a fragment thereof which immunospecifically binds to a one of the disclosed polypeptides.

[0028] 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.

[0029] We also produced transgenic tobacco and *Arabidopsis* that overexpressed AtPAP15, a PAP with phosphatase activity, which does not carry any C-terminal motif and was found to be secreted into extracellular growth medium. Significant secretion of phosphatase activity was observed in the transgenic plants and the transgenic plants showed larger biomass than the control plants in agar and soil supplemented with exogenous phytate. Higher P content was also obtained in overexpressed transgenic lines in phytate treatment. However, the growth of transgenic plants overexpressing AtPAP15 did not show any difference in growth phenotypes when it was compared with the wild-type, under treatments of K—P or No P, or in soil.

[0030] In conclusion, this disclosure is the first report to show that overexpressing a phosphatase with a C-terminal motif in transgenic plant is able to speed up the growth of the plants, to increase the sugar contents of plants, and to increase the yield of plants, by altering the carbon metabolism of the plants.

3.1 Definitions

[0031] The term "acidic" or "acid pH" as used herein refers to a pH value of less than about 6.0.

[0032] The term "homologue" as used herein refers to a polypeptide that possesses a similar or identical function to polypeptides encoded by SEQ ID NOS: 2, 4, 6, 8, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45 and 47, and/or a fragment of these polypeptides, that do not have an identical amino acid sequence of these polypeptides and/or a fragment of these polypeptides. A polypeptide that has a similar amino acid sequence included in the definition of the term "homologue" includes a polypeptide that satisfied at least one of the following: (i) polypeptide having an amino acid sequence that

is one or more of at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, and at least about 98% identical. (ii) a polypeptide encoded by a nucleotide sequence that is one or more of at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, and at least about 98% identical and/or conservatively substituted to one or more of the nucleotide sequences encoding the polypeptides of SEQ ID NOS: 2, 4, 6, 8, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45 and 47, and/or a fragment of the these polypeptides; (iii) a polypeptide encoded by a nucleotide sequence that hybridizes under stringent conditions as defined herein to one or more of nucleotide sequences SEQ ID NOS: 1, 3, 5, 7, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44 and 6; (iv) a polypeptide having an amino acid sequence that is one or more of at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90, and at least about 98% identical and/or conservatively substituted; (v) a nucleic acid sequence encoding an amino acid sequence that is one or more of at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, and at least about 98% identical and/or conservatively substituted; (vi) a fragment of any of the polypeptides or nucleic acid sequences described in (i) through (v) having one of at least 20 amino acid residues, at least 25 amino acid residues, at least 40 amino acid residues, at least 80 amino acid residues, at least 90 amino acid residues, at least 100 amino acid residues, at least 125 amino acid residues, at least 150 amino acid residues, at least 175 amino acid residues, at least 200 amino acid residues, at least 225 amino acid residues, at least 250 amino acid residues, at least 275 amino acid residues, at least 300 amino acid residues, at least 325 amino acid residues, at least 350 amino acid residues, or at least 375 amino acid residues; (vii) a polypeptide with similar structure and function or a nucleotide sequence encoding a polypeptide with similar structure and function, exhibiting the antigenicity, immunogenicity, catalytic activity, and other readily assayable activities, to polypeptides encoded by SEQ ID NOS: 2, 4, 6, 8, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45 and 47, and/or a fragment of these polypeptides, refers to a polypeptide that has a similar secondary, tertiary, or quaternary structure of these polypeptides, or a fragment of these polypeptides. 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. The term "homologue" is used herein to describe a sequence that has sequence homology. A sequence having sequence homology can be made using standard molecular biology techniques including site-directed mutagenesis including insertion or deletion of sequences. The term "homologue" is not limited to homologous genes or proteins originating from different species and expressly includes artificial modification to the sequences disclosed herein.

[0033] The term "conservatively substituted variant" refers to a polypeptide or a nucleic acid sequence encoding a homologue polypeptide in which one or more amino acid residues or codons have been modified by conservative substitution with an amino acid residue or a codon coding for an amino acid residue of similar chemical-type, as described below.

[0034] The term "an antibody or an antibody fragment which immunospecifically binds to polypeptides encoded by SEQ ID NOS: 2, 4, 6, 8, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45 and 47," as used herein refers to an antibody or a fragment thereof that immunospecifically binds to polypeptides encoded by SEQ ID NOS: 2, 4, 6, 8, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45 and 47, or a fragment of these polypeptide and does not non-specifically bind to other polypeptides. An antibody or a fragment thereof that immunospecifically binds to polypeptides encoded by SEQ ID NOS: 2, 4, 6, 8, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45 and 47, or a fragment of these polypeptide, may crossreact with other antigens. Preferably, an antibody or a fragment thereof that immunospecifically binds to polypeptides encoded by SEQ ID NOS: 2, 4, 6, 8, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45 and 47, or a fragment of these polypeptides, does not cross-react with other antigens. An antibody or a fragment thereof that immunospecifically binds to polypeptides encoded by SEQ ID NOS: 2, 4, 6, 8, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45 and 47, 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 which immunospecifically binds polypeptides encoded by SEQ ID NOS: 2, 4, 6, 8, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45 and 47, may be interchangeably referred to as "anti-

[0035] The term "derivative" as used herein refers to a given peptide or protein that is otherwise modified, e.g., by covalent attachment of any type of molecule, preferably having bioactivity, to the peptide or protein, including the incorporation of non-naturally occurring amino acids. The resulting bioactivity retains one or more biological activities of the peptide protein.

[0036] The term "fragment" as used herein refers to a fragment of a nucleic acid molecule containing one of at least about 25, at least about 30, at least about 35, at least about 40, at least about 45, at least about 100, at least about 150, at least about 200, at least about 250, at least about 300, at least about 350, 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, at least about 1350, from about 500 to about 2000, from about 1000 to about 2000 from about 200 to about 500, from about 500 to about 1000, form about 1000 to about 1500, and from about 1500 to about 2000 nucleic acid bases in length of the relevant nucleic acid molecule and having at least one functional feature of the nucleic acid molecule (or the encoded protein has one functional feature of the protein encoded by the nucleic acid molecule); or a fragment of a protein or a polypeptide containing one or more of at least about 5, at least about 10, at least about 15, at least about 20, at least about 25, at least about 30, at least about 35, at least about 40, at least about 45, at least about 50, at least about 55, at least about 60, at least about 65, at least about 70, at least about 75, at least about 80, at least about 90, at least about 100, at least about 120, at least about 140, 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, at least about 360, from about 250 to about 660, from about 350 to about 660, form about 450 to about 660, and form about 550 to about 660 amino acid residues in length of the relevant protein or polypeptide and having at least one functional feature of the protein or polypeptide, such functional features include ability to bind a Fe³⁺-Me²⁺ dimetal nuclear center and form a C-terminal motif.

[0037] An "isolated" nucleic acid molecule is one which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid molecule. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be 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, but excludes nucleic acid molecules present in recombinant DNA libraries. In a preferred embodiment, nucleic acid molecules encoding the disclosed polypeptides/proteins are isolated or purified.

[0038] 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.

[0039] The term "under stringent condition" 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, non-limiting example of stringent hybridization conditions is hybridization in 6× sodium chloride/sodium citrate (SSC), 0.5% SDS at about 68° C. followed by one or more washes in 2×SSC, 0.5% SDS at room temperature. Another preferred, non-limiting example of stringent hybridization conditions is hybridization in 6×SSC at about 45° C. followed by one or more washes in 0.2×SSC, 0.1% SDS at about 50-65° C.

[0040] The term "variant" as used herein refers either to a naturally occurring allelic variation of a given peptide or a recombinantly prepared variation of a given peptide or protein in which one or more amino acid residues have been modified by amino acid substitution, addition, or deletion.

[0041] The term "aligned" as used herein refers to a homology alignment between two or more sequences using a standard algorithm such as BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

[0042] The term "predicted to form a transmembrane motif by TMHMM analysis" or "predicted to form a C-terminal motif by TMHMM analysis" (http://www.cbs.dtu.dk/services/TMHMM/) herein refers to a probability that is equal to or greater than about 0.5.

BRIEF DESCRIPTION OF THE FIGURES

[0043] The following figures illustrate the embodiments and are not meant to limit the scope of the invention encompassed by the claims.

[0044] FIG. 1 shows the phylogenetic tree of PAP-like sequences in the *Arabidopsis* genome. Twenty-nine PAPs were aligned using ClustalX and the phylogenetic tree was created by the neighbor-joining algorithm of the MEGA4 program. The accession numbers of the PAP-like, transmem-

brane-like C-terminal motif containing, polypeptide from *Zea mays* (ZmPAP2) and *Oryza sativa* (OsPAP2) were ACG47621 and BAC15853.1, respectively.

[0045] FIG. 2A is the amino acid alignment of the C-terminal transmembrane-like motifs in AtPAP2 with other PAP sequences.

[0046] FIG. 2B is the amino acid alignment of AtPAP2 with other PAP sequences, showing the full length of each sequence. These sequences include homologous sequences from *B. napus* (BnPAP2), *G. max* (GmPAP2) and *Z. may* (ZmPAP2). The five conserved motifs (XDXX, XDXXY, GNH(D/E), XXXH, XHXH) are boxed. Residues in shades have low or no homology. Hydrophobic motifs at the C-termini of these polypeptides are underlined by a bar (614th-636th amino acid), which is absent from the sequence of AtPAP15. As shown, AtPAP15 does not have a C-terminal region corresponding to the other PAP sequences.

[0047] FIG. 3 shows that a unique hydrophobic motif is present at the C-termini of AtPAP2 and ZmPAP2 by TMHMM analysis. This transmembrane-like C-terminal motif is absent from AtPAP15.

[0048] FIG. 4 shows the characteristics of the T-DNA lines. The T-DNA line (Salk_013567) was obtained from TAIR. The AtPAP2 genomic sequence carries two exons and the T-DNA was inserted in exon 2 and causes a disruption of the AtPAP2 mRNA (a). Three PCR primers (A, B and C) were designed for the differentiation of the wild-type (WT) and the T-DNA line (atpap2-8) and they were used for PCR screening of genomic DNA extracted from WT and the T-DNA line (b). Total RNA was extracted from 10-day-old seedlings grown on MS with 2% sucrose using the TRIzol RNA isolation method and were used for RT-PCR (c). 50 µg of seedlings proteins were loaded for Western blotting studies, using the anti-AtPAP2 specific antiserum (Section 6.3).

[0049] FIG. 5 is the schematic diagram of the expression vector pBV-AtPAP2. CaMV 35S:35S promoter of the cauliflower mosaic virus; NOS: polyadenylation signal of nopaline synthase gene; aadA: bacterial streptomycin/spectinomycin resistance gene encoding aminoglycoside-3"-adenyltransferase; pNOS:BAR: bialaphos resistance gene under the control of the nopaline synthase promoter; born: basis of mobility from pBR322; ColE1: replication origin from pBR322; pVS1-REP: replication origin from pVS1; pVS1-STA: STA region from pVS1 plasmid; LB: left border T-DNA repeat; RB: right border T-DNA repeat. (Hajdukiewicz et al., 1994).

[0050] FIG. 6A shows the results of the Western blot analysis of the overexpression lines (OE), wild-type (WT), T-DNA and the complementation lines (CP) of AtPAP2 and FIG. 6B shows the results of the Western blot analysis of the overexpression lines (C-15) and wild-type (WT) of AtPAP15.

[0051] FIG. 7 shows the expression analysis of AtPAP2. The mRNA expression profile was analysed by the Spot History program of NASC (a). The protein expression profiles of 30 day old, soil-grown plant (b), seedlings germinated on MS agar (c) and 2 week old plants transferred to Pi-sufficient/Pideficient MS agar for 3 days (d), were analyzed by Western blotting using the anti-PAP2 antiserum.

[0052] FIG. 8 shows the growth performance of the wild-type, T-DNA and overexpression lines in soil. Seeds were germinated in MS agar with 2% sucrose for 10 days. Seedlings with 2 small visible rosette leaves (~1 mm) were transferred to soil and grown under 16 h/8 h light/dark cycles.

[0053] FIG. 9 shows the levels of sucrose and glucose in the rosette leaves of 21-day-old, soil grown seedlings.

[0054] FIG. 10 shows the recovery of various lines after prolonged darkness treatment. Seeds were germinated in MS agar with 2% sucrose for 10 days. Seedlings with 2 small visible rosette leaves (~1 mm) were transferred to soil and grown for 12 days under 16 h/8 h light/dark cycles. The lights of the growth chamber were then switched off for 12 days and the plants were allowed to recover under 16 h/8 h light/dark cycles for 1 week. n=9-12 per line.

[0055] FIG. 11. Detection of AtPAP2 protein in subcellular fractions by Western blotting. Mito.: Mitochondria; Chlorop.: Chloroplasts.

[0056] FIG. 12. shows a schematic representation of two vector constructs incorporating the AtPAP2 gene.

[0057] FIG. 13. shows Western blot analysis results for overexpression of AtPAP2 proteins missing the C-terminal motif

DETAILED DESCRIPTION

[0058] 5.1 Method of Speeding Up Plant Growth and Improving Crop Yield

[0059] The present disclosure provides a method that speeds up plant growth and elevates plant yields by introducing phosphatases with a C-terminal motif into plants. In an embodiment, the present disclosure relates to a class of genes of purple acid phosphates, and their respectively encoded protein products, as well as fragments, derivatives, homologues, and variants thereof. Methods for introducing this class of genes into plants to speed up the growth rate of plants, to increase the sugar contents of plants, and to increase of yield of plants, are provided.

[0060] A group of purple acid phosphatases (PAPs) which carry seven conserved amino acid residues (shown in bold face) in the five conserved motifs XDXX (example GDXG (SEQ ID NO: 48)), XDXXY (SEQ ID NO: 49), GNH(D/E) (SEQ ID NOS: 50-51), XXXH (example ZXGH (SEQ ID NO: 52)), XHXH (SEQ ID NO: 53), where X is any amino acid and Z is any amino acid selected from L, I, V, F, and M, and a transmembrane-like motif at their C-termini were identified in the genomes of a number of plants (FIGS. 1, 2A, and 2B). The presence of the C-terminal transmembrane-like motif enables the localization of this group of PAN to the membrane fraction (FIGS. 3 and 11). This property makes this group of PAPs differ from the other previously characterized PAPs because all previously characterized PAPs did not carry any C-terminal motif (FIGS. 2A, 2B, and 3). By using the protein sequence of a representative gene of this group, AtPAP2, to blast the NCBI database and various EST databases, a number of genomic or cDNA sequences were identified to encodes polypeptides that carry the five conserved motifs XDXX, XDXXY, GNH(D/E), XXXH, XHXH of PAPs and a transmembrane motif at their C-termini (FIG. 2B).

[0061] The introduction of a representative gene of this group of phosphatases, AtPAP2, into the genome of *Arabidopsis* by transgenic technology produced transgenic *Arabidopsis* that grew faster than the wild-type plants (FIG. 8), and the yield of seeds were elevated by approximately 40% (Table 3). However, transgenic plant that expressed AtPAP15 did not show these phenotypes. The sugar contents, including glucose and sucrose, in the leaf of the transgenic lines, were also found to be higher than that of the wild-types (FIG. 9).

[0062] Thus, this disclosure provides a method that speeds up plant growth and elevates plant yields by introducing phosphatases into plants. In an embodiment, a group of genes of purple acid phosphatases, and their respectively encoded protein products, as well as fragments, derivatives, homologues, and variants thereof are described.

[0063] 5.2 Homologues, Derivatives, and Variants of Phosphatases

[0064] In addition to the nucleic acid molecules (SEQ ID NOS: 1, 3, 5, 7, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44 and 46) and polypeptides (SEQ ID NOS: 2, 4, 8, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45 and 47) described in claims 9-16, the nucleic acid molecules and polypeptides also encompass those nucleic acid molecules and polypeptides having a common biological activity, similar or identical structural domain and/or having sufficient nucleotide sequence or amino acid identity (homologues) to those of the nucleic acid molecules and polypeptides described above.

[0065] Such common biological activities of the polypeptides include antigenicity, immunogenicity, catalytic activity especially phosphatase activity, ability to bind a Fe³⁺-Me²⁺ dimetal nuclear center, fold into or form a transmembrane-like C-terminal motif and other activities readily assayable by the skilled artisan.

[0066] A polypeptide that has a similar amino acid sequence (homologue) refers to a polypeptide that satisfied at least one of the following: (i) a polypeptide having an amino acid sequence that is one of at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, and at least about 95%, and at least about 98% identical and/or conservatively substituted to the amino acid sequence of a AtPAP2 (SEQ ID NO: 2) and/or other PAPs with a transmembrane-like C-terminal motifineluding SEQ ID NOS: 4, 6, 8, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45 and/or 47, a fragment of AtPAP2, and having at least one biological feature of the described polypeptides; (ii) a polypeptide encoded by a nucleotide sequence that is one of at least about 30%, at least about 40%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, and at least about 98% identical to the nucleotide sequence encoding AtPAP2 (SEQ ID NO: 1) and/or other PAPs with a transmembrane-like C-terminal motificeluding SEQ ID NOS: 3, 5, 7, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44 and/or 46, a fragment of AtPAP2 and having at least one structural and/or biological feature of AtPAP2; (iii) a polypeptide encoded by a nucleotide sequence that hybridizes under stringent conditions as defined herein to a nucleotide sequence encoding AtPAP2 (SEQ ID NO: 1) and/or other PAPs with a motif including SEQ ID NOS: 3, 5, 7, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44 and/or 46, a fragment of AtPAP2 and having at least one structural and/or biological feature of AtPAP2. A polypeptide with similar structure to AtPAP2, or a fragment of AtPAP2, refers to a polypeptide that has a similar secondary, tertiary, or quaternary structure of AtPAP2, a fragment of AtPAP2 and has at least one functional feature of a AtPAP2, including one or more of ability to bind a Fe³⁺-Me²⁺ dimetal nuclear center and fold into or form a transmembrane-like C-terminal motif. 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.

[0067] Those having skill in the art will readily recognized that mutations, deletions or insertions can be made in any of the sequences disclosed herein, including SEQ ID NOS: 1-8 and 18-47, without affecting function. Sequences useful in practicing the embodiments include sequences having homology to SEQ ID NOS: 1-8 and 18-47 and being a protein, polypeptide, or polynucleotide coding for such protein or peptide having functionality to bind a dimetal nuclear center (Fe³⁺-Me²⁺) and being a protein, polypeptide, or polynucleotide coding for such protein or peptide having a C-terminal motif. That is, those skilled in the art will recognize that many mutations can be made to any of SEO ID NOS: 1-8 and 18-47 without affecting the catalytic functionality nor interrupting the transmembrane-like C-terminal motif. Such modified sequences that maintain catalytic activity and a transmembrane-like C-terminal motif are defined as homologues to SEQ ID NOS: 1-8 and 18-47 and are including within the scope of useful sequences.

[0068] In one embodiment, such homologues can have about 30% or more identity to the sequences disclosed herein. In another embodiment, such homologues can have about 40% or more identity to the sequences disclosed herein. In yet another embodiment, such homologues can have about 50% or more identity to the sequences disclosed herein. In sill yet another embodiment, such homologues can have about 60% or more identity to the sequences disclosed herein. In even sill yet another embodiment, such homologues can have about 70% or more identity to the sequences disclosed herein. In a further embodiment, such homologues can have about 80% or more identity to the sequences disclosed herein. In yet a still further embodiment, homologues can have about 90% or more identity to the sequences disclosed herein. In a still further embodiment, homologues can have about 98% or more identity to the sequences disclosed herein.

[0069] Those having skill in the art will recognize that mutations can be made to proteins and peptides and/or to polynucleotides coding for protein and peptides or complementary thereto that substitute amino acid residue for other amino acids residues having similar chemical properties (conservative substitutions) and that such mutations are less likely to cause structural changes that affect functionality including catalytic activity and/or the function of a transmembrane-like C-terminal motif. Conservatively substituting amino acids are substituting an amino acid residue belong to any of the following 11 chemical groups with another amino acid from the same chemical 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; (11); amino acids having similar geometry and hydrogen bonding patterns such as aspartic acid, asparagine, glutamic acid and glutamine.

[0070] In one embodiment, homologues can have about 30% or more identity and/or conservative substitutions to the sequences disclosed herein. In another embodiment, homologues can have about 40% or more identity and/or conservative substitutions to the sequences disclosed herein. In yet another embodiment, homologues can have about 50% or more identity and/or conservative substitutions to the sequences disclosed herein. In still yet another embodiment, homologues can have about 60% or more identity and/or conservative substitutions to the sequences disclosed herein. In a further embodiment, homologues can have about 70% or more identity and/or conservative substitutions to the sequences disclosed herein. In a still further embodiment, homologues can have about 80% or more identity and/or conservative substitutions to the sequences disclosed herein. In still another embodiment, homologues can have about 90% or more identity and/or conservative substitutions to the sequences disclosed herein. In still another further embodiment, homologues can have about 98% or more identity and/ or conservative substitutions to the sequences disclosed herein.

[0071] Embodiments further provide isolated nucleic acid molecules which comprise or consist of one or more of at least about 25, at least about 30, at least about 35, at least about 40, at least about 45, at least about 100, at least about 150, at least about 200, at least about 250, at least about 300, at least about 350, 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, at least about 1350, from about 500 to about 2000, from about 1000 to about 2000, from about 200 to about 500, from about 500 to about 1000, form about 1000 to about 1500, and from about 1500 to about 2000 nucleotides of the nucleotide sequences of SEQ ID NOS: 1, 3, 5, 7, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44 and 46, or a complement thereof encoding a protein or polypeptide having one or more activity of the amino acid sequences of their encoded proteins (SEQ ID NOS: 2, 4, 6, 8, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45 and 47). The activity includes one or more of antigenicity, immunogenicity, catalytic activity (e.g., phosphatase activity), ability to bind a Fe³⁺-Me²⁺ dimetal nuclear center, fold into or form a transmembrane-like C-terminal motif, and other activities readily assayable.

[0072] Embodiments provide isolated polypeptides or proteins consisting of an amino acid sequence that contains one of about 5, at least about 10, at least about 15, at least about 20, at least about 25, at least about 30, at least about 35, at least about 40, at least about 45, at least about 50, at least about 55, at least about 60, at least about 65, at least about 70, at least about 75, at least about 80, at least about 90, at least about 100, at least about 120, at least about 140, 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, at least about 360, from about 250 to about 660, from about 350 to about 660, form about 450 to about 660, and form about 550 to about 660 amino acid bases in length of the relevant protein or polypeptide and having at least one functional feature of the protein or polypeptide, such functional features including ability to bind a Fe³⁺-Me²⁺ dimetal nuclear center and form a transmembrane-like C-terminal motif.

[0073] Additional embodiments are any of the phosphatases and homologues thereof with the identity and/or conservative substitutions to SEQ ID NOS: 1-8 and 18-47 described above that additionally consist of a protein, polypeptide, or polynucleotide encoding a protein having the five conserved motifs in purple acid phosphatases, including XDXX, XDXXY, GNH(D/E), XXXH, XHXH, where X is any amino acid. In one embodiment, the described phosphatases and homologues thereof consist of a protein, polypeptide, or polynucleotide encoding one of the sequences YHVCIGNHEYDF (SEQ ID NO: 54) and YHVCIGNHEYDW (SEQ ID NO: 55). In one embodiment, the described phosphatases and homologues thereof consist of a protein, polypeptide, or polynucleotide encoding a sequence of amino acid residues having one of the sequences YHVCIGNHEYD(W/F) (SEQ ID NO: 54) and YHVCIGN-HEYN(W/F) (SEQ ID NO: 55) or a protein, polypeptide, or polynucleotide encoding a homologue to one of the foregoing sequences with only conservative substitutions, as described above, to those sequences. In yet another embodiment, the described phosphatases and homologues thereof consist of a protein, polypeptide, or polynucleotide encoding a sequence of amino acid residues having one of the sequences GNHE (SEQ ID NO: 51) and GNHD (SEQ ID NO: 50). In still yet another embodiment, the described phosphatases and homologues thereof consist of a protein, polypeptide, or polynucleotide encoding a sequence of amino acid residues having one of the sequences GNHE (SEQ ID NO: 51) and GNHD (SEQ ID NO: 50) or a protein, polypeptide, or polynucleotide encoding a protein having a homologous sequence to one of the foregoing sequences SEQ 1N NOS: 50-51 with only conservative substitutions.

[0074] Additional embodiments are any of the phosphatases and homologues thereof with the identity and/or conservative substitutions to SEQ ID NOS: 1-8 and 18-47 described above that additionally consist of a protein, polypeptide, or polynucleotide encoding a sequence having at least about 70% or more identity and/or conservative substitutions to amino acid residues 302-315 of SEQ ID NO: 2 when such sequence is aligned with SEQ ID NO: 2. In another embodiment, the described phosphatases and homologues thereof consist of a protein, polypeptide, or polynucleotide encoding a protein having about 80% or more identity and/or conservative substitutions to amino acid residues 302-315 of SEQ ID NO: 2 when such sequence is aligned with SEQ ID NO: 2. In another embodiment, the described phosphatases and homologues consist of a protein, polypeptide, or polynucleotide encoding a sequence of amino acid residues having at least about 70% or more identity to the sequence HIGDISYARGYSW (SEQ ID NO: 56). In another embodiment, the described phosphatases and homologues thereof consist of a protein, polypeptide, or polynucleotide encoding a sequence of amino acid residues having the sequence HIGDISYARGYSW (SEQ ID NO: 56) or a protein, polypeptide, or polynucleotide encoding a protein having a homologous sequence to the foregoing sequences with only conservative substitutions, as described above, to those sequences.

[0075] In another embodiment, the described phosphatases and homologues thereof consist of a protein, polypeptide, or polynucleotide encoding a sequence of amino acid residues having at least about 70% or more identity to the sequences KEKLTVSFVGNHDGEVHD (SEQ ID NO: 57), KERLTL-SYVGNHDGEVHD (SEQ ID NO: 58), REKLTLTYVGN-HDGQVHD (SEQ ID NO: 59), and KEKLTLTYIGN-

HDGQVHD (SEQ ID NO: 60). In still yet another embodiment, the described phosphatases and homologues thereof consist of a protein, polypeptide, or polynucleotide encoding a sequence of amino acid residues having one or more of the sequences KEKLTVSFVGNHDGEVHD (SEQ ID NO: 57), KERLTLSYVGNHDGEVHD (SEQ ID NO: 59), and KEKLTLTYVGNHDGQVHD (SEQ ID NO: 59), and KEKLTLTYIGNHDGQVHD (SEQ ID NO: 60) or a protein, polypeptide, or polynucleotide encoding a protein having a homologous sequence to one of the foregoing sequences with only conservative substitutions, as described above, to those sequences.

[0076] In a further embodiment, the described phosphatases and homologues thereof consist of a protein, polypeptide, or polynucleotide encoding a sequence of amino acid residues having the sequence (F/Y)(V/I)GNHDGXXH (SEQ ID NOS: 61-64), where the first residue of the sequence can be F or Y and the second residue of the sequence can be V or I. In a still further embodiment, the described phosphatases and homologues thereof consist of a protein, polypeptide, or polynucleotide encoding a sequence of amino acid residues having the sequence (F/Y)(V/I)GNHDGXXH (SEQ ID NOS: 61-64), where the first residue of the sequence can be F or Y and the second residue of the sequence can be V or I, or a protein, polypeptide, or polynucleotide encoding a protein having a homologous sequence to the foregoing sequence with only conservative substitutions, as described above, to the foregoing sequence. In a yet still further embodiment, the described phosphatases and homologues thereof consist of a protein, polypeptide, or polynucleotide encoding a sequence of amino acid residues having the sequence (F/Y)(V/I)GN-HDGXXH (SEQ ID NOS: 61-64), where the first residue of the sequence can be F or Y and the second residue of the sequence can be V or I, or a protein, polypeptide, or polynucleotide encoding a protein having a homologous sequence having at least about 70% identity and/or conservative substitution, as described above, to the foregoing sequence.

[0077] In one embodiment, the described phosphatases and homologues thereof consist of a protein, polypeptide, or polynucleotide encoding a sequence of amino acid residues having at least about 60% or more identity and/or conservative substitutions to amino acid residues 614-636 of SEQ ID NO: 2 (SEQ ID NO: 65) and/or having at least about 60% or more identity and/or conservative substitutions to the sequence of 23 amino acid residues of SEQ ID NOS: 4, 6, 8, 19, 21, 23, 25, 27, 29, 31, 33 and 47 aligned with residues 614-636 of SEQ ID NO: 2 (SEQ ID NO: 65), and where amino acid residues aligned with amino acid residues 614-636 of SEO ID NO: 2 are predicted to form a transmembrane-like C-terminal motif by TMHMM analysis (http://www.cbs.dtu.dk/services/TM-HMM/). In one embodiment, the described phosphatases and homologues thereof consist of a protein, polypeptide, or polynucleotide encoding a sequence of amino acid residues having at least about 70% or more identity and/or conservative substitutions to amino acid residues 614-636 of SEQ ID NO: 2 (SEQ ID NO: 65) and/or having at least about 60% or more identity and/or conservative substitutions to the sequence of 23 amino acid residues of SEQ ID NOS: 4, 6, 8, 19, 21, 23, 25, 27, 29, 31, 33 and 47 aligned with residues 614-636 of SEQ ID NO: 2 (SEQ ID NO: 65), and where amino acid residues aligned with amino acid residues 614-636 of SEQ ID NO: 2 (SEQ ID NO: 65) are predicted to form a transmembrane-like C-terminal motif by TMHMM analysis (http://www.cbs.dtu. dk/services/TMHMM/). In one embodiment, the described

phosphatases and homologues thereof consist of a protein, polypeptide, or polynucleotide encoding a sequence of amino acid residues having at least about 80% or more identity and/or conservative substitutions to amino acid residues 614-636 of SEQ ID NO: 2 and/or having at least about 60% or more identity and/or conservative substitutions to the sequence of 23 amino acid residues of SEQ ID NOS: 4, 6, 8, 19, 21, 23, 25, 27, 29, 31, 33 and 47 aligned with residues 614-636 of SEQ ID NO: 2 (SEQ ID NO: 65), and where amino acid residues aligned with amino acid residues 614-636 of SEQ ID NO: 2 (SEQ ID NO: 65) are predicted to form a transmembrane-like C-terminal motif by TMHMM analysis (http://www.cbs.dtu.dk/services/TMHMM/). In one embodiment, the described phosphatases and homologues thereof consist of a protein, polypeptide, or polynucleotide encoding a sequence of amino acid residues having at least about 90% or more identity and/or conservative substitutions to amino acid residues 614-636 of SEQ ID NO: 2 and/or having at least about 90% or more identity and/or conservative substitutions to the sequence of 23 amino acid residues of SEQ ID NOS: 4, 6, 8, 19, 21, 23, 25, 27, 29, 31, 33 and 47 aligned with residues 614-636 of SEQ ID NO: 2, and where amino acid residues aligned with amino acid residues 614-636 of SEQ ID NO: 2 are predicted to form a transmembranelike C-terminal motif by TMHMM analysis (http://www.cbs. dtu.dk/services/TMHMM/).

[0078] In one embodiment, the described phosphatases or phosphatase genes consist of a protein, polypeptide, or polynucleotide encoding the sequence (L/M/V)-(L/MN)—Z-(G/A)-(V/A/L)-Z—Z-G—(F/Y)—X—Z-G (SEQ ID NO: 66), where Z is any of the hydrophobic residues L, I, V, F, and M. In another embodiment, the described phosphatase or phosphatase genes consist of a protein, polypeptide, or polynucleotide encoding the sequence (L/M/V)-(L/MN)—Z-(G/A)-(V/A/L)-Z—Z-G—(F/Y)—X—Z-G (SEQ ID NO: 66), or a protein, polypeptide, or polynucleotide encoding a sequence having at least 70% identity and/or conservative substitution to the foregoing sequence.

[0079] Embodiments also encompass 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.

[0080] In another aspect, an isolated nucleic acid molecule encodes a variant of a polypeptide in which the amino acid sequences have 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. In one aspect, these variants can act as either agonists or as antagonists. An agonist can retain substantially the same or a portion of the biological activities of the polypeptides and an antagonist can inhibit one or more of the activities of the polypeptides. Such modifications include amino acid substitution, deletion, and/ or insertion. Amino acid modifications can be made by any method known in the art and various methods are available to and routine for those skilled in the art.

[0081] For example, mutagenesis may be performed in accordance with any of the techniques known in the art including, but not limited to, synthesizing an oligonucleotide having one or more modifications within the sequence of a given polypeptide to be modified. Site-specific mutagenesis can be conducted using specific oligonucleotide sequences which encode the nucleotide sequence containing the desired mutations in addition to a sufficient number of adjacent nucleotides in the polypeptide. Such oligonucleotides can serve as primers which can form a stable duplex on both sides of the deletion junction being traversed. Typically, a primer of about 15 to about 75 nucleotides or more in length is preferred, with about 10 to about 25 or more residues on both sides of the junction of the sequence being altered. A number of such primers introducing a variety of different mutations at one or more positions can be used to generate a library of mutants.

[0082] The technique of site-specific mutagenesis is well known in the art, as described in various publications (e.g., Kunkel et al., Methods Enzymol., 154:367-82, 1987, which is hereby incorporated by reference in its entirety). In general, site-directed mutagenesis is performed by first obtaining a single-stranded vector or melting apart of two strands of a double stranded vector which includes within its sequence a DNA sequence which encodes the desired peptide. An oligonucleotide primer bearing the desired mutated sequence is prepared, generally synthetically. This primer is then annealed with the single-stranded vector, and subjected to DNA polymerizing enzymes such as T7 DNA polymerase, in order to complete the synthesis of the mutation-bearing strand. Thus, a heteroduplex is formed wherein one strand encodes the original non-mutated sequence and the second strand bears the desired mutation. This heteroduplex vector is then used to transform or transfect appropriate cells, such as E. coli cells, and clones are selected which include recombinant vectors bearing the mutated sequence arrangement. As will be appreciated, the technique typically employs a phage vector which exists in both a single stranded and double stranded form. Typical vectors useful in site-directed mutagenesis include vectors such as the M13 phage. These phages are readily commercially available and their use is generally well known to those skilled in the art. Double stranded plasmids are also routinely employed in site directed mutagenesis which eliminates the step of transferring the gene of interest from a plasmid to a phage.

[0083] Alternatively, the use of PCR with commercially available thermostable enzymes such as Taq DNA polymerase may be used to incorporate a mutagenic oligonucleotide primer into an amplified DNA fragment that can then be cloned into an appropriate cloning or expression vector. See, e.g., Tomic et al., *Nucleic Acids Res.*, 18(6):1656, 1987, and Upender et al., *Biotechniques*, 18(1):29-30, 32, 1995, for PCR-mediated mutagenesis procedures, which are hereby incorporated in their entireties. PCR employing a thermostable ligase in addition to a thermostable polymerase may also be used to incorporate a phosphorylated mutagenic oligonucleotide into an amplified DNA fragment that may then be cloned into an appropriate cloning or expression vector (see e.g., Michael, *Biotechniques*, 16(3):410-2, 1994, which is hereby incorporated by reference in its entirety).

[0084] Other methods known to those skilled in art of producing sequence variants of a given polypeptide or a fragment thereof can be used. For example, recombinant vectors encoding the amino acid sequence of the polypeptide or a

fragment thereof may be treated with mutagenic agents, such as hydroxylamine, to obtain sequence variants.

[0085] Optionally, the amino acid residues to be modified are surface exposed residues. Additionally, in making amino acid substitutions, preferably the amino acid residue to be substituted is a conservative amino acid substitution, for example, a polar residue is substituted with a polar residue, a hydrophilic residue with a hydrophilic residue, hydrophobic residue with a hydrophobic residue, a positively charged residue with a positively charged residue with a negatively charged residue. Moreover, the amino acid residue that can be modified is not highly or completely conserved across strains or species and/or is critical to maintain the biological activities of the protein.

[0086] Accordingly, included in the scope of the disclosure are nucleic acid molecules encoding a polypeptide of the invention that contains amino acid modifications that are not critical to its biological activity.

[0087] 5.3 Fusion Proteins

[0088] The present disclosure further encompasses fusion proteins in which the polypeptides 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, but may occur through linker sequences.

[0089] In one aspect, the fusion protein comprises a polypeptide which is 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 which is derived from a heterologous origin. Various signal sequences are commercially available.

[0090] 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 tags are 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 especially useful for purification of recombinantly produced polypeptides.

[0091] 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 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 which 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).

[0092] The nucleotide sequence coding for a fusion protein can be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence.

[0093] In a specific embodiment, the expression of a fusion protein is regulated by an inducible promoter.

[0094] 5.4 Preparation of Transgenic Plants

[0095] Carbon flow is a key process in plant biology and high energy carbon molecules (e.g. glucose) were harvested by plant through photosynthesis. The carbon molecules were then converted into more complicated carbohydrate molecules such as starch, cellulose, etc. Cellulose is the major component of cell wall and starch is the major storage form of glucose in plant cells and plant seeds. Therefore, the efficiency and/or the equilibrium of the carbon flow process become a limiting factor for plant growth and crop yield.

[0096] The present disclosure is based upon the discovery that overexpression of a membrane-bound phosphatase can enhance the growth performance of plants by altering its carbon metabolism, as indicated by, for example, a faster growth rate, a higher sugar contents, and a higher seed yield. [0097] In an embodiment, the present disclosure provides a transgenic plant containing a nucleic acid molecule that encodes and expresses a phosphatase having a C-terminal transmembrane-like domain. The transgenic plants disclosed herein have faster growth rate, and higher seed yield to comparable unengineered plants i.e. same species (strain). In a specific embodiment, such a phosphatase is from a plant species having a phosphatase activity and a C-terminal motif. In another embodiment, a transgenic plant disclosed herein comprises a nucleic acid molecule encoding phosphatase and expresses AtPAP2 (SEQ ID NO: 2) and/or other PAPs with a C-terminal motifincluding one or more of SEQ ID NOS: 4, 6, 8, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45 and 47. In another embodiment, the phosphatase is expressed on cellular membrane, for example, the ER or the Golgi apparatus. Such a membrane expression of a phosphatase in plants can be achieved by fusing onto the C-terminus with a nucleotide sequence encoding a C-terminal motif peptide which can efficiently attach the phosphatase upon translation thereof from the cells of a given plant. Accordingly, in another embodiment, a transgenic plant comprises a nucleic acid molecule encoding phosphatase and expresses AtPAP2 (SEQ ID NO: 2) and/or other PAPs with a C-terminal motif including SEQ ID NOS: 4, 6, 8, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45 and 47, except that 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, of SEQ ID NO: 2 or 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, of SEQ ID NOS: 4, 6, 8, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45 or 47, are replaced by a heterologous plant signal peptide by genetic engineering. In such a transgenic plant, the phosphatases are directed to various organelles/compartments of the cells. In another embodiment, a transgenic plant comprises a nucleic acid molecule encoding phosphatase and expresses homologues, derivatives, and/or fragments thereof having at least one functional feature and/or structural feature of a phosphatase polypeptide. In all embodiments where all or a portion of the N-terminal portion of SEQ ID NOS: 4, 6, 8, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45 and/or 47 are replaced, the embodiments include homologues to such sequences, as described above, having at least one functional feature and/or structural feature of a phosphatase polypeptide. In yet another embodiment, a transgenic plant comprises a nucleic acid molecule that hybridizes under stringent conditions, as defined herein, to a nucleic acid molecule having the sequence of SEQ

ID NOS: 1, 3, 5, 7, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44 or 46, or a complement thereof, and encodes a protein or polypeptide that exhibits at least one structural and/or functional feature of the disclosed phosphatase polypeptides. Specifically, the production of transgenic plant that overexpressed a membrane-bound phosphatase, which contributes to improving plant physiology, such as plant growth rate and characteristics, for example, in seed yield, is provided.

[0098] Accordingly, also provided are chimeric gene constructs for genetic modification of plants to increase their growth rate and improve the yield. The chimeric gene constructs comprise a sequence that encodes substantially solely for a phosphatase enzyme that carry a C-terminal transmembrane-like motif. Such a phosphatase enzyme can be derived from the purple acid phosphatase family. In a specific embodiment, the chimeric gene constructs comprise a nucleic acid having the sequence of SEQ ID NOS: 1, 3, 5, 7, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44 or 46. In another embodiment, the chimeric gene constructs comprise a nucleic acid molecule that encodes a homologue or fragment thereof having at least one functional feature and/or structural feature of a phosphatase polypeptide. In another specific embodiment, the chimeric gene constructs comprise a sequence that hybridizes under stringent conditions, as defined herein, to a nucleic acid having the sequence of SEQ ID NOS: 1, 3, 5, 7, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44 or 46, or a complement thereof, wherein the sequence encodes a protein or a polypeptide that exhibits at least one structural and/or functional feature of the phosphatase polypeptides. Furthermore, the phosphatases encoded by the nucleic acid molecules contained in the chimeric gene constructs can be any other phosphatases that have similar structural characteristics, such as having a C-terminal transmembrane-like motif, to those of the phosphatases described herein. Such phosphatase include, but not limited to, the following polypeptides: Purple acid phosphatases from Zea mays (Accession No: ACG47621); and Oryza sativa (Accession No: BAC15853.1).

[0099] The phosphatase-coding sequence is operatively linked to upstream and downstream regulatory components, preferably heterologous to the phosphatase sequence; for example CMV 35S promoter, which acts to cause expression of the gene (production of the enzyme) in plant cells (see Section 6.2). When a construct containing a gene for a phosphatase according to this disclosure, 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 successfully 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 described herein also favors the growth rate of the plant, resulting in faster growth rate and higher yield. Thus, 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 the cells of such whole plants a chimeric gene construct.

[0100] In specific embodiments, *Arabidopsis* (see Section 6) was adopted as the model system. An overexpression construct the gene coding for phosphatase were introduced into *Arabidopsis*.

[0101] In an embodiment, the phosphatase from *Arabidopsis* is used. The results obtained with this disclosure indicate that the growth rate and the seed yield of transgenic *Arabidopsis* were enhanced by overexpressing this gene (see Section 6.5 and FIG. 8 and Table 3).

[0102] While any plant species can be modified using the expression cassette and methods described herein, preferably included without limitation are species from the following genera with representative species in parentheses:

[0103] 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), Zea (corn); and

[0104] 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, faba bean), Vitis (grape).

[0105] Genetic engineering of plants can be achieved in several ways. The most common method is Agrobacteriummediated transformation. In this method, A. tumefaciens, which in nature infects plants by inserting tumor causing genes into a plant's genome, is altered. Selected genes are 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, that are then allowed to 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 place in liquid culture medium with Agrobacterium, then hormones are used to induce rooting thereby regenerate plantlets which are grown on selection media. A third 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 S J and Bent A F, 1998. Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. Plant J 16:735-43).

[0106] 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. It has been demonstrated 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).

[0107] 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 has entered the protoplast it can be integrated into the cells genome. Standard tissue culture techniques are then used to regenerate transgenic plants.

[0108] 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.

[0109] The method chosen for genetically engineering plants is most often dependent on the targeted plant species and which methods have been proven effective therein.

[0110] 5.5 Preparation of Antibodies

[0111] Antibodies which specifically recognize one of the described phosphatase polypeptides or fragments thereof can be used for detecting, screening, and isolating the polypeptide of the invention or fragments thereof, or similar sequences that might encode similar enzymes from the other organisms. For example, in one specific embodiment, an antibody which immunospecifically binds AtPAP2 or 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 of the invention or fragments, derivatives, homologues, or variants thereof, or similar molecules having the similar enzymatic activities as the phosphatase polypeptides, in samples, for example, a biological material, including plant cells, plants, food, drinks, or any materials derived from plants.

[0112] Antibodies specific for the described phosphatase polypeptides can be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of-interest can be produced by various procedures well known in the art. For example, an antigen derived from the phosphatase polypeptide can be administered to various host animals including,

but not limited to, rabbits, mice, rats, etc., to induce the production of antisera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete) adjuvant, mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful adjuvants for humans such as BCG (Bacille Calmette-Guerin) and Corynebacterium parvum. Such adjuvants are also well known in the art.

[0113] Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: Monoclonal Antibodies and T-Cell Hybridomas, pp. 563-681 (Elsevier, N.Y., 1981) (both of which are incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

[0114] Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art. In a non-limiting example, mice can be immunized with an antigen of interest or a cell expressing such an antigen. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells. Hybridomas are selected and cloned by limiting dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding the antigen. Ascites fluid, which generally contains high levels of antibodies, can be generated by inoculating mice intraperitoneally with positive hybridoma clones.

[0115] Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')₂ fragments may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')₂ fragments). F(ab')₂ fragments contain the complete light chain, and the variable region, the CH1 region and the hinge region of the heavy chain.

[0116] The antibodies or fragments thereof can be also produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

[0117] The nucleotide sequence encoding an antibody may be obtained from any information available to those skilled in the art (i.e., from Genbank, the literature, or by routine cloning). If a clone containing a nucleic acid encoding a particular antibody or an epitope-binding fragment thereof is not available, but the sequence of the antibody molecule or epitope-binding fragment thereof is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA

library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

[0118] Once the nucleotide sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., supra; and Ausubel et al., eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence by, for example, introducing amino acid substitutions, deletions, and/or insertions into the epitope-binding domain regions of the antibodies or any portion of antibodies which may enhance or reduce biological activities of the antibodies.

[0119] Recombinant expression of an antibody requires construction of an expression vector containing a nucleotide sequence that encodes the antibody. Once a nucleotide sequence encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art as discussed in the previous sections. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The nucleotide sequence encoding the heavy-chain variable region, light-chain variable region, both the heavy-chain and light-chain variable regions, an epitope-binding fragment of the heavy- and/or light-chain variable region, or one or more complementarity determining regions (CDRs) of an antibody may be cloned into such a vector for expression. Thus-prepared expression vector can be then introduced into appropriate host cells for the expression of the antibody. Accordingly, embodiments include host cells containing a polynucleotide encoding an antibody specific for the disclosed phosphatase polypeptides or fragments thereof.

[0120] The host cell can be co-transfected with two expression vectors, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides or different selectable markers to ensure maintenance of both plasmids. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, 1986, *Nature*, 322:52; and Kohler, 1980, *Proc. Natl. Acad. Sci. USA*, 77:2197). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

[0121] In another embodiment, antibodies can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains, such as Fab and Fv or disulfide-bond stabilized Fvs, expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phages used in these methods are typically filamentous phage, including fd and M13. The antigen binding domains are expressed as a recombinantly fused protein to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the immunoglobulins, or fragments thereof, include those disclosed in Brinkman et al., 1995, J. Immunol. Methods 182:41-50; Ames et al., 1995, J. Immunol. Methods 184:177-186; Kettleborough et al., 1994, Eur. J. Immunol., 24:952-958; Persic et al., 1997, Gene, 187:9-18; Burton et al., 1994, Advances in Immunology 57:191-280; PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Pat. Nos. 5,698,426; 5,223,409; 5,403,484; 5,580, 717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

[0122] As described in the above documents, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired fragments, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab)2 fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., 1992, Bio-Techniques 12(6):864-869; and Sawai et al., 1995, AJRI 34:26-34; and Better et al., Science, 240:1041-1043, 1988 (each of which is incorporated by reference in its entirety). Examples of techniques which can be used to produce singlechain Fvs and antibodies include those described in U.S. Pat. Nos. 4,946,778 and 5,258,498; Huston et al., 1991, Methods in Enzymology 203:46-88; Shu et al., 1993, PNAS 90:7995-7999; and Skerra et al., 1988, Science 240:1038-1040.

[0123] Once an antibody molecule has been produced by any methods described above, 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. [0124] 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. Pat. No. 5,474,981; Gillies et al., 1992, PNAS 89:1428-1432; and Fell et al., 1991, *J. Immunol.* 146:2446-2452, which are incorporated herein by reference in their entireties.

[0125] 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, polystyrene, polyvinyl chloride or polypropylene.

[0126] 5.6 Detection Assays

[0127] An exemplary method for detecting the presence or absence of an overexpressed phosphatase polypeptide or an inserted phosphatase-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 polypeptides. The nucleic acid probe can be, for example, a full-length cDNA, such as the nucleic acid of SEQ ID NOS: 1,3, 5, 7, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44 or 46, 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.

[0128] An exemplary agent for detecting an over-expressed phosphatase polypeptide is an antibody capable of binding to a phosphatase polypeptide product of an inserted phosphatase gene, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or F(ab')₂) can be used. See also the detailed descriptions about antibodies in Section 5.5.

[0129] 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.

[0130] In a specific embodiment, the methods further involve obtaining a control sample from a control subject, 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 comparing the presence of the phosphatase polypeptide or mRNA or genomic DNA encoding the polypeptide in the control sample with the presence of the polypeptide or mRNA or genomic DNA encoding endogenous phosphatase polypeptides in the test sample.

[0131] Embodiments also encompass kits for detecting the presence of a heterologous polypeptide or nucleic acid in a test sample.

[0132] The kit, for example, can comprise a labeled compound or agent capable of detecting the polypeptide or mRNA encoding the polypeptide in a test sample and means for determining the amount of the polypeptide or mRNA in the sample (e.g., an antibody which binds the polypeptide or an oligonucleotide probe which binds to DNA or mRNA encoding the polypeptide). Kits can also optionally include instructions for use.

[0133] For antibody-based kits, the kit can comprise, for example: (1) a first antibody (e.g., attached to a solid support) which binds to a phosphatase polypeptide; and, optionally, (2) a second, different antibody which binds to either the polypeptide or the first antibody and is conjugated to a detectable agent.

[0134] For oligonucleotide-based kits, the kit can comprise, for example: (1) an oligonucleotide, e.g., a detectably labeled oligonucleotide, which hybridizes to a nucleic acid sequence encoding an inserted phosphatase polypeptide or (2) a pair of primers useful for amplifying a nucleic acid molecule encoding an inserted phosphatase polypeptide. The kit can also comprise, e.g., a buffering agent, a preservative, or a protein stabilizing agent. The kit can also comprise components necessary for detecting the detectable agent (e.g., an enzyme or a substrate). The kit can also contain a control sample or a series of control samples which can be assayed and compared to the test sample contained. Each component of the kit is usually enclosed within an individual container and all of the various containers are within a single package along with instructions for use.

[0135] 5.7 Commercial Application of Transgenic Plants [0136] The transgenic plants generated can have many useful applications, including food, feed, biomass, biofuels (starch, cellulose, seed lipids) and wood pulp. The enhanced growth rate of the transgenic plants may provide additional carbon dioxide fixation per hectare of land per year and thus generate carbon credits.

6. EXAMPLES

[0137] The following examples illustrate the cloning of AtPAP2, its overexpression in transgenic *Arabidopsis*, and the characterization of the transgenic plants. These examples should not be construed as limiting. The following examples illustrate some embodiments. Unless otherwise indicated in the following examples and elsewhere in the specification and

claims, all parts and percentages are by weight, all temperatures are in degrees Centigrade, and pressure is at or near atmospheric pressure.

6.1 Sequence Alignment and Phylogenetic Analysis

[0138] PAP2 locus and its genomic organization, including its intron/exon boundaries, were identified in the *Arabidopsis* Col-0 ecotype (http://www.arabidopsis.org). Sequence alignment and phylogenetic tree were conducted using MEGA4 (Kumar et al., 2004) and ClustalW program (http://www.ebi. ac.uk/Tools/clustalw2/index.html). Amino acid sequence comparisons were performed using CLC Sequence Viewer 5.1.1 (www.cicbio.com).

[0139] Twenty nine PAP-like sequences were identified from the *Arabidopsis* genome and a phylogenetic tree was produced by neighbor-joining algorithm (FIG. 1). The gene locus of AtPAP2 (At1g13900) composes of two exons and the coding region is 1971 bp in length (SEQ ID NO: 1), which is predicted to encode a polypeptide of ~73.7-KD. Among the twenty nine PAP-like protein sequences, only AtPAP2 and AtPAP9 carry a unique hydrophobic motif at their C-termini by TMHMM analysis (http://www.cbs.dtu.dk/services/TM-HMM-2.0/) (FIG. 3). AtPAP2 was found to share 72% sequence identity in amino acid sequence with AtPAP9. Two sequences from *Zea mays* (Accession No: ACG47621) and *Oryza sativa* (Accession No: BAC15853.1) were found to share 58% and 57% a.a. identity with AtPAP2, respectively. Their sequences were aligned in FIG. 2.

[0140] AtPAP2-like sequences from other plant species that carry a hydrophobic motif at their C-termini were retrieved by tblastn program from Plant GDB database (http://www.plantgdb.org/) and NCBI database (http://blast. ncbi.nlm.nih.gov/Blast.cgi) using the amino acid sequence of AtPAP2 as the search sequence. cDNA and protein sequences that share high homology with that of AtPAP2 were identified in Zea mays (SEQ ID NOs: 7 and 8), Brassica rapa (SEQ ID NOs: 18 and 19), Hordeum vulgare (SEQ ID NOs: 20 and 21), Medicago truncatula (SEQ ID NOs: 22 and 23), Physcomitrella patens (SEQ ID NOs: 24 and 25), Populus trichocarpa (SEQ ID NOs: 26 and 27), Saccharum officinarum (SEQ ID NOs: 28 and 29), Solanum tuberosum (SEQ ID NOs: 30 and 31), Vitis vinifera (SEQ ID NOs: 32 and 33), Oryza sativa (SEQ ID NOs: 34 and 35), Gossypium hirsutum (SEQ ID NOs: 36 and 37) Panicum virgatum (SEQ ID NOs: 38 and 39), Solanum lycopersicum (SEQ ID NOs: 40 and 41), Sorghum bicolor (SEQ ID NOs: 42 and 43) and Triticum aestivum (SEQ ID NOs: 44 and 45).

[0141] The cDNA sequences of AtPAP-like sequences were amplified from a local *Glycine max* variety (SEQ ID NO: 5) and the *Brassica napus* cultivar Westar (SEQ ID NO: 46) by RT-PCR using primers designed from corresponding EST sequences, which were retrieved from the Plant GDB database (http://www.plantgdb.org/).

6.2 Screening of T-DNA Line and Production of Overexpression Lines and Complementation Lines in *Arabidopsis*

[0142] T-DNA insertion lines of PAP2 gene (Arabidopsis genomic locus name: Salk_013567), in the Col ecotype were obtained from *Arabidopsis* Biological Resources Center (Alonso et al., 2003). Homologous T-DNA lines were identified by genomic PCR screening from SIGnAl database (http://signal.salk.edu/cgi-bin/tdnaexpress) by using the

primers (LBa1, 5'-TGGTTCACGTAGTGGGCCATCG-3', SEQ ID NO: 9) and PAP2 specific forward primer (P2LP, 5'-TTGAAGTTTAACATGCCTGGG-3, SEQ ID NO: 10) and reverse primer (P2RP, 5'-TCCAATGCTCGA TTGATT-AGC-3', SEQ ID NO: 11). The PCR product was sequenced and the T-DNA insertion site was confirmed. To exclude the possibility that another T-DNA locus interferes with the PAP2 mutant site, homologous pap2 mutant lines were backcrossed to the wild-type to dilute the potential T-DNA sites. The produced heterozygous pap2 mutants were grown on the MS plates containing 50 mg/ml Kanamycin. The ratio of the resistant to sensitive plants was about 3:1. These results demonstrated a single insertion locus site of the T-DNA line (pap2-8) lines.

[0143] The inability of the T-DNA line to express full length AtPAP2 mRNA was confirmed by RT-PCR. Total RNA was extracted from 10-day-old seedlings grown on MS with 2% (w/v) sucrose using the ThIzol RNA isolation method (Invitrogen) with DNase I treatment. cDNAs were generated using Superscript III reverse transcriptase (Invitrogen, Carlsbad, Calif., USA) using an oligo dT primer. Two gene-specific primers, P2YF (5'-GGCCGTCGACAT-GATCGTTAATT TCTCTTTC-3'SEQ ID NO: 12) and P2NR (5'-CCGGACTAGTTCATGTCTCCTCGTTCTTGAC-3' SEQ ID NO: 13), were used to amplify a 1971 bp coding region of AtPAP2. For each sample, 1 µg of cDNA was amplified for 30 cycles, with an annealing temperature of 50° C. and using elongation factor (EF) primers, EF-1 (5'-GTTTCACATCAACATTGTGGTCA TTGG-3, SEQ ID NO: 14) and EF-2 (5'-GAGTACTTGGGGGTAGTG-GCATCC-3, SEQ ID NO: 15) (Axelos et al., 1989) for control experiment.

[0144] The inability of the T-DNA line to express protein was confirmed by Western blotting analysis (FIG. 4). Antiserum specific to AtPAP2 was raised in rabbit as described in Section 6.3.

[0145] To create transgenic AtPAP2 overexpressing lines or expressing this gene in the knockout mutants, the full length coding region of the AtPAP2 cDNA was amplified by PCR using primers P2YF (SEQ ID NO: 12) and P2NR (SEQ ID NO: 13). A SalI site and a SpeI site were engineered into P2YF and P2NR, respectively. The resulting product (1976 bp) was inserted into the Xhol/Spe I sites of a binary vector, immediately downstream to the cauliflower mosaic virus (CaMV) 35S promoter (FIG. 5).

[0146] 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 PAP2-overexpressing lines or into homologous pap2 plants (T-DNA lines) to generate complementation lines. Through 2 generations of selection on MS agar plate with 50 mg/l Basta (Riedel-deHaen), homologous 35S:PAP2 transgenic lines were obtained. The resistant plants were transferred to soil to grow to maturity, and their transgenic status was further confirmed by PCR and immunoblot analyses. As shown in FIG. 6A, AtPAP2 protein was overexpressed in OE lines but was absence from the T-DNA line. The homozygous T3 seeds of the transgenic plants were used for further analysis.

[0147] To create transgenic AtPAP15 overexpression lines, the cDNA of AtPAP15 was also amplified by RT-PCR and then subcloned into a plant binary vector which bared a kanamycin-resistant gene and a cauliflower mosaic virus 35S promoter (CaMV). This expression construct named was then

mobilized into *Agrobacterium tumefaciens* strain EHA105 by freeze-thaw transformation (Hofgen and Willmitzer, 1988) and transformed into *Arabidopsis*. Transgenic status was further confirmed by PCR and immunoblot analyses using an anti-AtPAP15 antiserum. As shown in FIG. 6B, AtPAP15 protein was overexpressed in OE lines. The homozygous T3 seeds of the transgenic plants were used for further analysis.

6.3 Production of PAP2 Polycolonal Antiserum and Western Blots Analysis

[0148] A fragment of AtPAP2 cDNA corresponding to the N terminal 120 amino acids (from 21 to 141) was amplified using forward primer P2AF (5'-GGTTGAGCTCGAT-TCTAAAGCGACCATTTC-3', SEQ ID NO: 16) and reverse primer P2AR (5'-TTTTGGTACCTCAGGATCCGAA AGT-CAGC-3', SEQ ID NO: 17). The PCR product was cleaved by SacI and KpnI and cloned into the pRsetA vector (Invitrogen) so that the coding sequence of the first 120 a.a. of AtPAP2 was fused to a His-tag sequence. The resulting plasmid was transformed into Escherichia coli strain BL21 (DE3). The BL21 cells were induced at 30° C. by 0.1 mM isopropylthio-β-Dgalactoside for 4 h and resuspended in 100 mM NaCl and 50 mM Tris-HCl, pH 7.5, 2 mM phenylmethylsulphonyl fluoride (PMSF). The lysates were sonicated 5 times for 30 s each. The overexpressed His-AtPAP2 fusion proteins in inclusion bodies were centrifuged at 5000×g for 15 min, and the pellets were solubilized in 150 mM NaCl, 8 M urea, and 20 mM Tris-HCl, pH 7.5. The fusion proteins were purified on a HisTrap FF (GE Healthcare) column and were used for standard immunization protocols in rabbits.

6.4 Expression Analysis of AtPAP2 mRNA and its Protein Levels

[0149] The mRNA expression level of AtPAP2 was analyzed by the Spot History program (http://affymetrix.arabidopsisinfo/narrays/spothistory.pl) that presented the expression levels of a given gene in thousands of microarray (Affymetrix ATH1 microarray) database. Spot history analysis indicated that the expression of AtPAP2 was constitutive but is relative low in most experimental circumstances (FIG. 7a). To determine AtPAP2 expression levels, different tissues of wild-type *A. thaliana* (Col-0) were collected.

[0150] The expression level of proteins were also studied by western blotting, using the anti-AtPAP2 antiserum generated from Section 6.3. Total plant soluble protein was extracted from wild-type A. thaliana, T-DNA line, AtPAP2overexpress lines in grinding buffer (Tris-HCl 50 mM, pH7.4 containing 150 mM NaCl, 1 mM EDTA, 0.2 mM PMSF) on ice. Protein extracts were centrifuged at 16000xg and supernatants were collected for Bradford protein concentration determination assay. Equal amount of protein samples (50-90 μg/lane in different experiments) were loaded and separated in 12% (w/v) SDS-PAGE. The separated proteins were transferred to Hybond C-Extra membranes (Amersham Biosciences) (400 mA, 1 h). Membranes were blocked with 5% (w/v) non-fat milk in TTBS washing buffer (pH 7.6) for 2 hours and probed with specific anti-AtPAP2 antiserum for 3 hours or overnight at an 1: 1000 dilution at 4° C. After rinsing the membrane with three changes of TTBS washing buffer (20 mM Tris-HCl, pH7.6, 136 mM NaCl, 0.1% Tween20) in half an hour, HRP-labeled secondary antibody, diluted 1:10, 000 in TTBS washing buffer was added. After 2 hours, the

membrane was washed thrice before the bands were visualized by Enhanced Chemiluminescence method (Amersham Biosciences). As shown in FIG. 7b, AtPAP2 protein was expressed in all tissues tested (Leaf, Flower, Stem, Root, Silique) at equal levels. The protein expression level of AtPAP2 during germination was very stable too (FIG. 7c) and was independent of phosphorus status (FIG. 7d).

6.5 Growth Phenotypes Of WT, T-DNA Line and OE Lines

[0151] Arabidopsis seeds were soaked in water at 4° C. for 3 days. The seeds were surface sterilized and sown on Murashige and Skoog (MS) medium supplemented with 2% (w/v) sucrose for 10 days. Seedlings with 2 rosette leaves of the same size were transferred to soil under Long Day (16 h light at 22° C./8 h dark at 18° C.) or Short Day (8 h light at 22° C./16 h dark at 18° C.) conditions in a plant growth chamber. Flowering time was started to be measured by scoring the number of rosette leaves and cauline leaves when the primary inflorescence florescence reached 1 cm above the rosette leaves. Ten to 20 plants were scored for each line (Liu et al., 2008; Wu et al., 2008).

[0152] The inflorescences of OE lines of AtPAP2 emerged earlier (5-6 days for Long Day, 14-16 days for Short Day) than that of the WT and T-DNA lines (Table 1). Under Long Day conditioning, the number of rosette leaves of the OE lines were less (5-6 leaves) than the WT during the emergence of inflorescence (Table 1 and FIG. 8). At day 28 (Long Day), the OE lines of AtPAP2 had more cauline leaves and inflorescences than the WT and T-DNA lines, but had less rosette leaves (Table 2.). This phenotype observation was repeated at least four times and the results of one of the experiments were shown here.

TABLE 1

A	AtPAP2 OE lines flowered at an earlier developmental stage.									
	Long Day (16 h/8 h)					Short Day (8 h/16 h)				
Lines	AEI	SD	NRL	SD	AEI	SD	NRL	SD		
Col-0 T-DNA OE7 OE21	26.9 25.7 20.0* 20.8*	1.2 0.7 1.1 0.6	13.0 11.6 6.4* 6.5*	0.8 1.1 0.5 0.7	41.0 40.7 25.6* 26.0*	4.7 4.9 1.3 1.1	18.0 15.0 5.3* 5.4*	3.0 3.0 0.5 0.5		

AEI: Average date of emergence of inflorescence

NRL: No. of rosette leaves at the first appearance of inflorescence

*Statistically (p \leq 0.001) different from the wild-type (n = 15)

TABLE 2

Phenotypes of AtPAP2 OE lines at Day 28 (Long Day).										
Lines	No. of Rosette Leaf	SD	No. of Cauline Leaf	SD	No. of Inflorescence	SD				
Col-0	14.5	1.2	1.6	0.5	1.0	0.0				
T-DNA	16.7	1.7	1.9	0.6	1.0					
OE7	9.9*	1.0	6.0*	1.2	3.6*	0.7				
OE21	10.2*	1.8	7.2*	1.6	3.7*	1.1				

^{*}Statistically (p < 0.001) different from the wild-type (n = 15).

[0153] At maturity (Long Day), the number of siliques and the total weight of seeds harvested from each line were recorded. Two separate experimental trials are shown in Tables 3A and 3B. Our results showed that overexpression of

AtPAP2 resulted in increase number of siliques per plant and the seed yield per plant. Compared to that of the wild-type, the seed yield of the two overexpression lines shown in Table 3A increased 38-40%. Compared to that of the wild-type, the seed yield of the two overexpression lines shown in Table 3B increased 54-58%.

TABLE 3A

OE lines produced more siliques and seeds (Trial 1).										
Lines	No. of siliques/plant	SD	Weight of seeds (g)/plant	SD	N					
Col-0 T-DNA OE7 OE21	327.4 236.6* 453.2** 498.2#	53.3 60.2 62.1 52.5	0.188 0.121* 0.264** 0.260**	0.047 0.040 0.039 0.049	5 7 5 7					

Statistically (p < 0.02*, p < 0.01**, p < 0.001**) different from the wild-type.

TABLE 3B

OE lines produced more siliques and seeds (Trial 2).									
Lines	No. of siliques/plant	SD	Weight of seeds (g)/plant	SD	N				
Col-0	396.4	89.5	0.225	0.058	13				
T-DNA	386.3	70.4	0.240	0.049	12				
OE7	610.9*	76.6	0.351*	0.050	7				
OE21	624.9*	94.7	0.355*	0.066	11				

Statistically (p < 0.0001*) different from the wild-type.

[0154] However, the OE lines of AtPAP15 grew normally and were not different from the wild-types. Therefore, the enhanced growth performance was due to the overexpression of AtPAP2, which bears a transmembrane-like motif at its C-terminus (FIGS. 2 and 3).

6.6 Growth Phenotypes of Truncated AtPAP2 Constructs

[0155] An alternate vector construct employing the sequence for AtPAP2 was also constructed using analogous techniques to those described above. As shown in FIG. 12, a construct equivalent to the OE lines of AtPAP2 missing the C-terminal motif (residues 614-636 of SEQ ID NO: 2) was constructed(P2C lines). Transgenic plants were generated using substantially identical techniques to those described above. Western blot analysis was used to confirm the overexpression of the AtPAP2 fragment proteins in transformed plant lines. Performance of Western blot analysis was identical to that reported above. As show in FIG. 13, the P2C lines were strongly overexpressed. The growth phenotype of the P2C lines appeared to be indifferent from the wild-type, which is indicative of the importance of the C-terminal domain of AtPAP2 in developing an increased growth phenotype.

6.7 MS/MS Analysis of Sucrose and Glucose Levels in Leaf

[0156] Rosette leaves of plants of various developmental stages were harvested at the end of the light period of 21-day-old plants. Soluble sugars were extracted from *Arabidopsis* using chloroform/methanol method (Lunn et al., 2006; Antonio et al., 2007; Luo et al., 2007). 100 mg plant tissues were ground to a fine powder in liquid nitrogen and mixed and

vortexed with 250 μ l ice-cold chloroform:methanol (3:7, v/v). Soluble metabolites were then extracted at -20° C. overnight. 200 μ l water was added to the mixture with repeated shaking. The extracts were centrifuged at $16000\times g$ for 10 min and the supernatant was collected. The pellet was re-extracted by 200 μ l water and the supernatant was collected by centrifugation as described above. The combined supernatant was evaporated to dryness using a SpeedVac and the pellet was redissolved in 200 μ l water. Finally, debris was removed by centrifugation at $16000\times g$ for 30 min.

[0157] 20 µl filtered samples were analyzed by an API-3000 triple-quadrupole mass spectrometer (Applied Biosystems) via an electrospray ionization source. The parameters, optimized by 0-40 μg/ml glucose and sucrose standards, were as following: curtain gas (CUR) 25, nebulizer gas (GS1) 50, auxiliary gas (GS2) 30, ionspray voltage -4.5 kV, temperature 400° C., declustering potential (DP) -106 V, entrance potential (EP) -8.5 V, collision cell entrance potential (CEP) -46.7 V, collision energy (CE) 20 V. The peaks were identified by comparison with glucose and sucrose standards and the amount of sugars were quantified by standard curves of these sugars. The Analyst 1.3.1 software (Applied Biosystems) was used for data acquisition, peak integration, and calculation. The amount of sucrose and glucose at the end of day in the shoots of 21-day-old soil grown plants were shown in FIG. 9. It was found that the levels of both sugars were significantly higher than that in WT.

6.8 Recovery of Plants after Prolonged Darkness Treatment

[0158] Seeds of wild-type, T-DNA, OE7 and OE21 lines were germinated in MS (2% sucrose) medium for 10 days. Seedlings with 2 small visible rosette leaves (1 mm) of the same size were transferred to soil for another 12 days in normal growth conditions (LDs, 16 h/light (22° C.)/8 h darkness (18° C.)). The light source of the growth chamber was then switched off for 12 days. Then the plants were allowed to recover under the 16 h/light (22° C.)/8 h darkness (18° C.) cycle for 10 days. The plants that stayed green and that continued to emerge inflorescence were recorded in Table 4.

TABLE 4

Surviving rate and flowering ratio after prolonged darkness treatment.									
	Flowering after recovery	Recovery (leaf greening)							
Wild-type	8/12	11/12							
wind-type	0/12								
T-DNA	5/12	8/12							
V 1									

[0159] Extended darkness could induce carbohydrate starvation (Thompson et al., 2005). Our data showed that the OE lines exhibited 100% recovery rate under prolonged (12 days) darkness treatment, which was higher than that of the WT and the T-DNA line (FIG. 10). This could be attributed to a higher endogenous sugar levels (FIG. 9) in the OE lines.

6.9 Phenotypes of Plants Under NaCl and ABA Treatments

[0160] 5-day-old seedlings grown on MS agar were transferred to MS agar with NaCl (50 mM, 100 mM, 150 mM), ABA (0.1 uM, 0.2 uM. 0.5 uM, 1 uM, 2 uM) or sorbitol (300

mM, 400 mM, 500 mM). Alternatively, seeds were directly germinated on the treatment media. Wild-type, T-DNA and OE lines did not show remarkable phenotypic differences under the above conditions.

6.10 Subcellular Fractionation

[0161] Rosette leaves of three-week-old wild-type (Col-0) Arabidopsis were harvested and stored at -80° C. freezer until use. Tissue (4-5 g) were ground to fine powder in liquid nitrogen using a mortar with a pestle. The powder was transferred into 10 ml grinding buffer (0.3 M sucrose, 40 mM Tris-HCl (pH 7.8), 5 mM MgCl₂, 1 mM PMSF) and swelled on ice for 5 min. Homogenization was performed for two 30-second pulses at high-speed setting. The homogenate was filtered through two layers of Miracloth (Tetko, Elmsford, N.Y., USA). Subsequently, the homogenate was separated by centrifugation at 350 g for 10 min at 4° C. The pellet (crude nuclear) was further layered onto 1 ml of 2.3 M sucrose, 50 mM Tris-HCl (pH 8.8), 5 mM MgCl₂ in an Eppendorf tube for centrifugation at 15,000 g 10 min at 4° \hat{C} , to obtain the nuclear fraction in the derived pellet. Supernatants from the first low-speed centrifugation (350 g) were centrifuged at 12,000×g for 20 min at 4° C. The pellet contained large particles including mitochondria, chloroplasts and peroxisomes. The supernatant was further centrifuged at 100,000×g for 1 h at 4° C. to yield the soluble cytosol fraction in the resulting supernatant. The pellet representing the membrane fraction was resuspended in 0.1 ml grinding buffer. Protein concentration in the extract was determined following the method of Bradford (Bradford, 1976) using the Bio-Rad Protein Assay Kit I.

[0162] To isolate cell wall, leaf tissues were homogenized in grinding buffer (62.5 mM Tris-HCl, pH 7.5, 5 mM MT, 1% (v/v) bovine serum albumin, 2 mM phenylmethylsulfonyl fluoride, 2 μ g/ml leupeptin, 2 μ g/ml E-64, 2 μ g/ml pepstatin A) using a Polytron (full speed, 3×10 s). The homogenate was centrifuged at 1,000×g for 3 min. The pellet was washed with ice-cold grinding buffer (without 1% BSA) 10 times. Finally the (cell wall) pellet was washed by resuspending in 500 mM CaCl₂, 20 mM NaCl, 62.5 mM Tris-HCl, pH 7.5, and spinning at 10,000×g for 15 min (He et al., 1996).

[0163] The subcellular fractions were run in a SDS-PAGE gel and were probed with anti-AtPAP2 antiserum. AtPAP2 was detected in membrane and soluble protein fractions but not in nucleus, mitochondria nor chloroplasts (FIG. 11).

[0164] In summary, *Arabidopsis* plants transformed with the AtPAP2 gene have the following phenotypes when they were compared with the wild-type: (1) Faster growth rate (Tables 1 and 2); (2) Higher sucrose content (FIG. 9); (3) Higher glucose content (FIGS. 9); and (4) Higher crop yield (Table 3).

[0165] Those skilled in the art will recognize, or be able to ascertain many equivalents to the specific embodiments described herein using no more than routine experimentation. Such equivalents are intended to be encompassed by the following claims.

[0166] With respect to any figure or numerical range for a given characteristic, a figure or a parameter from one range may be combined with another figure or a parameter from a different range for the same characteristic to generate a numerical range.

[0167] All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each

individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference.

[0168] Citation or discussion of a reference herein shall not be construed as an admission that such is prior art to the present patent application.

[0169] While the embodiments have been explained in relation to certain embodiments, it is to be understood that various modifications thereof will become apparent to those skilled in the art upon reading the specification. Therefore, it is to be understood that the embodiments disclosed herein are intended to cover such modifications as fall within the scope of the appended claims. Features of two or more of any of the above embodiments can be combined to form additional embodiments.

[0170] Other than in the operating examples, or where otherwise indicated, all numbers, values and/or expressions referring to quantities of ingredients, reaction conditions, etc., used in the specification and claims are to be understood as modified in all instances by the term "about."

6.11 Assays of enzymes involved in sucrose metabolism

[0171] Sucrose phosphate synthesis (SPS), sucrose synthesis (SuSy), cytosolic invertase and cell wall invertase activities in the shoot of 20-day-old plants were determined. Samples were collected 8 h after the light and dark period (Long Day). SPS activity was measured under both optimal (Vmax) and limiting (V limit) assay conditions (Park et al., 2008). SuSy, cytosolic invertase and insoluble cell wall invertase activities were also determined (Doehlert, 1987). The assays were repeated three times and the SPS (Vmax and V limit) activities of both independent lines were significantly higher than that of the wild-type and T-DNA lines in all three repeated experiments. The data of a representative experiment is shown in table 5. In contrast to SPS, SuSy, cytosolic invertase and cell wall invertase activities were not different among the lines.

TABLE 5

Enzyme assays									
Plant line	WT	T-DNA	OE7	OE21					
Sucrose phosphate synthase (µM sucrose/µg enzyme extracts/hour)									
Vmax (Day)	108.8 ± 18.1	116.1 ± 11.9	157.9 ± 21.7**	159.5 ± 19.0**					
Vlimit (Day)	63.6 ± 6.4	69.5 ± 2.5	90.8 ± 18.5**	79.8 ± 13.7*					
Vmax (Night)	118.3 ± 11.4	104.9 ± 14.4	150.9 ± 19.4**	136.5 ± 15.1*					
Vlimit (Night)	74.9 ± 6.3	56.7 ± 3.3	93.4 ± 3.6**	97.3 ± 10.9**					
	Sucrose synthas	se (µM glucose/	μg enzyme extrac	ets/hour)					
Day Night	249.2 ± 4.6 249.4 ± 7.2 ytosolic inverta	247.0 ± 24.0 252.7 ± 8.8 ase (μM glucose	248.6 ± 4.5 250.8 ± 8.7 e/µg enzyme extra	255.2 ± 5.4 258.8 ± 5.5 ccts/hour)					
Day (Acid)	14.3 ± 2.3	12.1 ± 5.4	18.3 ± 9.1	18.0 ± 0.8					
Night (Acid)	14.0 ± 6.8	26.1 ± 10.9	17.6 ± 4.3	13.8 ± 0.8					
Day (Alkaline)	169.7 ± 9.8	161.2 ± 32.3	160.9 ± 27.9	178.8 ± 16.9					
Night (Alkaline)	130.0 ± 8.2	105.1 ± 12.6	136.1 ± 13.6	136.6 ± 1.4					

TABLE 5-continued

Enzyme assays									
Plant line	WT	T-DNA	OE7	OE21					
Cell wall invertase (µM sucrose/µg enzyme extracts/hour)									
Day (Acid)	22.3 ± 3.9	18.0 ± 4.9	23.5 ± 6.3	24.0 ± 6.6					
Night (Acid)	21.6 ± 10.1	17.0 ± 4.3	28.3 ± 4.2	22.3 ± 3.3					
Day (Alkaline)	121.7 ± 2.8	127.1 ± 2.2	101.8 ± 6.4	105.5 ± 2.0					
Night (Alkaline)	138.9 ± 6.3	151.5 ± 8.7	123.4 ± 5.6	135.4 ± 14.7					

(**P < 0.01; *P < 0.05)

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SEQUENCE LISTING

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<213> ORGANISM: Arabidopsis thaliana

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<212> TYPE: PRT

<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 4

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Arg Ser Gly Asp Ile Val Val Ile Lys Trp Ser Gly Val Glu Ser Pro $35 \hspace{1.5cm} 40 \hspace{1.5cm} 45 \hspace{1.5cm}$

Ser Asp Leu Asp Trp Leu Gly Ile Tyr Ser Pro Pro Asp Ser Pro His 50 $\,$ 60 $\,$

Asp His Phe Ile Gly Tyr Lys Phe Leu Ser Asp Ser Pro Thr Trp Gln 65 70 70 80

Ser Gly Ser Gly Ser Ile Ser Leu Pro Leu Thr Asn Leu Arg Ser Asn \$85\$ 90 \$95\$

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Gly Glu Pho 545	e Gly Tyr Il 55	-		Asn Lys G 555	Slu Arg Lev	Thr 560
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Glu Gly Al. 610	a Ser Val Me	t Val Val 615	Gly Val	Ile Phe G 620	Sly Tyr Phe	e Val
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<212> TYPE: DNA

<213> ORGANISM: Glycine max

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1920 1965

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Asn Asp Asn Val Asn Gly Gly Ile Gly Ser Ala Lys Pro Glu Gly Gln 600 Ile Lys Glu Ser Thr Leu Ser Trp Tyr Val Gln Gly Gly Ser Val Leu 615 Val Leu Gly Ala Phe Met Gly Tyr Ile Leu Gly Phe Val Ser His Ala 625 630 635 Arg Lys Lys Gln Pro Glu Ser Arg Ser Gly Phe Ser Pro Val Lys Thr 645 650 Glu Glu Thr <210> SEQ ID NO 24 <211> LENGTH: 2007 <212> TYPE: DNA <213> ORGANISM: Physcomitrella patens <400> SEQUENCE: 24 atgggatege aagtatteea ttttettetg gtgttetttg ggtaetttet geatggaget tcatcagaat ctgtgatttt ggacgcgaga cctacaatat tacaacattc aggagaaaat atcactcttg cttggaaggg tgtgaattta ccgacgaaat acgattggct gggtatatat acgcctccta cttctcctga cgaccagcat atcgggtaca tacttctctc ttcctgttca acatggacaa caggcgcctg ctccttgcag atccccttgg tcaacatgcg tgctccttac agtttccgaa ttttcagagg cgtgttcgta aatgtatctg caagtacaaa tgtgactgga 360 tcaaacaatg gggctacaac gatatcattg gatcgggagg gtaatcctct accagatgtc 420 acgaaacggt tagctgcaag cccagttgtt caattctcca attacaacga gccaacacaa 480 attcatctag ctctttcctc ggacgagact gctgttaggg ttatgtttgt cactagggat 540 cctctgagaa gccaagtaag attcggggaa gatggagatg aactgggcaa cacagttgat 600 gctacatcag tcacatactc tcaaattgat atgtgcgatg aacctgcaag ttcttatggg 660 tggagatctc cgggatacat acataatgtt gtgatggggg ggctgaatcc tgggagtcgc 720 tatttctatc gggtaggaag caatgtagga ggatggagct cgacctatag cttcatcgct 780 ccacatcctc qtqctqatqa aacaaatqct ctcatattcq qtqacatqqq tacttcqatt 840 ccttattcaa cqtatcaata cacqcaqaqc qaqaqcaaqa ataccqtqaa qtqqctcaca 900 cqqqacctaq aacaaataqq tqacaaacct aqcttcqtaq cqcacattqq tqacataaqc 960 tatgetegtg gtttatettg getetgggae aacttettea cecaaatega gecegtaget 1020 gcaaqatcac catatcatqt ttqcatqqqa aaccacqaat atqattqqcc tqqqcaacct 1080 ttcaagccag actggtcacc ataccaaaca gatggaggcg gagaatgtgg cgtgccatat agettacget teateatgee gggaaactee teettaceea etggaactae etceecagee accaaaaacc tctattattc cattgatgtt ggggttgtgc atttcctctt ctattctacc gaaaccgatt tccaggtagg ctccccccag tacactttta tagccaacga cttgagaaca gttgacagga acaagacgcc ctttgtggta tttttgggcc atcggccgct ctatacaacc 1380 gattaccgag ccttgttaga cacgatgaca cagaaattag ttcaaacttt tgagcctttg 1440 ttgatagata ccaatgtcac tgtagccttt tgtggccatg tccataagta cgagcgaatg 1500 tgccccttga aaaattacac ctgtattgaa ccatctaagg caaacggtga gcttccaatt 1560 catatggtgg tgggaatggg aggtgctgat caccaaccca ttgatgaccc tctccccagt 1620

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tata	atcaç	ggc t	cacat	tgcaa	ac ga	agaca	atcto	c ato	gacga	attt	cata	atgti	tgg t	caaco	cacgat	1740
ggga	aaggt	ege a	atgat	tgtt	gt c	gaaat	tcca	a gtt	ctg	gatg	atat	caa	gtc 1	ggag	gcatat	1800
gtt	gagto	ga g	ggga	gtcti	t t	ttga	acact	gad	cage	ggag	tgca	aaata	acc 1	tgtg	ggcagg	1860
tctç	gagaa	ata t	tgta	agcat	tt co	etgtt	tgtt	tta	agcgt	tgg	gtt	gtgga	atg (gggg	gegget	1920
gcta	actct	tt t	ttt	catgo	cg ga	aggca	agcaç	g agg	gaago	caga	tttg	ggca	gcc 1	gtca	accgt	1980
gag	gaago	cta ç	gttci	ttct	ca at	tata	aa									2007
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					Dho	шіа	Dho	Lou	Lou	Wal.	Dho	Dho	Clv	Тиги	Pho	
nec 1	GIY	ser	GIII	5 5	Pne	HIS	Pne	ьeu	10	vai	Pne	Pne	GIY	Tyr 15	Pne	
Leu	His	Gly	Ala 20	Ser	Ser	Glu	Ser	Val 25	Ile	Leu	Asp	Ala	Arg 30	Pro	Thr	
Ile	Leu	Gln 35	His	Ser	Gly	Glu	Asn 40	Ile	Thr	Leu	Ala	Trp 45	Lys	Gly	Val	
Asn	Leu 50	Pro	Thr	Lys	Tyr	Asp 55	Trp	Leu	Gly	Ile	Tyr 60	Thr	Pro	Pro	Thr	
Ser 65	Pro	Asp	Asp	Gln	His 70	Ile	Gly	Tyr	Ile	Leu 75	Leu	Ser	Ser	Cys	Ser 80	
Thr	Trp	Thr	Thr	Gly 85	Ala	CÀa	Ser	Leu	Gln 90	Ile	Pro	Leu	Val	Asn 95	Met	
Arg	Ala	Pro	Tyr 100	Ser	Phe	Arg	Ile	Phe 105	Arg	Gly	Val	Phe	Val 110	Asn	Val	
Ser	Ala	Ser 115	Thr	Asn	Val	Thr	Gly 120	Ser	Asn	Asn	Gly	Ala 125	Thr	Thr	Ile	
Ser	Leu 130	Asp	Arg	Glu	Gly	Asn 135	Pro	Leu	Pro	Asp	Val 140	Thr	Lys	Arg	Leu	
Ala 145	Ala	Ser	Pro	Val	Val 150	Gln	Phe	Ser	Asn	Tyr 155	Asn	Glu	Pro	Thr	Gln 160	
Ile	His	Leu	Ala	Leu 165	Ser	Ser	Asp	Glu	Thr 170	Ala	Val	Arg	Val	Met 175	Phe	
Val	Thr	Arg	Asp 180	Pro	Leu	Arg	Ser	Gln 185	Val	Arg	Phe	Gly	Glu 190	Asp	Gly	
Asp	Glu	Leu 195	Gly	Asn	Thr	Val	Asp 200	Ala	Thr	Ser	Val	Thr 205	Tyr	Ser	Gln	
Ile	Asp 210	Met	Сув	Asp	Glu	Pro 215	Ala	Ser	Ser	Tyr	Gly 220	Trp	Arg	Ser	Pro	
Gly 225	Tyr	Ile	His	Asn	Val 230	Val	Met	Gly	Gly	Leu 235	Asn	Pro	Gly	Ser	Arg 240	
Tyr	Phe	Tyr	Arg	Val 245	Gly	Ser	Asn	Val	Gly 250	Gly	Trp	Ser	Ser	Thr 255	Tyr	
Ser	Phe	Ile	Ala 260	Pro	His	Pro	Arg	Ala 265	Asp	Glu	Thr	Asn	Ala 270	Leu	Ile	
Phe	Gly	Asp 275	Met	Gly	Thr	Ser	Ile 280	Pro	Tyr	Ser	Thr	Tyr 285	Gln	Tyr	Thr	
a1.	a	a 1.	a	T	7	m1	**- 7	T	m	T	m1	7	7	T	a 1	

Gln Ser Glu Ser Lys Asn Thr Val Lys Trp Leu Thr Arg Asp Leu Glu

Gln Ile Gly Asp Lys Pro Ser Phe Val Ala His Ile Gly Asp Ile Ser Tyr Ala Arg Gly Leu Ser Trp Leu Trp Asp Asn Phe Phe Thr Gln Ile 330 Glu Pro Val Ala Ala Arg Ser Pro Tyr His Val Cys Met Gly Asn His 345 Glu Tyr Asp Trp Pro Gly Gln Pro Phe Lys Pro Asp Trp Ser Pro Tyr 360 Gln Thr Asp Gly Gly Glu Cys Gly Val Pro Tyr Ser Leu Arg Phe Ile Met Pro Gly Asn Ser Ser Leu Pro Thr Gly Thr Thr Ser Pro Ala Thr Lys Asn Leu Tyr Tyr Ser Ile Asp Val Gly Val Val His Phe Leu 410 Phe Tyr Ser Thr Glu Thr Asp Phe Gln Val Gly Ser Pro Gln Tyr Thr 425 Phe Ile Ala Asn Asp Leu Arg Thr Val Asp Arg Asn Lys Thr Pro Phe 435 440 445Val Val Phe Leu Gly His Arg Pro Leu Tyr Thr Thr Asp Tyr Arg Ala Leu Leu Asp Thr Met Thr Gln Lys Leu Val Gln Thr Phe Glu Pro Leu 475 Leu Ile Asp Thr Asn Val Thr Val Ala Phe Cys Gly His Val His Lys 490 Tyr Glu Arg Met Cys Pro Leu Lys Asn Tyr Thr Cys Ile Glu Pro Ser 505 Lys Ala Asn Gly Glu Leu Pro Ile His Met Val Val Gly Met Gly Gly 520 Ala Asp His Gln Pro Ile Asp Asp Pro Leu Pro Ser Gln Ser Gln Pro 535 Ile Phe Pro Gln Pro Ser Trp Ser Val Phe Arg Thr Phe Glu Trp Gly 550 555 Tyr Ile Arg Leu His Ala Thr Arg His Leu Met Thr Ile Ser Tyr Val $_{565}$ $_{570}$ Gly Asn His Asp Gly Lys Val His Asp Val Val Glu Ile Pro Val Leu $580 \hspace{1.5cm} 585 \hspace{1.5cm} 585 \hspace{1.5cm} 590 \hspace{1.5cm}$ Asp Asp Ile Lys Ser Gly Ala Tyr Val Glu Ser Arg Glu Ser Phe Phe Asp Thr Ala Ser Gly Val Gln Ile Pro Cys Gly Arg Ser Glu Asn Ile Val Ala Phe Leu Phe Val Leu Ala Leu Gly Cys Gly Cys Gly Ala Ala Ala Thr Leu Phe Phe Met Arg Arg Gln Gln Arg Lys Gln Ile Trp Gln Pro Val Asn Arg Glu Glu Ala Ser Ser Ser Gln Leu <210> SEQ ID NO 26 <211> LENGTH: 1944

<212> TYPE: DNA

<213> ORGANISM: Populus trichocarpa

<400> SEQUENCE: 26

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<210> SEQ ID NO 27

<211> LENGTH: 647

<212> TYPE: PRT

<213> ORGANISM: Populus trichocarpa

<400> SEQUENCE: 27

Met Lys Leu Pro Ile Phe Leu Leu Leu Leu Leu Leu Ser Leu Ile Thr 1 $$ 5 $$ 10 $$ 15

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Gln	Lys	Ser 35	Gly	Asp	Thr	Val	Thr 40	Ile	Ser	Trp	Ser	Asn 45	Val	Asp	Ser
Pro	Ser 50	Lys	Leu	Asp	Trp	Leu 55	Gly	Leu	Tyr	Ser	Pro 60	Pro	Asp	Ser	Pro
His 65	Asp	His	Phe	Ile	Gly 70	Tyr	Lys	Phe	Leu	Ser 75	Ser	Ser	Pro	Ser	Trp 80
Gln	Ser	Gly	Ser	Gly 85	Ser	Ile	Ser	Leu	Pro 90	Ile	Thr	Asn	Leu	Arg 95	Ser
Asn	Tyr	Ser	Phe 100	Arg	Ile	Phe	His	Trp 105	Thr	Glu	Ser	Glu	Ile 110	Asn	Pro
Lys	Arg	His 115	Asp	His	Asp	His	Asn 120	Pro	Leu	Pro	Gly	Thr 125	Ala	His	Phe
Leu	Ala 130	Glu	Ser	Asp	Val	Val 135	Gly	Phe	Glu	Ser	Gly 140	His	Gly	Pro	Glu
Gln 145	Ile	His	Leu	Ala	Tyr 150	Thr	Asp	Asp	Glu	Asp 155	Glu	Met	Arg	Val	Met 160
Phe	Val	Val	Gly	Asp 165	Gly	Glu	Glu	Arg	Gly 170	Val	Lys	Trp	Gly	Glu 175	Arg
Asp	Gly	Glu	Trp 180	Ser	His	Val	Ser	Gly 185	Ala	Arg	Val	Val	Arg 190	Tyr	Glu
Arg	Glu	Asp 195	Met	Cys	Asp	Ala	Pro 200	Ala	Asn	Gly	Ser	Ile 205	Gly	Trp	Arg
Asp	Pro 210	Gly	Trp	Ile	His	Asp 215	Gly	Val	Met	Lys	Asp 220	Leu	Lys	Lys	Gly
Val 225	Arg	Tyr	Tyr	Tyr	Gln 230	Val	Gly	Ser	Asp	Ser 235	Lys	Gly	Trp	Ser	Thr 240
Thr	Arg	Ser	Phe	Val 245	Ser	Arg	Asn	Gly	Asp 250	Ser	Asp	Glu	Thr	Ile 255	Ala
Phe	Leu	Phe	Gly 260	Asp	Met	Gly	Thr	Ser 265	Thr	Pro	Tyr	Ala	Thr 270	Phe	Ile
Arg	Thr	Gln 275	Asp	Glu	Ser	Ile	Ser 280	Thr	Met	Lys	Trp	Ile 285	Leu	Arg	Asp
Ile	Glu 290	Ala	Ile	Gly	_	Lys 295		Ala	Phe		Ser 300		Ile	Gly	Asp
Ile 305	Ser	Tyr	Ala	Arg	Gly 310	Tyr	Ser	Trp	Leu	Trp 315	Asp	His	Phe	Phe	Thr 320
Gln	Val	Glu	Pro	Val 325	Ala	Ser	Lys	Val	Pro 330	Tyr	His	Val	Cys	Ile 335	Gly
Asn	His	Glu	Tyr 340	Asp	Trp	Pro	Leu	Gln 345	Pro	Trp	Lys	Pro	Asp 350	Trp	Ala
Asn	Ala	Val 355	Tyr	Gly	Thr	Asp	Gly 360	Gly	Gly	Glu	Сув	Gly 365	Val	Pro	Tyr
Ser	Leu 370	Lys	Phe	Asn	Met	Pro 375	Gly	Asn	Ser	Ser	380 380	Ser	Thr	Gly	Thr
Arg 385	Ala	Pro	Ala	Thr	Arg 390	Asn	Leu	Tyr	Tyr	Ser 395	Phe	Asp	Thr	Gly	Ala 400
Val	His	Phe	Val	Tyr 405	Ile	Ser	Thr	Glu	Thr 410	Asn	Phe	Val	Ala	Gly 415	Ser
Ser	Gln	Tyr	Asn	Phe	Ile	Lys	Gln	Asp	Leu	Glu	Ser	Val	Asp	Arg	Ser

											-	con	tin	ued		
			420					425					430			
ГÀа	Thr	Pro 435	Phe	Val	Val	Val	Gln 440	Gly	His	Arg	Pro	Met 445	Tyr	Thr	Thr	
Ser	Asn 450	Glu	Asn	Arg	Asp	Ala 455	Pro	Met	Arg	Asn	Lys 460	Met	Leu	Glu	His	
eu 65	Glu	Pro	Leu	Phe	Thr 470	Lys	Tyr	Asn	Val	Thr 475	Leu	Ala	Leu	Trp	Gly 480	
lis	Val	His	Arg	Tyr 485	Glu	Arg	Phe	CÀa	Pro 490	Val	Asn	Asn	Phe	Ile 495	Cha	
Gly	Ser	Thr	Trp 500	Lys	Gly	Phe	Pro	Val	His	Ala	Val	Ile	Gly 510	Met	Ala	
Gly	Gln	Asp 515	Trp	Gln	Pro	Ile	Trp 520	Glu	Pro	Arg	Ser	Asp 525	His	Pro	Asn	
Asp	Pro 530		Phe	Pro	Gln	Pro 535		Arg	Ser	Met	Phe 540	Arg	Gly	Gly	Glu	
Phe 545		Tyr	Thr	ГÀа	Leu 550	Val	Ala	Thr	Lys	Glu 555	ГÀз	Leu	Thr	Leu	Thr 560	
	Val	Gly	Asn	His 565		Gly	Lys	Met	His 570		Met	Val	Glu	Phe 575		
Ala	Ser	Gly	Glu 580		Leu	Ser	Gly	Asp 585		Ser	Ile	Ser	Val		Ala	
Gly	Ala	Arg 595		Gly	Val	Val	Asp		Thr	Phe	Ser	Trp		Val	Lys	
Gly	Ala 610		Val	Leu	Val	Leu 615		Ala	Phe	Val	Gly 620	Tyr	Thr	Leu	Gly	
Tyr 625		Ser	His	Ser	Arg		Gln	Asn	Gly	Asn 635		Ala	Ser	Trp	Thr 640	
	Val	Lys	Ser	Glu 645		Ile										
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gccg	geg	ggg (ctgc	ggcg	gg c	acca	ccct	c ac	egeg.	accc	tct	ccag	cga (ccaga	atcaaç	g
atco	gcto	gga (cagg	cctc	cc g	gccc	cgga	gg(cctc	gact	acg	tegg	cat (ctact	cgccg	g
ccgt	cct	ccc (gcga	ccgc	ga c	ttcci	cgg	c ta	cctc	ttcc	tca	acgg	ctc (egeet	cctg	g
cgcg	geg	gct o	cagg	ggag	ct c	tccct	caaq	g cg	cctc	ccga	ccc.	tgcg	ege (gccct	accaç	g
ttcc	gcct	ct t	teeg	ctgg	cc c	gccaa	acga	g ta	ctcc	tacc	acc.	acat	cga (ccato	gaccgo	g
															ggcgad	
															gggt	
															gaagad	
gaga	agga	agt (ggaa	ggag	gt g	ggca	cggat	gt	gagc	acgt	aca	agca	aaa (gcaca	atgtgo	С

660 720

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tcatcaagct	tccaatactc	tggtgctcct	gtgcatcttg	tgattgggat	gggcggggca	1560
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atattttccg	gcctggtatc	tagtaacagt	agtgttgctg	aggtggtgga	tgatactaaa	1800
catggcacag	gagtcagcac	cgtgcgaaaa	atatctccgt	tgtacttgga	aatcggaggc	1860
agtgtattgt	ttgcactgct	tctgggattt	tcctttggat	ttcttatcag	gagaaagaaa	1920
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<210> SEQ ID NO 29

<211> LENGTH: 653

<212> TYPE: PRT

<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 29

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Ala Ala Val Ala Ala Gly Gly Ala Ala Ala Gly Thr Thr Leu Thr Ala $20 \hspace{1.5cm} 25 \hspace{1.5cm} 30 \hspace{1.5cm}$

Thr Leu Ser Ser Asp Gln Ile Lys Ile Arg Trp Thr Gly Leu Pro Ala \$35\$

Asp Arg Asp Phe Leu Gly Tyr Leu Phe Leu Asn Gly Ser Ala Ser Trp 65 70 75 80

Arg Gly Gly Ser Gly Glu Leu Ser Leu Pro Arg Leu Pro Thr Leu Arg 85 90 95

Ala Pro Tyr Gln Phe Arg Leu Phe Arg Trp Pro Ala Asn Glu Tyr Ser 100 105 110

Tyr His His Ile Asp His Asp Arg Asn Pro Leu Pro His Gly Lys His 115 \$120\$

Arg Val Ala Val Ser Ala Asp Val Ser Val Gly Asp Pro Ala Arg Pro 130 \$130\$

Glu Gln Val His Leu Ala Phe Ala Asp Gly Ile Asp Glu Met Arg Val

145			150					155					160
Leu Phe ¹	Val C	ys Gly 165	Asp	Arg	Gly	Lys	Arg 170	Val	Val	Arg	Tyr	Gly 175	Leu
Gln Lys (sp Glu 80	Lys	Glu	Trp	Lys 185	Glu	Val	Gly	Thr	Asp 190	Val	Ser
Thr Tyr I	Lys G 195	ln Lys	His	Met	Сув 200	Asp	Trp	Pro	Pro	Asn 205	Ser	Ser	Val
Ala Trp 2	Arg A	sp Pro	Gly	Phe 215	Val	Phe	Asp	Gly	Leu 220	Met	Lys	Gly	Leu
Glu Pro 0 225	Gly A	rg Arg	Tyr 230	Phe	Tyr	Lys	Val	Gly 235	Ser	Asp	Thr	Gly	Gly 240
Trp Ser (Glu I	le Tyr 245	Ser	Phe	Ile	Ser	Arg 250	Asp	Ser	Glu	Ala	Asn 255	Glu
Thr Asn		he Leu 60	Phe	Gly	Asp	Met 265	Gly	Thr	Tyr	Val	Pro 270	Tyr	His
Thr Tyr :	Ile A 275	rg Thr	Gln	Asp	Glu 280	Ser	Leu	Ser	Thr	Val 285	Lys	Trp	Ile
Leu Arg 2	Asp I	le Glu	Ala	Leu 295	Gly	Asp	Lys	Pro	Ala 300	Phe	Ile	Ser	His
Ile Gly 2	Asp I	le Ser	Tyr 310	Ala	Arg	Gly	Tyr	Ser 315	Trp	Val	Trp	Asp	His 320
Phe Phe	Ser G	ln Ile 325	Glu	Pro	Ile	Ala	Ala 330	Asn	Thr	Pro	Tyr	His 335	Val
Cys Ile (sn His 40	Glu	Tyr	Asp	Trp 345	Pro	Ser	Gln	Pro	Trp 350	Lys	Pro
Trp Trp 7	Ala T 355	hr Tyr	Gly	ГÀа	Asp 360	Gly	Gly	Gly	Glu	Сув 365	Gly	Ile	Pro
Tyr Ser \ 370	Val L	ys Phe	Arg	Met 375	Pro	Gly	Asn	Ser	Ile 380	Leu	Pro	Thr	Gly
Asn Gly (Gly P	ro Asp	Thr 390	Arg	Asn	Leu	Tyr	Tyr 395	Ser	Phe	Asp	Ser	Gly 400
Val Val I	His P	he Val 405	Tyr	Met	Ser	Thr	Glu 410	Thr	Asn	Phe	Val	Gln 415	Gly
Ser Asp (yr Asn 20	Phe	Leu	Tàa	Ala 425	Asp	Leu	Glu	TÀa	Val 430	Asn	Arg
Ser Arg	Thr P 435	ro Phe	Val	Val	Phe 440	Gln	Gly	His	Arg	Pro 445	Met	Tyr	Thr
Ser Ser 2 450	Asp G	lu Thr	Arg	Asp 455	Ala	Ala	Leu	Arg	Gln 460	Gln	Met	Leu	Gln
His Leu (465	Glu P	ro Leu	Leu 470	Val	Thr	Tyr	Ser	Val 475	Thr	Leu	Ala	Leu	Trp 480
Gly His V	Val H	is Arg 485	Tyr	Glu	Arg	Phe	Сув 490	Pro	Met	Lys	Asn	Phe 495	Gln
Cys Val A		hr Ser 00	Ser	Ser	Phe	Gln 505	Tyr	Ser	Gly	Ala	Pro 510	Val	His
Leu Val :	Ile G 515	ly Met	Gly	Gly	Ala 520	Asp	Trp	Ala	Thr	Ile 525	Trp	Gln	Pro
Arg Pro 2 530	Asp H	is Pro	Asp	Val 535	Pro	Ile	Phe	Pro	Gln 540	Pro	Glu	Arg	Ser
Met Tyr 2 545	Arg G	ly Gly	Glu 550	Phe	Gly	Tyr	Thr	Arg 555	Leu	Ala	Ala	Thr	Arg 560

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1200

1260

1320

1380

1440

1500

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cacatggagt ctaagttctc atggtatgta aaggttggaa gtgtgctaat gcttggagct	1860
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Ile Ser Ser Ser Ser Ser Ser Gln Ile Ser Ile Ser Val Thr Pro Lys 20 25 30	
Thr Leu Ser Lys Ser Gly Asp Phe Val Thr Ile Lys Trp Thr Gly Ile 35 40 45	
Pro Ser Pro Ser Lys Leu Asp Phe Leu Gly Ile Tyr Ser Pro Pro Ser 50 55 60	
Ser Leu His Asp Asn Phe Ile Gly Tyr Ile Phe Leu Ser Ser Thr Pro 65 70 75 80	
Glu Trp Glu Ser Gly Ser Gly Ser Ile Ser Ile Pro Leu Val Asn Leu 85 90 95	
Arg Ser Gly Tyr Gln Phe Arg Ile Phe Arg Trp Thr Glu Ser Glu Ile 100 105 110	
Val Pro Asp Leu Val Asp His Asp His Asn Pro Leu Pro Gln Thr Lys 115 120 125	
His Ile Leu Ala Val Ser Glu Glu Val Gly Phe Val Ser Gly Arg Gly 130 135 140	
Pro Glu Gln Val His Leu Ala Leu Thr Gly Phe Glu Asp Glu Met Arg 145 150 155 160	
Val Met Phe Val Thr Pro Asp Gly Lys Glu Ser Tyr Val Arg Tyr Gly 165 170 175	
Leu Thr Arg Gly Arg Leu Gly Arg Val Val Lys Thr Arg Val Val Arg 180 185 190	
Tyr Glu Lys Glu Asp Leu Cys Asp Ala Pro Ala Asn Ser Ser Ile Gly 195 200 205	
Trp Arg Asp Pro Gly Tyr Ile His Asp Gly Val Met Leu Asn Leu Lys 210 215 220	
Lys Gly Lys Lys Tyr Tyr Gln Val Gly Ser Asp Ser Gly Gly Trp 225 230 235 240	
Ser Thr Ile Tyr Ser Phe Val Ser Gln Asn Arg Asp Ser Gly Glu Thr 245 250 255	
Phe Ala Phe Leu Phe Gly Asp Met Gly Thr Ala Thr Pro Tyr Leu Thr 260 265 270	
Phe Leu Arg Thr Gln Asp Glu Ser Lys Ser Thr Ile Lys Trp Ile Ser 275 280 285	

Arg Asp Ile Glu Ala Leu Gly Asn Lys Pro Ala Leu Ile Ser His Ile 295 Gly Asp Ile Ser Tyr Ala Arg Gly Tyr Ser Trp Leu Trp Asp Asn Phe 310 Phe Thr Gln Val Glu Pro Val Ala Ser Arg Val Pro Tyr His Val Cys 330 Ile Gly Asn His Glu Tyr Asp Trp Pro Leu Gln Pro Trp Lys Pro Asp Trp Ser Ser Tyr Gly Lys Asp Gly Gly Glu Cys Gly Val Pro Tyr 360 Arg Ser Tyr Phe His Met Pro Arg Asn Ser Ser Val Pro Thr Gly Met 375 His Ala Pro Ala Thr Arg Asn Leu Tyr Tyr Ser Phe Asp Ser Gly Pro 395 Val His Phe Val Tyr Met Ser Thr Glu Thr Asn Phe Leu Pro Gly Ser 410 Asn Gln Tyr Asp Phe Leu Lys His Asp Leu Glu Ser Val Asp Arg Val Lys Thr Pro Phe Val Val Phe Gln Gly His Arg Pro Met Tyr Ser Ser Ser Ser Gly Ala Lys Asp Ile Ser Leu Arg Lys Arg Met Met Glu Tyr Leu Glu Pro Leu Leu Val Lys Asn Asn Val Asn Leu Val Leu Trp Gly His Val His Arg Tyr Glu Arg Phe Cys Pro Leu Asn Asn Phe Thr Cys Gly Ser Leu Ala Leu Asn Gly Lys Glu Gln Lys Ala Phe Pro Val Gln 505 Ile Val Ile Gly Met Ala Gly Gln Asp Trp Gln Pro Ile Trp Ala Pro 520 Arg Glu Asp His Pro Thr Asp Pro Ile Phe Pro Gln Pro Leu Gln Ser 535 Leu Tyr Arg Gly Ser Glu Phe Gly Tyr Val Arg Leu His Ala Thr Lys 550 Lys Lys Leu Thr Leu Ser Tyr Val Gly Asn His Asp Gly Glu Val His Asp Lys Val Glu Phe Leu Ala Ser Gly Leu Leu Leu Ser Ala Gly Ile 585 Arg Asp Gly Pro Ala Asp Ala Val His Met Glu Ser Lys Phe Ser Trp 600 Tyr Val Lys Val Gly Ser Val Leu Met Leu Gly Ala Phe Met Gly Tyr Ile Val Gly Phe Leu Ser His Ala Arg Lys Asn Ser Ala Asp Lys Gly Trp Arg Pro Ile Lys Thr Glu Glu Ile <210> SEQ ID NO 32

<211> LENGTH: 1959

<212> TYPE: DNA

<213> ORGANISM: Vitis vinifera

<400> SEQUENCE: 32

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ccagcgaatg	agagtgttgg	gtggagagat	ccgggtttta	ttcaagatgc	ggtgatgagg	660	
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ggggctttta	tgggctatgt	tattgggttc	gtatcacatg	ccaggagaga	agctgccttg	1920	
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<210> SEQ ID NO 33 <211> LENGTH: 652 <212> TYPE: PRT <213> ORGANISM: Vitis vinifera

<400> SEQUENCE: 33

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Ser	Pro 50	Ser	Asp	Leu	Asp	Trp 55	Leu	Gly	Ile	Tyr	Ser 60	Pro	Pro	Ser	Ser
Ala 65	His	Asp	Asn	Phe	Ile 70	Gly	Tyr	Val	Phe	Leu 75	Ser	Ser	Cys	Pro	Thr 80
Trp	Glu	Ser	Gly	Ser 85	Gly	Ser	Ile	Ser	Leu 90	Pro	Leu	Val	Asn	Leu 95	Arg
Ala	Asn	Tyr	Ser 100	Phe	Arg	Ile	Phe	Arg 105	Trp	Ser	Arg	Ser	Glu 110	Val	Asp
Pro	Thr	Arg 115	Met	Asp	His	Asp	His 120	Asn	Pro	Leu	Pro	Gly 125	Thr	Thr	His
Leu	Val 130	Ala	Glu	Ser	Gly	Glu 135	Val	Gly	Phe	Gly	Gly 140	Gly	Gly	Gly	Pro
Glu 145	Gln	Ile	His	Leu	Ala 150	Tyr	Thr	Asp	Arg	Glu 155	Asp	Glu	Met	Arg	Val 160
Met	Phe	Val	Thr	Gly 165	Asp	Ala	Gly	Val	Arg 170	Thr	Val	Arg	Tyr	Gly 175	Leu
Ser	Arg	Asp	Ala 180	Met	His	Arg	Val	Val 185	Thr	Ala	Ala	Val	Gly 190	Arg	Tyr
Glu	Arg	Glu 195	Asp	Met	Cys	Asp	Ser 200	Pro	Ala	Asn	Glu	Ser 205	Val	Gly	Trp
Arg	Asp 210	Pro	Gly	Phe	Ile	Gln 215	Asp	Ala	Val	Met	Arg 220	Asn	Leu	Lys	TÀa
Gly 225	Lys	Arg	Tyr	Tyr	Tyr 230	Lys	Val	Gly	Ser	Asp 235	Ser	Gly	Gly	Trp	Ser 240
Ala	Ile	His	Asn	Phe 245	Met	Ser	Arg	Asp	Met 250	Asp	Ser	Glu	Lys	Thr 255	Ile
Ala	Phe	Leu	Phe 260	Gly	Asp	Met	Gly	Thr 265	Ala	Thr	Pro	Tyr	Ser 270	Thr	Phe
Leu	Arg	Thr 275	Gln	Glu	Glu	Ser	Lys 280	Ser	Thr	Val	Lys	Trp 285	Ile	Leu	Arg
Asp	Ile 290	Glu	Ala	Leu	Asp	Asp 295	Asn	Pro	Ala	Phe	Ile 300	Ser	His	Ile	Gly
Asp 305	Ile	Ser	Tyr	Ala	Arg 310	Gly	Tyr	Ser	Trp	Leu 315	Trp	Asp	Asn	Phe	Phe 320
Thr	Gln	Val	Glu	Pro 325	Ile	Ala	Ser	Arg	Leu 330	Pro	Tyr	His	Val	335 235	Ile
Gly	Asn	His	Glu 340	Tyr	Asp	Trp	Pro	Leu 345	Gln	Pro	Trp	Lys	Pro 350	Asp	Trp
Ser	Ser	Thr 355	Val	Tyr	Gly	Thr	Asp 360	Gly	Gly	Gly	Glu	Сув 365	Gly	Val	Pro
Tyr	Ser 370	Leu	Lys	Phe	ГЛа	Met 375	Pro	Gly	Asn	Ser	Ser 380	Glu	Leu	Thr	Gly
Thr 385	Arg	Ala	Pro	Ala	Thr 390	Arg	Asn	Leu	Phe	Tyr 395	Ser	Phe	Asp	Thr	Lys 400
Ala	Val	His	Phe	Val 405	Tyr	Ile	Ser	Thr	Glu 410	Thr	Asn	Phe	Leu	Pro 415	Gly
Ser	Ser	Gln	Tyr 420	Asp	Phe	Ile	Lys	Gln 425	Asp	Leu	Glu	Ser	Val 430	Asp	Arg

Lys Lys Thr Pro Phe Val Val Gln Gly His Arg Pro Met Tyr Thr Thr Ser Asn Glu Leu Arg Asp Ala Pro Val Arg Glu Arg Met Leu Lys 455 Tyr Leu Glu Pro Leu Phe Val Lys Asn Asn Val Thr Leu Ala Leu Trp 470 Gly His Val His Arg Tyr Glu Arg Phe Cys Pro Ile Asn Asn Phe Thr Cys Gly Asn Met Gly Leu Asn Gly Glu Tyr Leu Gly Gly Leu Pro Val His Ile Val Ile Gly Met Ala Gly Gln Asp Trp Gln Pro Thr Trp Glu Pro Arg Pro Asp His Pro Lys Asp Pro Val Tyr Pro Gln Pro Lys Trp Ser Leu Tyr Arg Gly Gly Glu Phe Gly Tyr Thr Arg Leu Val Ala Thr Lys Glu Lys Leu Thr Leu Ser Tyr Val Gly Asn His Asp Gly Glu Val His Asp Thr Val Glu Ile Leu Ala Ser Gly Gln Val Leu Ser Gly Val Gly Glu Asp Asp Ala Gln Pro Arg Val Glu Val Ala Glu Tyr Thr Phe Ser Trp Tyr Val Lys Gly Ala Ser Ile Leu Val Leu Gly Ala Phe Met 615 Gly Tyr Val Ile Gly Phe Val Ser His Ala Arg Arg Glu Ala Ala Leu 630 Arg Lys Asn Trp Thr Pro Val Lys Ile Glu Asp Ser 645 <210> SEQ ID NO 34 <211> LENGTH: 1962 <212> TYPE: DNA <213> ORGANISM: Oryza sativa <400> SEOUENCE: 34 60 atgettetet teeteetett eeteetegee geeggegagg eegeggegge ggeggegge accacgetea eegegacgee ggegaagete acceagteeg accgegagat cacgateegg 120 180 tgqtcqqqcc tcccqqaccc qqacqqcctc qactacqtcq qcatctactc qccqccqacc tectecgace gegacttect eggetacete ttecteaaeg geteggeeae etggegeaeg 240 qqcaccqqcq aqctcaccct cccqcqcctc cccaacctqc qcqcqcccta ccaqttccqc ctcttccgct ggcccgcgag ggagtactcc taccaccaca tcgaccacga cgggaacccg ctccccacg gccgccaccg cgtcgccgcc tccggtgagg tcgccttcga ctcccctcc cgccccgacc aggtgcacct ctcgttcgcc gacggggtcg acgagatgcg ggtcatgttc 540 gtctgcggcg acggcgggag gagggtggtg aggtacgggc cggcgaagga ggaggggag ggctggaagg aggtggccgc ggaggtgagg acgtacgagc agaagcacat gtgcgactcg 600 ccggcgaact cctccgtcgg gtggagggat ccagggttcg tcttcgatgg actcatgaag ggattggagc ccgggaggag gtacttctac aaggttggta gcaactcttc aggatggagc 720 gatacgtaca gcttcatttc acgtgacaac gaagccaatg aaactattgc atttctcttt

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<400> SEQUENCE: 35

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Ser Asp Arg Glu Ile Thr Ile Arg Trp Ser Gly Leu Pro Asp Pro Asp 35 40

Gly Leu Asp Tyr Val Gly Ile Tyr Ser Pro Pro Thr Ser Ser Asp Arg $50 \\ 0 \\ 0 \\ 0 \\ 0$

Asp Phe Leu Gly Tyr Leu Phe Leu Asn Gly Ser Ala Thr Trp Arg Thr 65 70 70 80

Gly Thr Gly Glu Leu Thr Leu Pro Arg Leu Pro Asn Leu Arg Ala Pro

Tyr Gln Phe Arg Leu Phe Arg Trp Pro Ala Arg Glu Tyr Ser Tyr His 105

His Ile Asp His Asp Gly Asn Pro Leu Pro His Gly Arg His Arg Val 120

Ala Ala Ser Gly Glu Val Ala Phe Asp Ser Pro Ser Arg Pro Asp Gln 135 140

Val His Leu Ser Phe Ala Asp Gly Val Asp Glu Met Arg Val Met Phe

Val	Сла	Gly	Asp	Gly 165	Gly	Arg	Arg	Val	Val 170	Arg	Tyr	Gly	Pro	Ala 175	Lys
Glu	Glu	Gly	Glu 180	Gly	Trp	Lys	Glu	Val 185	Ala	Ala	Glu	Val	Arg 190	Thr	Tyr
Glu	Gln	Lys 195	His	Met	Сув	Asp	Ser 200	Pro	Ala	Asn	Ser	Ser 205	Val	Gly	Trp
Arg	Asp 210	Pro	Gly	Phe	Val	Phe 215	Asp	Gly	Leu	Met	Lys 220	Gly	Leu	Glu	Pro
Gly 225	Arg	Arg	Tyr	Phe	Tyr 230	Lys	Val	Gly	Ser	Asn 235	Ser	Ser	Gly	Trp	Ser 240
Asp	Thr	Tyr	Ser	Phe 245	Ile	Ser	Arg	Asp	Asn 250	Glu	Ala	Asn	Glu	Thr 255	Ile
Ala	Phe	Leu	Phe 260	Gly	Asp	Met	Gly	Thr 265	Tyr	Ile	Pro	Tyr	Asn 270	Thr	Tyr
Val	Arg	Thr 275	Gln	Asp	Glu	Ser	Leu 280	Ser	Thr	Val	Lys	Trp 285	Ile	Leu	Arg
Asp	Ile 290	Gln	Ala	Leu	Gly	Asp 295	Lys	Pro	Ala	Phe	Ile 300	Ser	His	Ile	Gly
Asp 305	Ile	Ser	Tyr	Ala	Arg 310	Gly	Tyr	Ala	Trp	Val 315	Trp	Asp	His	Phe	Phe 320
Asn	Gln	Ile	Glu	Pro 325	Ile	Ala	Ala	Asn	Thr 330	Pro	Tyr	His	Val	Cys 335	Ile
Gly	Asn	His	Glu 340	Tyr	Asp	Trp	Pro	Leu 345	Gln	Pro	Trp	Lys	Pro 350	Trp	Trp
Ala	Thr	Gly 355	Ile	Tyr	Gly	Thr	Asp 360	Gly	Gly	Gly	Glu	Сув 365	Gly	Ile	Pro
Tyr	Ser 370	Val	Lys	Phe	Arg	Met 375	Pro	Gly	Asn	Ser	Phe 380	Val	Pro	Thr	Gly
Asn 385	Gly	Ala	Pro	Asp	Thr 390	Arg	Asn	Leu	Tyr	Tyr 395	Ser	Phe	Asp	Ser	Gly 400
Val	Val	His	Phe	Val 405	Tyr	Met	Ser	Thr	Glu 410	Thr	Asn	Phe	Val	Gln 415	Gly
Ser	Asp	Gln	Tyr 420	Asn	Phe	Ile	Lys	Ala 425	Asp	Leu	Glu	Lys	Val 430	Asn	Arg
Ser	Arg	Thr 435		Phe	Ile		Phe 440		Gly	His	Arg	Pro 445		Tyr	Thr
Ser	Ser 450	Asn	Glu	Ala	Arg	Asp 455	Phe	Ala	His	Arg	Gln 460	Gln	Met	Leu	Gln
Asn 465	Leu	Glu	Pro	Leu	Leu 470	Val	Thr	Tyr	Lys	Val 475	Thr	Leu	Ala	Leu	Trp 480
Gly	His	Val	His	Arg 485	Tyr	Glu	Arg	Phe	Сув 490	Pro	Met	ГЛа	Asn	Phe 495	Gln
CAa	Val	Asn	Met 500	Ser	Ser	Ser	Phe	Val 505	Tyr	Pro	Gly	Ala	Pro 510	Val	His
Leu	Val	Ile 515	Gly	Met	Gly	Gly	Gln 520	Asp	Tyr	Gln	Pro	Phe 525	Trp	Gln	Pro
Arg	Lys 530	Asp	His	Pro	Asp	Val 535	Pro	Val	Tyr	Pro	Gln 540	Pro	Glu	Arg	Ser
Met 545	Tyr	Arg	Gly	Gly	Glu 550	Phe	Gly	Tyr	Thr	Lув 555	Leu	Val	Ala	Thr	Lys 560
Glu	Lys	Leu	Thr	Leu	Thr	Tyr	Ile	Gly	Asn	His	Asp	Gly	Gln	Val	His

-continued	
565 570 575	
Asp Met Val Glu Ile Phe Ser Gly Gln Val Ser Asn Asn Asn Gly Val 580 585 590	
Pro Glu Val Ile Asp Asp Thr Lys Leu Ser Thr Gly Val Ser Thr Lys 595 600 605	
Leu Lys Ile Pro Leu Phe Ser Leu Glu Ile Val Gly Ser Val Met Phe 610 615 620	
Ala Leu Val Leu Gly Phe Ser Leu Gly Phe Leu Ile Arg Arg Lys Lys 625 630 635 640	
Glu Ala Ala Gln Trp Thr Pro Val Lys Asn Glu Glu Thr 645 650	
<210> SEQ ID NO 36 <211> LENGTH: 738 <212> TYPE: DNA <213> ORGANISM: Gossypium hirsutum <400> SEOUENCE: 36	
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gcacgagcgg gaagcagcca atatgacttt ctgaagcatg atctagagtc ggttgatcgg atgaagaccc cttttgttgt agttcaaggg catagaccaa tgtacactac aagtttcgaa	60 120
agtagggacg cccattgag agagaaaatg cttgagcatt tggaaccttt atttgtgaaa	180
agtagggacg coocartgag agagaaaatg ottgagcart tggaaccttt attrgtgaaa aacaatgtga accttgcatt atggggccat gttcatcggt acgagaggtt ttgtccattg	240
aagaacttca catgtggaag catggggcag aaggggaagg attgggaggc atttccagtt	300
catqttqtqa ttqqqatqqc aqqacaaqac tqqcaaccaa catqqqaacc tcqaccaqac	360
catccaacga teeggtetae ceacaacee aagaggtett tgtacegeae aggegagttt	420
gggtacacta gattaattgc tacaaaagag aaacttacac tatcgttcgt aggaaaccat	480
gacggggagg tgcatgacat ggttgagatt ttggcatctg ggcaagttct caatggtggt	540
gatgataaca atggtaaagt cggagcagtc cataaggttg atgatgtgac acggtactca	600
ttttcacact atgtctgggg tggtagtgtc ttggtgcttg gtggttttgt tggctatgtt	660
ctgggtttcg tttcacatgc taggagacaa attgcaacag aaagaggctg gacttccttg	720
aaaaccgagg agcaatga	738
<210> SEQ ID NO 37 <211> LENGTH: 245 <212> TYPE: PRT <213> ORGANISM: Gossypium hirsutum	
<400> SEQUENCE: 37	
Ala Arg Ala Gly Ser Ser Gln Tyr Asp Phe Leu Lys His Asp Leu Glu 1 10 15	
Ser Val Asp Arg Met Lys Thr Pro Phe Val Val Val Gln Gly His Arg 20 25 30	
Pro Met Tyr Thr Thr Ser Phe Glu Ser Arg Asp Ala Pro Leu Arg Glu 35 40 45	
Lys Met Leu Glu His Leu Glu Pro Leu Phe Val Lys Asn Asn Val Asn 50 55 60	
Leu Ala Leu Trp Gly His Val His Arg Tyr Glu Arg Phe Cys Pro Leu 65 70 75 80	

Lys Asn Phe Thr Cys Gly Ser Met Gly Gln Lys Gly Lys Asp Trp Glu 85 90 95

<400> SEQUENCE: 39

-continued

Ala Phe Pro Val His Val Val Ile Gly Met Ala Gly Gln Asp Trp Gln 100 105 Pro Thr Trp Glu Pro Arg Pro Asp His Pro Thr Ile Pro Ser Thr His Asn Pro Lys Arg Ser Leu Tyr Arg Thr Gly Glu Phe Gly Tyr Thr Arg 135 Leu Ile Ala Thr Lys Glu Lys Leu Thr Leu Ser Phe Val Gly Asn His 150 Asp Gly Glu Val His Asp Met Val Glu Ile Leu Ala Ser Gly Gln Val 170 Leu Asn Gly Gly Asp Asp Asn Asn Gly Lys Val Gly Ala Val His Lys 185 Val Asp Asp Val Thr Arg Tyr Ser Phe Ser His Tyr Val Trp Gly Gly Ser Val Leu Val Leu Gly Gly Phe Val Gly Tyr Val Leu Gly Phe Val Ser His Ala Arg Arg Gln Ile Ala Thr Glu Arg Gly Trp Thr Ser Leu Lys Thr Glu Glu Gln <210> SEQ ID NO 38 <211> LENGTH: 930 <212> TYPE: DNA <213> ORGANISM: Panicum virgatum <400> SEQUENCE: 38 tggccatcac aaccttggaa accatggtgg gctacatatg gaaaggacgg tgggggtgaa 60 tgtggaatac catacagtgt caagttcaga atgcctggca attcagttct acctactggt 120 aatggtggtc cagacaccag gaatctttat tactcctttg attcaggtgt ggtgcatttc gtgtacatgt caactgaaac taattttctt cagggcagtg accagtacaa cttcttaaaa 240 gcggaccttg agaaggtgaa ccgaactaga acaccattcg ttgtttttca gggccaccgt 300 cccatgtaca cctcaagtga tgaaaccagg gatgctgctt tgaaacagca gatgctccag 360 aatttqqaac cactqctqqt qacatacaat qtqacccttq cactctqqqq acatqtccac 420 aggtatgaga ggttctgccc catgaagaac ttccaatgtg ttaacacttc gtcaagcttc 480 caataccctg gcgcccctgt gcatcttgtg atcgggatgg gtggtcaaga ctggcaacct 540 atatqqcaac caaqqcctqa tcaccctqat qttcccatct ttccqcaqcc tqaqaqqtct atgtaccgtg gtggtgttt tggatacaca agacttgtag ctacaaggga gaagctaaca ctaacgtatg tggggaacca tgatgggcaa gtccatgata tggtggagat attttctggc caagtatcca gcaacagcag tgttgctgag gctgttgatg gtgcaaaact cagcacagga gtcagcaccg tgcgaaaaat gcctcctttg tacttggaaa tcggaggcag tgtgatgttt gcactactgc tggggtttgg ttttggattt cttgtcagga gaaagaaaga agctgcacaa 900 tgggctccgg taaagaacga ggaatcttaa 930 <210> SEQ ID NO 39 <211> LENGTH: 309 <212> TYPE: PRT <213 > ORGANISM: Panicum virgatum

Trp 1	Pro	Ser	Gln	Pro 5	Trp	Lys	Pro	Trp	Trp 10	Ala	Thr	Tyr	Gly	Lys 15	Asp		
Gly	Gly	Gly	Glu 20	Cys	Gly	Ile	Pro	Tyr 25	Ser	Val	ГЛа	Phe	Arg 30	Met	Pro		
Gly	Asn	Ser 35	Val	Leu	Pro	Thr	Gly 40	Asn	Gly	Gly	Pro	Asp 45	Thr	Arg	Asn		
Leu	Tyr 50	Tyr	Ser	Phe	Asp	Ser 55	Gly	Val	Val	His	Phe 60	Val	Tyr	Met	Ser		
Thr 65	Glu	Thr	Asn	Phe	Leu 70	Gln	Gly	Ser	Asp	Gln 75	Tyr	Asn	Phe	Leu	Lys		
Ala	Asp	Leu	Glu	Lys	Val	Asn	Arg	Thr	Arg 90	Thr	Pro	Phe	Val	Val 95	Phe		
Gln	Gly	His	Arg 100	Pro	Met	Tyr	Thr	Ser 105	Ser	Asp	Glu	Thr	Arg 110	Asp	Ala		
Ala	Leu	Lys 115	Gln	Gln	Met	Leu	Gln 120	Asn	Leu	Glu	Pro	Leu 125	Leu	Val	Thr		
Tyr	Asn 130	Val	Thr	Leu	Ala	Leu 135	Trp	Gly	His	Val	His 140	Arg	Tyr	Glu	Arg		
Phe 145	Cys	Pro	Met	Lys	Asn 150	Phe	Gln	Cys	Val	Asn 155	Thr	Ser	Ser	Ser	Phe 160		
Gln	Tyr	Pro	Gly	Ala 165	Pro	Val	His	Leu	Val 170	Ile	Gly	Met	Gly	Gly 175	Gln		
Asp	Trp	Gln	Pro 180	Ile	Trp	Gln	Pro	Arg 185	Pro	Asp	His	Pro	Asp 190	Val	Pro		
Ile	Phe	Pro 195	Gln	Pro	Glu	Arg	Ser 200	Met	Tyr	Arg	Gly	Gly 205	Val	Phe	Gly		
Tyr	Thr 210	Arg	Leu	Val	Ala	Thr 215	Arg	Glu	Lys	Leu	Thr 220	Leu	Thr	Tyr	Val		
Gly 225	Asn	His	Asp	Gly	Gln 230	Val	His	Asp	Met	Val 235	Glu	Ile	Phe	Ser	Gly 240		
Gln	Val	Ser	Ser	Asn 245	Ser	Ser	Val	Ala	Glu 250	Ala	Val	Asp	Gly	Ala 255	Tàa		
Leu	Ser	Thr	Gly 260	Val	Ser	Thr	Val	Arg 265	Lys	Met	Pro	Pro	Leu 270	Tyr	Leu		
Glu	Ile	Gly 275	Gly	Ser	Val	Met	Phe 280	Ala	Leu	Leu	Leu	Gly 285	Phe	Gly	Phe		
Gly	Phe 290	Leu	Val	Arg	Arg	Lys 295	Lys	Glu	Ala	Ala	Gln 300	Trp	Ala	Pro	Val		
305	Asn	Glu	Glu	Ser													
<211 <212	L> LE 2> TY	ENGTI PE:	NO H: 11 DNA [SM:	L79	anum	lyco	pers	sicum	1								
< 400)> SE	EQUE	ICE :	40													
		_			at g	ggad	etget	acg	jccat	act	tgad	attt	ct t	cgta	ıcacag	60	
gaag	gaaag	gta a	aatca	acga	at ta	agto	gata	ago	cgto	jata	ttga	agct	ct t	ggta	ataag	120	
ccts	geeet	ta t	ctca	cata	at to	gaga	atato	ago	tacç	jcca	gagg	gatao	ctc t	tggt	tgtgg	180	
~~~	20++	++ +	taat	aaaa	+ ~		tatt		+ ~ ~ ~		++ ~	12 t 20	+	at at	aasta	240	

gacaactttt ttactcaggt ggaacctgtt gcatccagag ttccatacca tgtatgcatc

ggaaaccatg	aatatgattg	gccacttcaa	ccttggaagc	ctgattggtc	aagctacggg	300
aaagatgggg	gaggtgaatg	tggtgtaccc	tacagtcata	agttccatat	gccaggaaac	360
tcttcagtgc	cgactggaat	gcatgctcct	gcaactcgga	atctttatta	ctcatttgat	420
tctgggcccg	ttcactttgt	ctatatgtca	actgaaacaa	atttcctgcc	aggtagtaac	480
cagtatgact	ttttaaagca	tgacttggaa	tcagttgatc	gagtaaaaac	tccttttgtc	540
gtctttcaag	ggcacagacc	aatgtacagt	tcaagtagcg	gaacaaaaga	tatatctttg	600
aggaagagaa	tggttgagta	tttggaacct	cttcttgtga	agaacaatgt	gaatcttgta	660
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agcttggcct	tgaacgggaa	ggagcaaaag	gctttccctg	ttcaaattgt	gatcgggatg	780
gcaggacagg	actggcagcc	tatctgggca	ccaagagaag	accaccctac	ggatcctatt	840
ttcccacagc	ctctgcaatc	tctgtaccgt	gggagtgaat	ttggatacat	gaggetgeat	900
gccacaaagg	aaaagcttac	actttcttat	gtaggaaacc	atgacggaga	ggtgcatgat	960
aaggtggagt	tcctagcttc	aggacaactt	ctcaatgctg	gtatccgtga	tggtcctgca	1020
gatacagtac	acatggagtc	taacttctca	tggtatgtaa	aggttggaag	tgtgctaatg	1080
cttggagctt	tgatgggtta	catagttgga	ttcatatctc	atgctcggaa	aaattctgct	1140
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<210> SEQ ID NO 41

<211> LENGTH: 392

<212> TYPE: PRT

<213> ORGANISM: Solanum lycopersicum

<400> SEQUENCE: 41

Ala Phe Leu Phe Gly Asp Met Gly Thr Ala Thr Pro Tyr Leu Thr Phe 1  $\phantom{\bigg|}$  10  $\phantom{\bigg|}$  15

Leu Arg Thr Gln Glu Glu Ser Lys Ser Thr Ile Lys Trp Ile Ser Arg  $20 \\ 25 \\ 30 \\$ 

Asp Ile Glu Ala Leu Gly Asn Lys Pro Ala Leu Ile Ser His Ile Gly \$35\$ \$40\$ \$45\$

Thr Gln Val Glu Pro Val Ala Ser Arg Val Pro Tyr His Val Cys Ile 65 70 75 80

Gly Asn His Glu Tyr Asp Trp Pro Leu Gln Pro Trp Lys Pro Asp Trp 85  $\phantom{0}$  90  $\phantom{0}$  95

Ser Ser Tyr Gly Lys Asp Gly Gly Gly Glu Cys Gly Val Pro Tyr Ser 100 105 110

His Lys Phe His Met Pro Gly Asn Ser Ser Val Pro Thr Gly Met His 115 120 125

Ala Pro Ala Thr Arg Asn Leu Tyr Tyr Ser Phe Asp Ser Gly Pro Val

His Phe Val Tyr Met Ser Thr Glu Thr Asn Phe Leu Pro Gly Ser Asn 145 150 150 155 160

Gln Tyr Asp Phe Leu Lys His Asp Leu Glu Ser Val Asp Arg Val Lys \$165\$ \$170\$ \$175\$

Thr Pro Phe Val Val Phe Gln Gly His Arg Pro Met Tyr Ser Ser Ser 180  $$185\$ 

Ser Gly Thr Lys Asp Ile Ser Leu Arg Lys Arg Met Val Glu Tyr Leu

-continued	
195 200 205	
Glu Pro Leu Leu Val Lys Asn Asn Val Asn Leu Val Leu Trp Gly His 210 215 220	
Val His Arg Tyr Glu Arg Phe Cys Pro Leu Asn Asn Phe Thr Cys Gly 225 230 230	
Ser Leu Ala Leu Asn Gly Lys Glu Gln Lys Ala Phe Pro Val Gln Ile 245 250 255	
Val Ile Gly Met Ala Gly Gln Asp Trp Gln Pro Ile Trp Ala Pro Arg 260 265 270	
Glu Asp His Pro Thr Asp Pro Ile Phe Pro Gln Pro Leu Gln Ser Leu 275 280 285	
Tyr Arg Gly Ser Glu Phe Gly Tyr Met Arg Leu His Ala Thr Lys Glu 290 295 300	
Lys Leu Thr Leu Ser Tyr Val Gly Asn His Asp Gly Glu Val His Asp 305 310 315 320	
Lys Val Glu Phe Leu Ala Ser Gly Gln Leu Leu Asn Ala Gly Ile Arg	
Asp Gly Pro Ala Asp Thr Val His Met Glu Ser Asn Phe Ser Trp Tyr 340 345 350	
Val Lys Val Gly Ser Val Leu Met Leu Gly Ala Leu Met Gly Tyr Ile 355 360 365	
Val Gly Phe Ile Ser His Ala Arg Lys Asn Ser Ala Asp Asn Gly Trp	
370 375 380  Arg Pro Ile Lys Thr Glu Val Ile 385 390	
<210> SEQ ID NO 42 <211> LENGTH: 406 <212> TYPE: DNA <213> ORGANISM: Sorghum bicolor <400> SEQUENCE: 42	
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acagcctgag aggtccatgt accgtggcgg tgagtttgga tacacaaggc ttgtagcaac 12	0
aagggagaag ctaacattaa cctatgtggg gaaccatgat gggcaagtcc atggtatggt	0
ggagatattt tetggeetgg tateeagtaa eagtagtgtt getgtggeag tgeatgaeae 24	0
caaacttggc acagaagtca gcaccgtgcg aaaaatatct ccattgtact tggaaatcgg 30	0
aggcagtgta ttgtttgcac tgctcctggg attttccttt ggatttctta tcaggagaaa 36	0
ggaagaagct gcacagtgga ctccagtaaa gaacgaggaa tcataa 40	6
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<400> SEQUENCE: 43	
Pro Ile Trp Gln Pro Arg Pro Asp His Pro Asp Val Pro Ile Phe Pro 1 5 10 15	
Gln Pro Glu Arg Ser Met Tyr Arg Gly Gly Glu Phe Gly Tyr Thr Arg 20 25 30	
Leu Val Ala Thr Arg Glu Lys Leu Thr Leu Thr Tyr Val Gly Asn His 35 40 45	

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Asp Gly Gln Val His Gly Met Val Glu Ile Phe Ser Gly Leu Val Ser
                        55
Ser Asn Ser Ser Val Ala Val Ala Val His Asp Thr Lys Leu Gly Thr
                    70
Glu Val Ser Thr Val Arg Lys Ile Ser Pro Leu Tyr Leu Glu Ile Gly
                                   90
Gly Ser Val Leu Phe Ala Leu Leu Leu Gly Phe Ser Phe Gly Phe Leu
                               105
Ile Arg Arg Lys Glu Glu Ala Ala Gln Trp Thr Pro Val Lys Asn Glu
                           120
Glu Ser
    130
<210> SEQ ID NO 44
<211> LENGTH: 960
<212> TYPE: DNA
<213> ORGANISM: Triticum aestivum
<400> SEQUENCE: 44
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tctacatatg gaaaggatgg tggaggtgaa tgcggaatac catacagtgt caagttcagg
atgcctggga attctgttct acctactggc aatggagctc cggacacacg gaatctctat
tactcttttg attcaggtgt tgtgcatttt gtgtacatgt cgactgaaac taatttcgtt
cagggcagcg accaacacaa tttcctaaaa gctgacctag agaaggtgaa ccgaagtaga
                                                                     300
accccatttg ttgtgtttca gggccaccgg cccatgtata cctcgagcaa cgaagccagg
gattttgcca tgagacagca gatgatccag catcttgaac tgctcttggt gatgtacaat
                                                                     420
gtgaccettg ccetgtgggg acatgtccat aggtatgaga ggttctgccc catgaagaat
                                                                     480
tcacagtgtc tgaacacatc atcaagcttc atataccctg gtgcccctgt tcatgttgtg
                                                                     540
atcgggatgg ccggacaaga ctggcaaccg atctggcaac caaggcgtga tcatccagat
                                                                     600
gttcccatct ttccacagcc tgggatctcc atgtaccgtg gtggtgagtt cgggtacaca
                                                                     660
                                                                     720
aaactggtag ctaccaggga gaagctaacg ctgatgtacg tcgggaacca tgacggacaa
qtccatqaca tqqtqqaqat attctctqqa caaacatcta ctqaaqctaq tqctaccqaq
                                                                     780
geggteaate aaacaaaget eggeteggga accagegeea agetgaagat tteeceatta
                                                                     840
tacttggaaa ttggaggtag tgtgatgttg gcattgctgc ttggttttgc cttgggattt
                                                                     900
ctcctcagga agaagagaa agcggcacaa tggactccgg tgaagaacga ggaatcctaa
<210> SEQ ID NO 45
<211> LENGTH: 319
<212> TYPE: PRT
<213> ORGANISM: Triticum aestivum
<400> SEQUENCE: 45
His Val Cys Ile Gly Asn His Glu Tyr Asp Trp Pro Ser Gln Pro Trp
                                   10
Lys Pro Ser Trp Ser Thr Tyr Gly Lys Asp Gly Gly Glu Cys Gly
Ile Pro Tyr Ser Val Lys Phe Arg Met Pro Gly Asn Ser Val Leu Pro
Thr Gly Asn Gly Ala Pro Asp Thr Arg Asn Leu Tyr Tyr Ser Phe Asp
```

Ser 65	Gly	Val	Val	His	Phe 70	Val	Tyr	Met	Ser	Thr 75	Glu	Thr	Asn	Phe	Val 80	
Gln	Gly	Ser	Asp	Gln 85	His	Asn	Phe	Leu	Lys	Ala	Asp	Leu	Glu	Lув 95	Val	
Asn	Arg	Ser	Arg 100	Thr	Pro	Phe	Val	Val 105	Phe	Gln	Gly	His	Arg 110	Pro	Met	
Tyr	Thr	Ser 115	Ser	Asn	Glu	Ala	Arg 120	Asp	Phe	Ala	Met	Arg 125	Gln	Gln	Met	
Ile	Gln 130	His	Leu	Glu	Leu	Leu 135	Leu	Val	Met	Tyr	Asn 140	Val	Thr	Leu	Ala	
Leu 145	Trp	Gly	His	Val	His 150	Arg	Tyr	Glu	Arg	Phe 155	Сув	Pro	Met	Lys	Asn 160	
Ser	Gln	Cys	Leu	Asn 165	Thr	Ser	Ser	Ser	Phe 170	Ile	Tyr	Pro	Gly	Ala 175	Pro	
Val	His	Val	Val 180	Ile	Gly	Met	Ala	Gly 185	Gln	Asp	Trp	Gln	Pro 190	Ile	Trp	
Gln	Pro	Arg 195	Arg	Asp	His	Pro	Asp 200	Val	Pro	Ile	Phe	Pro 205	Gln	Pro	Gly	
Ile	Ser 210	Met	Tyr	Arg	Gly	Gly 215	Glu	Phe	Gly	Tyr	Thr 220	Lys	Leu	Val	Ala	
Thr 225	Arg	Glu	Lys	Leu	Thr 230	Leu	Met	Tyr	Val	Gly 235	Asn	His	Asp	Gly	Gln 240	
Val	His	Asp	Met	Val 245	Glu	Ile	Phe	Ser	Gly 250	Gln	Thr	Ser	Thr	Glu 255	Ala	
Ser	Ala	Thr	Glu 260	Ala	Val	Asn	Gln	Thr 265	Lys	Leu	Gly	Ser	Gly 270	Thr	Ser	
Ala	Lys	Leu 275	Lys	Ile	Ser	Pro	Leu 280	Tyr	Leu	Glu	Ile	Gly 285	Gly	Ser	Val	
Met	Leu 290	Ala	Leu	Leu	Leu	Gly 295	Phe	Ala	Leu	Gly	Phe 300	Leu	Leu	Arg	Lys	
305	Arg	Glu	Ala	Ala	Gln 310	Trp	Thr	Pro	Val	Lys 315	Asn	Glu	Glu	Ser		
<210	)> SI	EQ II	ои с	46												
<212	1> LF 2> TY 3> OF	PE:	DNA		ssica	a naj	ous									
< 400	)> SI	EQUEI	NCE :	46												
atga	atcgt	cg a	actto	ctcta	ac ct	tcat	ccto	tto	catct	ccg	tctt	cati	ttc (	ctcaç	gctaac	60
gcca	aaago	caa o	cctta	atcca	at ct	ccc	ccaaa	a act	ctaa	agcc	gato	ccgg	cga 1	tcca	atcctc	120
atca	aaato	ggt (	ccaa	cgtc	ga ct	ctc	cctcc	gat	ctc	gact	ggct	agg	cat (	ctact	cccc	180
cca	gacto	ctc (	ccca	cgac	ca ct	tcat	cggo	tac	caaat	tcc	tcaa	acgt	ctc (	ccca	ecgtgg	240
caat	ccg	gct o	ccgg	egega	at ct	ccct	caaa	c cto	cacca	acc	tcc	gatc	gaa (	ctaca	acgttc	300
cgta	atctt	caa (	gatg	gacg	ca gt	ccga	agato	c aat	ccga	agc	acaa	agga	cca (	cgaco	agaat	360
ccct	taco	gg (	gaac	gaag	ca co	ettei	ggcg	g gaa	atcg	gagc	aggt	ggg	gtt (	eggat	cegee	420
ggc	gtggg	gga (	ggcc	ggag	ca ga	atcca	atttç	g gcg	gttc	gagg	ataa	aggti	taa (	cagga	atgegg	480
gtca	acgtt	cg t	tagct	ggg	ga to	gggg	aagaa	a agg	gttc	gtga	ggta	acgga	aga 🤅	gggga	aggac	540
gcgt	tgg	cga a	actco	cgcg	gc gg	gege	geggg	g att	aggt	acg	agag	ggga	gca 1	tatgt	gtaat	600

gctccggcta	attccaccgt	gggatggaga	gatcccgggt	ggatttttca	taccgttatg	660
aagaatttga	acggtggcgt	taggtattat	tatcaggttg	ggagtgattc	aaagggatgg	720
agtgagatcc	acagctttat	cgctcgagat	atctactcag	aagaaaccat	agctttcatg	780
ttcggagaca	tgggttgcgc	tacaccttac	aataccttta	tccggacgca	ggacgagagt	840
atctcaacag	ttaagtggat	actccgcgac	atcgaagctc	ttggtgacaa	gccagctctt	900
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gggggagagt	gcggtgtgcc	gtatagtctc	aagttcaaca	tgcctggaaa	ctcgtcggaa	1140
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ggacacagac	cgatgtacac	cacgagcaac	gaggtgagag	acgcgatgat	taggcaaaag	1380
atggtggagc	atttggagcc	gctgtttgtg	gagaacaacg	tgacgcttgc	tctgtgggga	1440
catgttcata	gatacgagag	gttttgtccg	ataagcaaca	acacgtgtgg	gaaacagtgg	1500
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cagccgagac	cgaaccatcc	gggtcttcct	atattccctc	agcctgaaca	gtcgatgtac	1620
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tttgtgggta	accatgatgg	agaagttcat	gatagtgttg	agatettage	gtctggggaa	1740
gtaatcagtg	ggaggaaaga	ggaaactatt	aagaccgttc	ctgtatctgc	aacacttgtg	1800
gggaaacctg	agtctgatgt	cttatggtat	gttaaaggag	caggettgtt	ggttatgggt	1860
gtgcttttag	ggttccttat	agggttttt	acaaggggga	agaaaggatc	ttcttcatct	1920
gataaccgtt	ggatcccagt	caagaacgag	gagacatga			1959

<210> SEQ ID NO 47

<211> LENGTH: 652

<212> TYPE: PRT

<213> ORGANISM: Brassica napus

<400> SEQUENCE: 47

Met Ile Val Asp Phe Ser Thr Phe Ile Leu Phe Ile Ser Val Phe Ile 1 5 10 15

Ser Ser Ala Asn Ala Lys Ala Thr Leu Ser Ile Ser Pro Lys Thr Leu  $20 \hspace{1.5cm} 25 \hspace{1.5cm} 30$ 

Ser Arg Ser Gly Asp Ser Ile Leu Ile Lys Trp Ser Asn Val Asp Ser 35  $\phantom{\bigg|}40\phantom{\bigg|}$ 

Pro Ser Asp Leu Asp Trp Leu Gly Ile Tyr Ser Pro Pro Asp Ser Pro 50 60

His Asp His Phe Ile Gly Tyr Lys Phe Leu Asn Val Ser Pro Thr Trp 65 70 70 80

Gln Ser Gly Ser Gly Ala Ile Ser Leu Pro Leu Thr Asn Leu Arg Ser  $85 \hspace{0.5cm} 690 \hspace{0.5cm} 95 \hspace{0.5cm} 95 \hspace{0.5cm}$ 

Asn Tyr Thr Phe Arg Ile Phe Arg Trp Thr Gln Ser Glu Ile Asn Pro  $100 \ \ \, 100 \ \ \, 105$ 

Lys His Lys Asp His Asp Gln Asn Pro Leu Pro Gly Thr Lys His Leu \$115\$ \$120\$ \$125\$

Leu	Ala 130	Glu	Ser	Glu	Gln	Val 135	Gly	Phe	Gly	Ser	Ala 140	Gly	Val	Gly	Arg
Pro 145	Glu	Gln	Ile	His	Leu 150	Ala	Phe	Glu	Asp	Lys 155	Val	Asn	Arg	Met	Arg 160
Val	Thr	Phe	Val	Ala 165	Gly	Asp	Gly	Glu	Glu 170	Arg	Phe	Val	Arg	Tyr 175	Gly
Glu	Gly	Lys	Asp 180	Ala	Leu	Ala	Asn	Ser 185	Ala	Ala	Ala	Arg	Gly 190	Ile	Arg
Tyr	Glu	Arg 195	Glu	His	Met	Сув	Asn 200	Ala	Pro	Ala	Asn	Ser 205	Thr	Val	Gly
Trp	Arg 210	Asp	Pro	Gly	Trp	Ile 215	Phe	His	Thr	Val	Met 220	Lys	Asn	Leu	Asn
Gly 225	Gly	Val	Arg	Tyr	Tyr 230	Tyr	Gln	Val	Gly	Ser 235	Asp	Ser	Lys	Gly	Trp 240
Ser	Glu	Ile	His	Ser 245	Phe	Ile	Ala	Arg	Asp 250	Ile	Tyr	Ser	Glu	Glu 255	Thr
Ile	Ala	Phe	Met 260	Phe	Gly	Asp	Met	Gly 265	Сла	Ala	Thr	Pro	Tyr 270	Asn	Thr
Phe	Ile	Arg 275	Thr	Gln	Asp	Glu	Ser 280	Ile	Ser	Thr	Val	Lys 285	Trp	Ile	Leu
Arg	Asp 290	Ile	Glu	Ala	Leu	Gly 295	Asp	Lys	Pro	Ala	Leu 300	Val	Ser	His	Ile
Gly 305	Asp	Ile	Ser	Tyr	Ala 310	Arg	Gly	Tyr	Ser	Trp 315	Val	Trp	Asp	Glu	Phe 320
Phe	Ala	Gln	Ile	Glu 325	Pro	Ile	Ala	Ser	Arg 330	Val	Pro	Tyr	His	Val 335	CÀa
Ile	Gly	Asn	His 340	Glu	Tyr	Asp	Phe	Pro 345	Thr	Gln	Pro	Trp	Lys 350	Pro	Asp
Trp	Gly	Thr 355	Tyr	Gly	Asn	Asp	Gly 360	Gly	Gly	Glu	Cys	Gly 365	Val	Pro	Tyr
Ser	Leu 370	Lys	Phe	Asn	Met	Pro 375	Gly	Asn	Ser	Ser	Glu 380	Pro	Thr	Gly	Thr
Lys 385	Ala	Pro	Pro	Thr	Arg 390	Asn	Leu	Tyr	Tyr	Ser 395	Tyr	Asp	Met	Gly	Ser 400
Val	His	Phe	Leu	Tyr 405		Ser	Thr		Thr 410		Phe	Leu	Lys	Gly 415	-
Arg	Gln	Tyr	Glu 420	Phe	Ile	Lys	Arg	Asp 425	Leu	Glu	Ser	Val	Asn 430	Arg	Glu
Lys	Thr	Pro 435	Phe	Val	Val	Val	Gln 440	Gly	His	Arg	Pro	Met 445	Tyr	Thr	Thr
Ser	Asn 450	Glu	Val	Arg	Asp	Ala 455	Met	Ile	Arg	Gln	Lys 460	Met	Val	Glu	His
Leu 465	Glu	Pro	Leu	Phe	Val 470	Glu	Asn	Asn	Val	Thr 475	Leu	Ala	Leu	Trp	Gly 480
His	Val	His	Arg	Tyr 485	Glu	Arg	Phe	Сув	Pro 490	Ile	Ser	Asn	Asn	Thr 495	Cys
Gly	Lys	Gln	Trp 500	Arg	Gly	Ser	Pro	Val 505	His	Leu	Val	Ile	Gly 510	Met	Gly
Gly	Gln	Asp 515	Trp	Gln	Pro	Ile	Trp 520	Gln	Pro	Arg	Pro	Asn 525	His	Pro	Gly
Leu	Pro	Ile	Phe	Pro	Gln	Pro	Glu	Gln	Ser	Met	Tyr	Arg	Thr	Gly	Glu

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Phe Gly Tyr Thr Arg Leu Val Ala Asn Lys Glu Lys Leu Thr Val Ser
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Phe Val Gly Asn His Asp Gly Glu Val His Asp Ser Val Glu Ile Leu
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Ala Ser Gly Glu Val Ile Ser Gly Arg Lys Glu Glu Thr Ile Lys Thr
                                585
Val Pro Val Ser Ala Thr Leu Val Gly Lys Pro Glu Ser Asp Val Leu
                          600
Trp Tyr Val Lys Gly Ala Gly Leu Leu Val Met Gly Val Leu Leu Gly
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Ser Ser Ala His Ser Ile Pro Ser Thr Leu Asp Gly Pro Phe Val Pro
Val Thr Val Pro Leu Asp Thr Ser Leu Arg Gly Gln Ala Ile Asp Leu
Pro Asp Thr Asp Pro Arg Val Arg Arg Arg Val Ile Gly Phe Glu Pro
Glu Gln Ile Ser Leu Ser Leu Ser Ser Asp His Asp Ser Ile Trp Val
               70
Ser Trp Ile Thr Gly Glu Phe Gln Ile Gly Lys Lys Val Lys Pro Leu
             85
                               90
Asp Pro Thr Ser Ile Asn Ser Val Val Gln Phe Gly Thr Leu Arg His
           100
                               105
Ser Leu Ser His Glu Ala Lys Gly His Ser Leu Val Tyr Ser Gln Leu
```

Tyr	Pro 130	Phe	Asp	Gly	Leu	Leu 135	Asn	Tyr	Thr	Ser	Gly 140	Ile	Ile	His	His
Val 145	Arg	Ile	Thr	Gly	Leu 150	Lys	Pro	Ser	Thr	Ile 155	Tyr	Tyr	Tyr	Arg	Сув 160
Gly	Asp	Pro	Ser	Arg 165	Arg	Ala	Met	Ser	Lys 170	Ile	His	His	Phe	Arg 175	Thr
Met	Pro	Val	Ser 180	Ser	Pro	Ser	Ser	Tyr 185	Pro	Gly	Arg	Ile	Ala 190	Val	Val
Gly	Asp	Leu 195	Gly	Leu	Thr	Tyr	Asn 200	Thr	Thr	Asp	Thr	Ile 205	Ser	His	Leu
Ile	His 210	Asn	Ser	Pro	Asp	Leu 215	Ile	Leu	Leu	Ile	Gly 220	Asp	Val	Ser	Tyr
Ala 225	Asn	Leu	Tyr	Leu	Thr 230	Asn	Gly	Thr	Ser	Ser 235	Asp	Cys	Tyr	Ser	Cys 240
Ser	Phe	Pro	Glu	Thr 245	Pro	Ile	His	Glu	Thr 250	Tyr	Gln	Pro	Arg	Trp 255	Asp
Tyr	Trp	Gly	Arg 260	Phe	Met	Glu	Asn	Leu 265	Thr	Ser	ГÀа	Val	Pro 270	Leu	Met
Val	Ile	Glu 275	Gly	Asn	His	Glu	Ile 280	Glu	Leu	Gln	Ala	Glu 285	Asn	Lys	Thr
Phe	Glu 290	Ala	Tyr	Ser	Ser	Arg 295	Phe	Ala	Phe	Pro	Phe 300	Asn	Glu	Ser	Gly
Ser 305	Ser	Ser	Thr	Leu	Tyr 310	Tyr	Ser	Phe	Asn	Ala 315	Gly	Gly	Ile	His	Phe 320
Val	Met	Leu	Gly	Ala 325	Tyr	Ile	Ala	Tyr	Asp 330	Lys	Ser	Ala	Glu	Gln 335	Tyr
Glu	Trp	Leu	Lys 340	Lys	Asp	Leu	Ala	Lys 345	Val	Asp	Arg	Ser	Val 350	Thr	Pro
Trp	Leu	Val 355	Ala	Ser	Trp	His	Pro 360	Pro	Trp	Tyr	Ser	Ser 365	Tyr	Thr	Ala
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Glu	Arg	Ser	Asn	Arg 405	Val	Tyr	Asn	Tyr	Glu 410	Leu	Asp	Pro	Сув	Gly 415	Pro
Val	Tyr	Ile	Val 420	Ile	Gly	Asp	Gly	Gly 425	Asn	Arg	Glu	Lys	Met 430	Ala	Ile
Glu	His	Ala 435	Asp	Asp	Pro	Gly	Lys 440	Cys	Pro	Glu	Pro	Leu 445	Thr	Thr	Pro
Asp	Pro 450	Val	Met	Gly	Gly	Phe 455	CÀa	Ala	Trp	Asn	Phe 460	Thr	Pro	Ser	Asp
Lys 465	Phe	Сув	Trp	Asp	Arg 470	Gln	Pro	Asp	Tyr	Ser 475	Ala	Leu	Arg	Glu	Ser 480
Ser	Phe	Gly	His	Gly 485	Ile	Leu	Glu	Met	Lys 490	Asn	Glu	Thr	Trp	Ala 495	Leu

Trp Thr Trp Tyr Arg Asn Gln Asp Ser Ser Ser Glu Val Gly Asp Gln 500 505 510

Ile Tyr Ile Val Arg Gln Pro Asp Arg Cys Pro Leu His His Arg Leu 515 520 525

What is claimed is:

- 1. A method to make a transgenic plant having increased rate of plant growth and elevate plant yields comprising:
  - introducing a gene coding for a phosphatase into a plant, the phosphatase gene encodes for a polypeptide comprising a C-terminal motif having the sequence of SEQ ID NO: 66 or a homologue thereof, and one or more sequences selected from the group consisting of SEQ ID NOS: 48-53.
- 2. The method of claim 1, wherein the phosphatase gene is a purple acid phosphatase gene comprising a nucleotide sequence encoding a polypeptide comprising an amino acid sequence of one or more selected from SEQ ID NOS: 2, 6, 8, 19, 21, 23, 25, 27, 29, 31, 33, 35, and 47, and homologues thereof.
- 3. The method of claim 1, wherein the phosphatase gene is a purple acid phosphatase gene comprising a nucleotide sequence selected from SEQ ID NOS: 1, 5, 7, 18, 20, 22, 24, 26, 28, 30, 32, 34, and 46 and homologues thereof.
- **4.** The method of claim **2**, wherein said nucleotide sequence comprises one or more selected from the group consisting of SEQ ID NOs 1, 5, 7, 18, 20, 22, 24, 26, 28, 30, 32, 34, and 46 and homologues thereof.
- 5. The method of claim 2, wherein said nucleotide sequence comprises a nucleotide sequence encoding a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 2 and conservatively substituted variants thereof.
- 6. The method of claim 2, wherein said nucleotide sequence comprises a nucleotide sequence encoding a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 54 and 55 and conservatively substituted variants thereof.
- 7. The method of claim 6, wherein said nucleotide sequence comprises a nucleotide sequence encoding a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 50 and 51.
- **8**. The method of claim **2**, wherein said nucleotide sequence comprises a nucleotide sequence encoding a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 57-60 and conservatively substituted variants thereof.
- **9.** The method of claim **8**, wherein said nucleotide sequence comprises a nucleotide sequence encoding a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 50 and 51.
- 10. The method of claim 2, wherein said nucleotide sequence comprises a nucleotide sequence encoding a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 61-64.
- 11. The method of claim 1, wherein introducing the phosphatase gene up-regulates the enzymatic activity of sucrose phosphate synthase in the transgenic plant relative to the wild-type plant.

- 12. The method of claim 1, wherein introducing the phosphatase gene up-regulates the sucrose and/or glucose level in the transgenic plant relative to the wild-type plant.
- 13. The method of claim 1, wherein introducing the phosphatase gene increases the growth rate of the transgenic plant relative to the wild-type plants.
- 14. The method of claim 1, wherein introducing the phosphatase gene results in a higher crop yield of the transgenic plant relative to the wild-type plants.
- 15. The method of claim 1, wherein the plant is a species selected from one of the group consisting of the following genera: 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, Solanum lycopersicon, Majorana, Malva, Gossypium, Manihot, Medicago, Nemesia, Nicotiana, Onobrychis, Pelargonium, Petunia, Ranunculus, Raphanus, Salpiglossis, Senecio, Sinapis, Solanum, Trifolium, Vigna, and Vitis.
- **16**. The method of claim **1**, wherein the plant is a species selected from the family *Brassica*.
  - 17. A transformed plant, comprising:
  - a plant comprising at least one additional gene coding for a phosphatase relative to a corresponding wild-type plant, the phosphatase gene encodes for a polypeptide comprising a C-terminal motif having the sequence of SEQ ID NO: 66 or a homologue thereof, and one or more sequences selected from the group consisting of SEQ ID NOS: 48-53.
- 18. The transformed plant of claim 17, wherein the phosphatase gene is a purple acid phosphatase gene comprising a nucleotide sequence encoding a polypeptide comprising an amino acid sequence of one or more selected from SEQ ID NOS: 2, 6, 8, 19, 21, 23, 25, 27, 29, 31, 33, 35, and 47, and homologues thereof.
- 19. The transgenic plant of claim 17, wherein the phosphatase gene is a purple acid phosphatase gene comprising a nucleotide sequence selected from SEQ ID NOS: 1, 5, 7, 18, 20, 22, 24, 26, 28, 30, 32, 34, and 46 and homologues thereof.
- **20**. The transgenic plant of claim **17**, wherein the phosphatase gene is a purple acid phosphatase gene comprising a nucleotide sequence selected from SEQ ID NO: 1 or a homologue thereof.
- 21. The transformed plant of claim 17, wherein the phosphatase gene is a purple acid phosphatase gene comprising a nucleotide sequence encoding a polypeptide comprising an amino acid sequence of one or more selected from SEQ ID NOS: 2 or a homologue thereof.
- 22. The transformed plant of claim 17, wherein said plant is of a monocotyledonous species.

- 23. The transformed plant of claim 17, wherein said plant is of a dicotyledonous species.
- 24. The transformed plant of claim 17, wherein the plant is a species selected from one of the group consisting of the following genera: 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, Solanum lycopersicon, Majorana, Malva, Gossypium, Manihot, Medicago, Nemesia, Nicotiana, Onobrychis, Pelargonium, Petunia, Ranunculus, Raphanus, Salpiglossis, Senecio, Sinapis, Solanum, Trifolium, Vigna, and Vitis.
- **25**. The transformed plant of claim **17**, wherein the plant is a species selected from the family *Brassica*.
- **26.** A vector comprising a plasmid comprising a phosphatase gene comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOS: 1, 5, 7, 18, 20, 22, 24, 26, 28, 30, 32, 34, and 46 and homologues thereof, wherein the plasmid is capable of transforming a bacterial cell
- 27. The vector of claim 26, wherein the bacterial cell is *Agrobacterium tumefaciens*.
- 28. The vector of claim 26, wherein said phosphatase gene encodes an amino acid sequence comprising one or more selected from the group consisting of SEQ ID NOS: 2, 6, 8, 19, 21, 23, 25, 27, 29, 31, 33, 35, and 47 and homologues thereof, except that all or an N-terminal portion of amino acid residues 1 to 50 of these amino acid sequences are replaced by a plant signal peptide such that said polypeptide is sorted to various organelles or compartments of plant cells upon expression of the phosphatase gene in a transformed host plant cell.
- **29**. The vector of claim **26**, wherein the phosphatase gene comprises a nucleic acid sequence selected from SEQ ID NO: 1 or a homologue thereof.
  - 30. A host cell comprising the vector of claim 26.

- 31. The host cell of claim 30, wherein the phosphatase gene comprising the vector is operably linked to a heterologous promoter.
- **32**. The host cell of claim **31**, wherein the heterologous promoter is a plant promoter.
- 33. The host cell of claim 31, wherein the heterologous promoter is a promoter derived from cauliflower mosaic virus
- 34. The host cell of claim 30, wherein the host cell is a plant species.
- **35.** A method for preparing a cell or progeny thereof capable of expressing a purple acid phosphatase in a host cell, comprising:

transforming a bacterial cell with the vector of claim **26** and transforming the host cell by transfer of DNA from the bacterial cell to the host cell.

- **36**. The method of claim **35**, wherein the bacterial cell is *Agrobacterium tumefaciens* and the host cell is a plant cell.
- 37. The method of claim 36, wherein the host cell is from the family *Brassica*.
- **38**. An expression cassette comprising a phosphatase gene comprising a nucleotide sequence encoding an enzyme having phosphatase activity, wherein said nucleotide sequence hybridizes to a nucleotide sequences selected from the group consisting of SEQ ID NOS: 1, 5, 7, 18, 20, 22, 24, 26, 28, 30, 32, 34, and 46, under stringent condition and is operably linked to regulatory nucleotide sequences such that said regulatory nucleotide sequences cause expression of the nucleotide sequence in plant cells.
- **39**. The expression cassette of claim **38**, wherein said nucleotide sequence hybridizes to the nucleotide sequence of SEQ ID NO: 1.
- **40**. The Expression cassette of claim **38**, wherein said regulator nucleotide sequence is a cauliflower mosaic virus promoter.
  - 41. A method of feeding animals, comprising: providing a feed comprising matter derived from the transformed plant of claim 17 to an animal for consumption by the animal.

* * * * *