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(54) Title: COMPOSITIONS AND METHODS FOR ENHANCED PLANT GROWTH AND SEED YIELD

(57) Abstract: A transgenic plant having enhanced photosynthesis and increased assimilate partitioning directed into fruits, seeds, and/or other plant part is disclosed. The transgenic plant comprises a heterologous bicarbonate transporter and is engineered to have reduced expression of cell wall invertase inhibitor (CWI) activity and/or increased expression of cell wall invertase (CWI) activity. Methods of making the transgenic plant and transgenic polynucleotide are disclosed.



COMPOSITIONS AND METHODS FOR ENHANCED PLANT GROWTH AND SEED YIELD

CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority to US application number 62/245,502 filed October 23, 2015, the disclosure of which is incorporated herein by reference in its entirety.

STATEMENT OF GOVERNMENT SUPPORT

[0001] This invention was made in part with government support from the United States Department of Energy. The government has certain rights in this invention.

SEQUENCE LISTING

[0002] A Sequence Listing, incorporated herein by reference, is submitted in electronic form as an ASCII text file, created October 16, 2015, of size 94KB, and named "7H65357.txt".

BACKGROUND

[0003] The relative inefficiency of photochemical conversion of light energy to fixed carbon during photosynthesis is a major factor limiting crop productivity. Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), the enzyme catalyzing the first phase of carbon fixation via the carboxylation of ribulose-1,5-bisphosphate (RuBP), is a foremost contributor to this inefficiency. O₂ effectively competes for CO₂ at the active site of Rubisco resulting in oxygenation of RuBP and non-productive photorespiration at the expense of the productive carboxylation reaction (Whitney SM, Houtz RL, & Alonso H (2011) *Plant Physiol* 155(1):27-35.). To date, efforts to increase the specificity and catalytic activity of Rubisco directly have met with limited success. As an alternative, considerable attention has been given to the engineering of carbon concentrating mechanisms (CCMs) of cyanobacteria and algae as potential solutions for increased carboxylation vs. oxygenation activity at Rubisco (Price GD, *et al.* (2013) *J Exp Bot* 64(3):753-768; Meyer M & Griffiths H (2013) *J Exp Bot* 64(3):769-786; Price GD, Badger MR, & von Caemmerer S (2011) *Plant Physiol* 155(1):20-26). These aquatic photosynthetic organisms evolved under CO₂-limiting conditions, and their CCMs sequester Rubisco and concentrate CO₂ at the enzyme, thereby increasing carbon assimilation by several orders of magnitude. One common element of

CCMs is the use of high affinity membrane bicarbonate transporters to increase intracellular or intraorganellar HCO_3^- . HCO_3^- is rapidly converted to CO_2 by endogenous carbonic anhydrases to significantly increase the concentration of the CO_2 at Rubisco. Although this general concept has been understood for many years, it has proven very challenging to implement this concept successfully in plants until the recent work described in WO2015103074.

[0004] The availability of CO_2 for carbon fixation via the carboxylation of RuBP by Rubisco is dependent upon the internal concentration of CO_2 in the intercellular air space (C_i) and the resistance to CO_2 diffusion through the liquid phase across cell walls and cellular membranes to the chloroplast stroma (Evans JR & Von Caemmerer S (1996) *Plant Physiol* 110(2):339-346; Tholen D & Zhu XG (2011) *Plant Physiol* 156(1):90-105). The chloroplast envelope is proposed to contribute upwards of 50% of the total resistance to diffusion through the liquid phase (Evans JR & Von Caemmerer S (1996) *Plant Physiol* 110(2):339-346). Although less well characterized than cyanobacterial transporters, algal bicarbonate transporters have the advantage over their bacterial counterparts because they are native chloroplast proteins and are predicted to be properly targeted and regulated when expressed in vascular plants. The CCM of the green algae, *Chlamydomonas reinhardtii*, contains at least two putative chloroplast HCO_3^- transporters, LCIA (Miura K, *et al.* (2004) *Plant Physiol* 135(3):1595-1607; Mariscal V, *et al.* (2006) *Protist* 157(4):421-433.) and CCP1/2 (Chen ZY, Lavigne LL, Mason CB, & Moroney JV (1997) *Plant Physiol* 114(1):265-273; Spalding MH & Jeffrey M (1989) *Plant Physiol* 89(1):133-137). These transporters are induced >1000-fold in response to low CO_2 environments and are localized to the chloroplast envelope (Moroney JV & Mason CB (1991) *Can J Bot* 69(5):1017-1024; Ramazanov Z, Mason CB, Geraghty AM, Spalding MH, & Moroney JV (1993) *Plant Physiol* 101(4):1195-1199). Demonstration of the use of the transporters to improve plant yield, and in particular seed yield, including the use of the LCIA and CCP1 genes alone to enhance photosynthesis in plants, is described in WO2015103074.

[0005] The positive impact on growth and yield observed in plants expressing algal putative bicarbonate transporters demonstrates that carbon concentrating mechanisms are effective means of increasing carbon assimilation at RuBisCO. Furthermore, the positive impact of increased CO_2 concentrations on photosynthesis have been demonstrated by experiments in which C_3 plants were grown under high CO_2 concentrations. In these conditions, photosynthesis is shown to increase, with increased growth and productivity (Ainsworth, E.A. and A. Rogers, (2007) *Plant, Cell & Environment*, 30(3): 258-270).

However, it's been shown that C₃ plants adapt to increased CO₂ by reducing photosynthetic capacity and/or carbon allocation, thereby limiting the overall yield increases below the calculated theoretical gains (Ainsworth, E.A. and A. Rogers,. (2007) *Plant, Cell & Environment*, 30(3): 258-270; Leakey, A.D., et al.. (2009) *J Exp Bot.*, 60(10):2859-76).

[0006] Thus, there is a need for transgenic plants achieving additional improvements in overall yield.

SUMMARY

[0007] Disclosed, in various embodiments, are transgenic plants having enhanced photosynthesis and increased assimilate partitioning directed into fruits, seeds and/or other plant part (e.g., roots and/or tubers), and/or increased seed, root and/or tuber size, or any combination thereof.

[0008] In an embodiment, the transgenic plant comprises a heterologous bicarbonate transporter and is engineered to have reduced expression of cell wall invertase inhibitor (CWII) activity and/or increased expression of cell wall invertase (CWI) activity.

[0009] In an embodiment the transgenic plant comprises a first polynucleotide operably linked to a promoter and encoding a heterologous bicarbonate transporter polypeptide; and at least one of a second polynucleotide comprising an engineered endogenous cell wall invertase inhibitor (CWII) gene, wherein said engineered CWII gene has reduced expression of CWII activity compared to the endogenous CWII gene, a third polynucleotide operably linked to a promoter and encoding a heterologous cell wall invertase (CWI), a fourth polynucleotide operably linked to a promoter and encoding a suppressor of an endogenous cell wall invertase inhibitor (CWII), and a fifth polynucleotide comprising an engineered endogenous cell wall invertase (CWI) gene, wherein said engineered endogenous CWI gene results in increased expression of CWI activity compared to the endogenous CWI gene.

[0010] Also disclosed herein are methods and compositions for producing the transgenic plants.

[0011] In an embodiment, the method comprises transforming a plant cell with a first polynucleotide operatively linked to a promoter and encoding a heterologous bicarbonate transporter polypeptide; and modifying the plant's endogenous cell wall invertase inhibitor gene to reduce cell wall invertase inhibitor activity of the modified gene, expressing in the plant a suppressor of a cell wall invertase inhibitor (CWII), and/or expressing or overexpressing a cell wall invertase (CWI) in the plant.

[0012] These and other embodiments, advantages and features of the invention become clear when detailed description and examples are provided in subsequent sections.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] The following is a brief description of the drawings wherein like elements are numbered alike and which are presented for the purposes of illustrating the exemplary embodiments disclosed herein and not for the purposes of limiting the same.

[0014] Figure 1 is a schematic diagram presenting the vector and gene constructs for stacked gene expression of LCIA and CCP1 in *Camelina sativa*.

[0015] Figure 2A shows two representative T1 transgenic plants transformed with the LCIA+CCP1 construct.

[0016] Figure 2B is a photograph of a gel showing the genotype confirmation that both genes are present in the transgene insertion in the transgenic lines shown in Figure 2A.

[0017] Figure 2C is a photograph of a gel showing representative RT-PCR results confirming expression of the CCP1 and LCIA transgenes by RT-PCR in one plant shown in (A). A. Labels above the figure indicate the specificity of the DNA primers used for PCR. Labels below the figures indicate the source of the cDNA used as the template. Wild type (WT) cDNA was used as a negative control.

[0018] Figures 3A-D shows schematic diagrams of the vectors to induce RNAi silencing of the cell wall invertase inhibitor 1 (CWII1) (Figs. 3A and 3B) and cell wall invertase inhibitor 2 (CWII2) (Figs. 3C and 3D) gene families in *Camelina*.

[0019] Figure 4 is a photograph of gels showing PCR confirmation of representative LCIA-CCP1 plants transformed with pEG-301-P2-IHP3 (P2-IHP3), pEG-301-P2-IHP2 (P2-IHP2), pEG-301-P1-IHP1 (P1-IHP1), and pEG-301-P1-IHP3 (P1-IHP3) vectors.

[0020] Figure 5 is a histogram showing Cell Wall Invertase Inhibitor 2 expression in P2S2 x LCIA-CCP1 T3 homozygous lines.

[0021] Figures 6A-C each show a histogram of plant height (growth) of P2S2xLCIA-CCP1 lines relative to wild type and LCIA-CCP1 parental control lines in the left panel and a photograph of the plants in the right panel at (A) 27, (B) 34, and (C) 41 days after sowing.

[0022] Figure 7 is a histogram of photosynthesis rates, measured as CO₂ assimilation, in P2S2xLCIA-CCP1 T3 homozygous lines. CO₂ assimilation measured at the No.8 leaf at DAS 30 with the Li-6400XT. Values are mean±SD (n=3). Tukey-Kramer test, P < 0.05.

[0023] Figure 8 is a histogram of seed yields from LCIA-CCP1-34 plants expressing the CWII2 RNAi construct. Plants grown under controlled growth chamber conditions. Seed yields were measured as total seed weight per plant. Each sample represents the average of 5 plants. The error bars represent standard deviation of the mean.

[0024] Figure 9 is a histogram of seed weights from LCIA-CCP1-34 plants expressing the CWII2 RNAi construct. Plants grown under controlled growth chamber conditions. Seed weights were measured as weight per 100 seeds. Each sample represents the average of 5 plants. The error bars represent standard deviation of the mean.

[0025] Figure 10 presents histograms showing Cell Wall Invertase Inhibitor 1 expression in P2S2 T3 homozygous lines. Quantitative real-time PCR analysis CWII1 mRNA levels. Actin2 was used as an endogenous reference. Relative quantification was carried out using the comparative Ct method. Values are mean $\pm$ SD (n=3). $P < 0.05$. 15 DAS plants.

DETAILED DESCRIPTION

[0026] Disclosed herein are transgenic plants having enhanced growth and yield, and methods for producing such plants. The improvements in plant growth and yield are achieved by combining the expression of proteins of one of the CO₂ concentrating mechanisms of organisms, such as cyanobacteria and algae, to enhance photosynthesis (carbon fixation) with a reduction in expression of cell wall invertase inhibitors (CWIIs) and/or enhancement of expression of cell wall invertases (CWIs), both of which play a significant role in allocation of fixed carbon to vegetative and seed tissues. The transgenic plants disclosed herein comprise a heterologous bicarbonate transporter and also have reduced expression of cell wall invertase inhibitor (CWII) activity and/or increased expression of cell wall invertase (CWI) activity.

[0027] Plant invertases and their respective inhibitors have been shown to have a significant role in carbohydrate partitioning to seeds and other carbon sink tissues. Invertases accomplish this through regulating phloem loading, unloading, and sucrose transport (Lammens, W., et al., (2008) J Mol Biol., **377**(2): 378-85). Plant invertases are a class of proteins that hydrolyze sucrose into fructose and glucose. Cell wall invertases (CWIs), located within the cell wall, play key roles in the unloading of sucrose from the apoplast to the sink tissues. Cell wall and vacuolar invertases are highly stable proteins due to the presence of glycans, and as a result their activity may be highly dependent on posttranslational regulation by its inhibitory protein. Cell wall invertases interact with

inhibitor proteins, the cell wall invertase inhibitors (CWIIs), which post-transcriptionally regulate their activity.

[0028] The cell wall invertase inhibitors are small peptides, with molecular masses (MW) ranging from 15 to 23 kD, and may be localized to either the cell wall or vacuole. The cell wall invertase inhibitors are expressed in pollen development, early developing seeds, and senescing leaves: indicative of assimilate allocation being a limiting factor at these stages of development.

[0029] Quantitative trait loci analysis for fruit size in tomato (Lin5), and grain size in rice (GIF1) and maize (MN1) identified mutations in cell wall invertases that led to reduction in its activity in pedicel/fruit tissues (Wang et al. (2008) *Nature Genetics*. 40(11):1370-1374; Fridman et al. (2004) *Science* 305(5691): 1786-1789; Cheng et al. (1996) *Plant Cell*. 8(6):971-983). Fruit-specific suppression of the cell wall invertase inhibitor (CWII) in tomato or rice led to increases in net seed/grain weight of 22% and 10%, respectively (Wang et al. (2008) *Nature Genetics*. 40(11): 1370-1374; Jin et al. *Plant Cell*. (2009) 21(7):2072-89).

[0030] Thus, two general approaches can be used to enhance assimilate flux into sink tissues: overexpression of CWI or repression of its inhibitor protein CWII. Methods using one or both of these general approaches for producing plants having an increased number of seeds and methods for producing plants having increased assimilate partitioning directed into fruits, seeds and/or other plant part (e.g., roots and/or tubers), and/or increased seed, root and/or tuber size, or any combination thereof and transgenic plants made by these methods were disclosed in WO2014209792.

[0031] Surprisingly it has been found that expression in transgenic plants of a heterologous bicarbonate transporter, e.g., from cyanobacteria or algae, in conjunction with genetic engineering of the plant to reduce expression of cell wall invertase inhibitor (CWII) activity and/or increase expression of cell wall invertase (CWI) activity results in increased carbon fixation and increased carbon/assimilate allocation into vegetative tissues and developing seeds.

[0032] Disclosed herein is a transgenic plant having enhanced photosynthesis and enhanced assimilate allocation into vegetative tissues and developing seeds.

[0033] In an embodiment, the transgenic plant comprises a heterologous bicarbonate transporter and is engineered to have reduced expression of cell wall invertase inhibitor (CWII) activity and/or increased expression of cell wall invertase (CWI) activity.

[0034] Reduced expression of CWII activity can be achieved by suppressing expression of endogenous CWII activity by transforming the plant with a polynucleotide operably linked to a promoter and encoding a suppressor of an endogenous cell wall invertase inhibitor (CWII) or by genome editing or mutation of the endogenous CWII gene such that the modified endogenous CWII gene has reduced expression of CWII activity compared to the unmodified endogenous CWII gene. The suppressor introduced into the plant can be an RNA interference (RNAi) nucleic acid or an antisense RNA. Increased expression of CWI activity in the transgenic plant can be achieved by genome editing or mutation of the endogenous CWI gene such that the edited or mutated endogenous CWI gene has increased expression of CWI activity compared to the unmodified endogenous CWI gene, by increasing copy number of an endogenous CWI gene, or by introducing a polynucleotide encoding at least one copy of a heterologous CWI operably linked to a promoter expressible in the plant. Transgenic plants having reduced expression of cell wall invertase inhibitor (CWII) activity and/or increased expression of cell wall invertase (CWI) activity and methods of producing such plants were disclosed in WO2014209792.

[0035] In an embodiment the transgenic plant comprises a first polynucleotide operably linked to a promoter and encoding a heterologous bicarbonate transporter polypeptide; and at least one of a second polynucleotide comprising an engineered endogenous cell wall invertase inhibitor (CWII) gene, wherein said engineered CWII gene has reduced expression of CWII activity compared to the endogenous CWII gene, a third polynucleotide operably linked to a promoter and encoding a heterologous cell wall invertase (CWI), a fourth polynucleotide operably linked to a promoter and encoding a suppressor of an endogenous cell wall invertase inhibitor (CWII), and a fifth polynucleotide comprising an engineered endogenous cell wall invertase (CWI) gene, wherein said engineered endogenous CWI gene results in increased expression of CWI activity compared to the endogenous CWI gene.

[0036] As used herein, the term "gene" refers to a nucleic acid molecule capable of being used to produce mRNA, antisense RNA, miRNA, and the like. Genes may or may not be capable of being used to produce a functional protein. Genes can include both coding and non-coding regions (e.g., introns, regulatory elements, promoters, enhancers, termination sequences and 5' and 3' untranslated regions). In some embodiments, gene refers to a coding sequence operably linked to a promoter.

[0037] In any of these embodiments, the expressed heterologous bicarbonate transporter is localized to a chloroplast envelope membrane or an inner mitochondrial

membrane. The first polynucleotide operatively linked to a promoter and encoding a heterologous bicarbonate transporter polypeptide can optionally further encode a chloroplast envelope targeting peptide operatively linked to the heterologous bicarbonate transporter. The first polynucleotide operatively linked to a promoter and encoding a heterologous bicarbonate transporter polypeptide can further comprise a sequence encoding an epitope tag to facilitate affinity capture or localization of the expressed bicarbonate transporter. Epitope tagging is a technique in which a known epitope is fused to a recombinant protein by means of genetic engineering. The first commercially available epitope tags were originally designed for protein purification. Additionally, by choosing an epitope for which an antibody is available, the technique makes it possible to detect proteins for which no antibody is available. Additional examples of epitope tags include FLAG, 6 × His, glutathione-S-transferase (GST), HA, cMyc, AcV5, or tandem affinity purification epitope tags.

[0038] Herein, a “bicarbonate transporter” protein is a protein which transports bicarbonate by any transport mechanism. Classes of bicarbonate transporters include anion exchangers and $\text{Na}^+/\text{HCO}_3^-$ symporters.

[0039] In any of the disclosed embodiments, the bicarbonate transporter can be a bicarbonate transporter from a cyanobacterium, e.g., a BicA polypeptide or a SbtA polypeptide, or from an algae, e.g., a CCP1 polypeptide, a CCP2 polypeptide, or an LCIA polypeptide. The cyanobacterium can be a *Synechocystis*, e.g., *Synechocystis* PCC6803, or a *Synechococcus*, e.g., *Synechococcus* PCC700. The algae can be a *Chlamydomonas* species, for example a *Chlamydomonas reinhardtii*. In a preferred embodiment, the heterologous bicarbonate transporter can be the green algal putative bicarbonate transporters LCIA and CCP1, which localize to the chloroplast envelope. Transgenic plants comprising heterologous bicarbonate transporters and methods of producing such plants were disclosed in WO2015103074.

[0040] An increase in CO_2 assimilation rate of a transgenic plant disclosed herein can be at least about, e.g. 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 105%, 110%, 120%, 125%, 130%, 135%, 140%, 145%, 150%, 155%, 160%, 165%, 170%, 175%, 180%, 185%, 190%, 195%, 200%, 205%, 210%, 215%, 220%, 225%, 230%, 235%, 240%, 245%, 250%, 255%, 260%, 265%, 270%, 275%, 280%, 285%, 290%, 296%, 300%, 310%, 320%, 330%, 340%, 350%, 375%, 400%, 450%, 500% or more, higher than the CO_2

assimilation rate of a control plant of the same species that does not comprise the heterologous bicarbonate transporter. Net photosynthetic rates (e.g., $\text{mmol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) are usually expressed on a leaf-area basis. The rate of CO_2 fixed by the leaf or a portion of the leaf within a sampling chamber is determined by measuring the change in the CO_2 concentration of the air flowing across the sampling chamber by infrared gas analysis. An exemplary instrument for measuring CO_2 assimilation rate is a LI-6400XT (Li-COR Inc., Lincoln, NE, USA).

[0041] A decrease in transpiration rate (e.g., $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) of a transgenic plant disclosed herein can be about, e.g. at least 5%, at least 10%, at least 15%, at least 20%, or at least 25%, lower than the transpiration rate of a control plant of the same species that does not comprise the heterologous bicarbonate transporter. Transpiration rate can also be measured by infrared gas analysis. An exemplary instrument for measuring transpiration rate is a LI-6400XT (Li-COR Inc., Lincoln, NE, USA).

[0042] An increase in water use efficiency (WUE) of a transgenic plant disclosed herein can be at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, or at least 40% higher than the WUE of a control plant of the same species that does not comprise the heterologous bicarbonate transporter. WUE is determined as in WO2015103074.

[0043] An increase in nitrogen use efficiency (NUE) of a transgenic plant disclosed herein can be at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, or at least 40% higher than the NUE of a control plant of the same species that does not comprise the heterologous bicarbonate transporter. NUE is determined as in WO2015103074.

[0044] An increase in maturation rate of a transgenic plant disclosed herein can be at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, or at least 40% higher than the maturation rate of a control plant of the same species that does not comprise the heterologous bicarbonate transporter. Maturation rate is determined by observation of the time (e.g., days) required for a mature plant to develop from a planted seed.

[0045] An increase in assimilate partitioning directed into seeds and fruits (or other plant parts (e.g., roots and/or tubers)) of a transgenic plant disclosed herein can be an increase of at least about, e.g., 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%,

47%, 48%, 49%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%), 200%), 300%, 400%), 500% or more, or any range therein, as compared to a control. The control is a plant of the same species not comprising the heterologous polynucleotide sequences, modified endogenous cell wall invertase gene, and/or modified endogenous cell wall invertase inhibitor gene. The products of assimilate can be measured directly from tissue (e.g., glucose, sucrose, fructose), or downstream products (e.g., starch, oil, protein), and the like. In particular embodiments, an increase in assimilate partitioning directed into seeds and fruits (or other plant parts) of a stably transformed plant disclosed herein can be an increase of at least about, e.g., 2% to about 60%, 5% to about 55%, about 5% to about 50%, about 5% to about 60%, about 10% to about 50%, about 10% to about 55%, about 10% to about 60%, about 15% to about 45%, about 15% to about 50%, about 15% to about 55%, about 15% to about 60%, about 20% to about 40%, about 20% to about 45%, about 20% to about 50%, about 20% to about 55%, about 20% to about 60%, and the like.

[0046] "Increased assimilate partitioning directed into seeds and fruits" or "increasing assimilate partitioning directed into seeds and fruits," "directed assimilate partitioning into seeds or fruits" refers to an increase in importing of sugars/assimilates into the fruit and seed/grain (e.g., phloem unloading into fruits/seeds/grains) of a plant that has been transformed or otherwise modified as disclosed herein (e.g., a plant comprising a first polynucleotide operably linked to a promoter and encoding a heterologous bicarbonate transporter polypeptide; and at least one of a second polynucleotide comprising an engineered endogenous cell wall invertase inhibitor (CWII) gene, having reduced expression of CWII activity compared to the endogenous CWII gene, a third polynucleotide operably linked to a promoter and encoding a heterologous cell wall invertase (CWI), a fourth polynucleotide operably linked to a promoter and encoding a suppressor of an endogenous cell wall invertase inhibitor (CWII), and a fifth polynucleotide comprising an engineered endogenous cell wall invertase (CWI) gene having increased expression of CWI activity compared to the endogenous CWI gene) as compared to a control plant (e.g., a plant of the same species not comprising a first polynucleotide operably linked to a promoter and encoding a heterologous bicarbonate transporter polypeptide; and at least one of a second polynucleotide comprising an engineered endogenous cell wall invertase inhibitor (CWII) gene having reduced expression of CWII activity compared to the endogenous CWII gene, a third polynucleotide operably linked to a promoter and encoding a heterologous cell wall invertase (CWI), a fourth polynucleotide operably linked to a promoter and encoding a suppressor of an endogenous cell wall invertase inhibitor (CWII), and a fifth polynucleotide comprising an

engineered endogenous cell wall invertase (CWI) gene having increased expression of CWI activity compared to the endogenous CWI gene). In some embodiments, the increased assimilate partitioning can be directed into other plant parts including but not limited to roots, modified (used here in the horticultural sense) roots (fusiform root, napiform root, conical root, etc.), leaves, stems, modified stems (tuber, rhizome, stolon, corm, etc.), and the like.

[0047] An increase in seed number produced by a transgenic plant disclosed herein can be at least about, e.g., 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 105%, 110%, 120%, 125%, 130%, 135%, 140%, 145%, 150%, 155%, 160%, 165%, 170%, 175%, 180%, 185%, 190%, 195%, 200%, 205%, 210%, 215%, 220%, 225%, 230%, 235%, 240%, 245%, 250%, 255%, 260%, 265%, 270%, 275%, 280%, 285%, 290%, 296%, 300%, 310%, 320%, 330%, 340%, 350%, 375%, 400%, 450%, 500% or more, or any range therein, as compared to a control as described herein. In other embodiments, an increased number of seeds can be an increase of about 20% to about 200%, about 20% to about 250%, about 20% to about 300%, about 20% to about 350%, about 30% to about 200%, about 30% to about 250%, about 30% to about 300%, about 30% to about 350%, about 40% to about 200%, about 40% to about 250%, about 40% to about 300%, about 40% to about 350%, about 50% to about 200%, about 50% to about 250%, about 50% to about 300%, about 50% to about 350%, about 75% to about 200%, about 75% to about 250%, about 75% to about 300%, about 75% to about 350%, about 100% to about 200%, about 100% to about 250%, about 100% to about 300%, about 100% to about 350%, and the like, as compared to a control. In some particular embodiments, the increase in number of seeds can be about 120% to about 320%, about 150% to about 200%, about 150% to about 250%, about 150% to about 300%, about 150% to about 350%, and the like, as compared to a control. "Increased number of seeds," "increasing the number of seeds," "increased seed production," "increasing seed production," "increased seed yield" or "increasing seed yield" as used herein refers to the production of a greater number of seeds in a plant that has been transformed or otherwise modified as disclosed herein compared to a plant that is not transformed or otherwise modified as disclosed herein. Seed number can be increased, for example, through increasing the number of seeds per seed bearing plant part (e.g., fruit, pod, loment, capsule, silique, follicle, achene, drupe, utricle, pome, and the like) and/or through increasing the number of seed bearing plant parts.

[0048] As used herein, "increased seed size" refers to an increase in seed weight and/or seed volume. An increase in seed size or volume of seed produced by a transgenic plant disclosed herein can be an increase of at least about, e.g., 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500% or more, or any range therein, as compared to a control.

[0049] As used herein, "increased tuber size" refers to an increase in tuber weight and/or tuber volume. An increase in tuber size or volume produced by a transgenic plant disclosed herein can be an increase of at least about, e.g., 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500% or more, or any range therein, as compared to a control.

[0050] Herein, "plant" refers to all genera and species of higher and lower plants of the Plant Kingdom. The term includes the mature plants, seeds, shoots and seedlings, and parts, propagation material, plant organ tissue, protoplasts, callus and other cultures, for example cell cultures, derived from them, and all other species of groups of plant cells giving functional or structural units. Mature plants refers to plants at any developmental stage beyond the seedling. Seedling refers to a young, immature-plant at an early developmental stage.

[0051] The term "plant part," as used herein, includes but is not limited to reproductive tissues (e.g., petals, sepals, stamens, pistils, receptacles, anthers, pollen, flowers, fruits, flower bud, ovules, seeds, embryos, nuts, kernels, ears, cobs and husks); vegetative tissues (e.g., petioles, stems, roots, root hairs, root tips, pith, coleoptiles, stalks, shoots, branches, bark, apical meristem, axillary bud, cotyledon, hypocotyls, and leaves); vascular tissues (e.g., phloem and xylem); specialized cells such as epidermal cells, parenchyma cells, chlorenchyma cells, schlerenchyma cells, stomates, guard cells, cuticle, mesophyll cells; callus tissue; and cuttings. The term "plant part" also includes plant cells, including plant cells that are intact in plants and/or parts of plants, plant protoplasts, plant tissues, plant organs, plant cell tissue cultures, plant calli, plant clumps, and the like. As used herein,

"shoot" refers to the above ground parts including the leaves and stems. As used herein, the term "tissue culture" encompasses cultures of tissue, cells, protoplasts and callus.

[0052] As used herein, "plant cell" refers to a structural and physiological unit of the plant, which typically comprise a cell wall but also includes protoplasts. A plant cell of the present invention can be in the form of an isolated single cell or can be a cultured cell or can be a part of a higher-organized unit such as, for example, a plant tissue (including callus) or a plant organ. In some embodiments, a plant cell can be an algal cell.

[0053] "Plant" encompasses all annual and perennial monocotyledonous or dicotyledonous plants and includes by way of example, but not by limitation, those of the genera Cucurbita, Rosa, Vitis, Juglans, Fragaria, Lotus, Medicago, Onobrychis, Trifolium, Trigonella, Vigna, Citrus, Linum, Geranium, Manihot, Daucus, Arabidopsis, Brassica, Raphanus, Sinapis, Atropa, Capsicum, Datura, Hyoscyamus, Lycopersicon, Nicotiana, Solarium, Petunia, Digitalis, Majorana, Cichorium, Helianthus, Lactuca, Bromus, Asparagus, Antirrhinum, Heterocallis, Nemesis, Pelargonium, Panieum, Pennisetum, Ranunculus, Senecio, Salpiglossis, Cucumis, Browaalia, Glycine, Pisum, Phaseolus, Lolium, Oryza, Zea, Avena, Hordeum, Secale, Triticum, Sorghum, Picea and Populus.

[0054] Preferred plants are those from the following plant families: Amaranthaceae, Asteraceae, Brassicaceae, Carophyllaceae, Chenopodiaceae, Compositae, Cruciferae, Cucurbitaceae, Labiatae, Leguminosae, Papilionoideae, Liliaceae, Linaceae, Malvaceae, Rosaceae, Rubiaceae, Saxifragaceae, Scrophulariaceae, Solanaceae, Sterculiaceae, Tetragoniaceae, Theaceae, Umbelliferae.

[0055] The invention can particularly be applied advantageously to dicotyledonous plant organisms. Preferred dicotyledonous plants are selected in particular from the dicotyledonous crop plants such as, for example, Asteraceae such as sunflower, tagetes or calendula and others; Compositae, especially the genus Lactuca, very particularly the species sativa (lettuce) and others; Cruciferae, particularly the genus Brassica, very particularly the species napus (oilseed rape), campestris (beet), oleracea cv Tastie (cabbage), oleracea cv Snowball Y (cauliflower) and oleracea cv Emperor (broccoli) and other cabbages; and the genus Arabidopsis, very particularly the species thaliana, and cress or canola and others; Cucurbitaceae such as melon, pumpkin/squash or zucchini and others; Leguminosae, particularly the genus Glycine, very particularly the species max (soybean), soya, and alfalfa, pea, beans or peanut and others; Rubiaceae, preferably the subclass Lamiidae such as, for example Coffea arabica or Coffea liberica (coffee bush) and others; Solanaceae, particularly the genus Lycopersicon, very particularly the species esculentum (tomato), the genus

Solanum, very particularly the species tuberosum (potato) and melongena (aubergine) and the genus Capsicum, very particularly the genus annuum (pepper) and tobacco or paprika and others; Sterculiaceae, preferably the subclass Dilleniidae such as, for example, Theobroma cacao (cacao bush) and others; Theaceae, preferably the subclass Dilleniidae such as, for example, Camellia sinensis or Thea sinensis (tea shrub) and others; Umbelliferae, particularly the genus Daucus (very particularly the species carota (carrot)) and Apium (very particularly the species graveolens dulce (celery)) and others; and linseed, cotton, hemp, flax, cucumber, spinach, carrot, sugar beet and the various tree, nut and grapevine species, in particular banana and kiwi fruit.

[0056] Also encompassed are ornamental plants, useful or ornamental trees, flowers, cut flowers, shrubs or turf. Plants which may be mentioned by way of example but not by limitation are angiosperms, bryophytes such as, for example, Hepaticae (liver flowers) and Musci (mosses); pteridophytes such as ferns, horsetail and clubmosses; gymnosperms such as conifers, cycads, ginkgo and Gnetatae, the families of the Rosaceae such as rose, Ericaceae such as rhododendron and azalea, Euphorbiaceae such as poinsettias and croton, Caryophyllaceae such as pinks, Solanaceae such as petunias, Gesneriaceae such as African violet, Balsaminaceae such as touch-me-not, Orchidaceae such as orchids, Iridaceae such as gladioli, iris, freesia and crocus, Compositae such as marigold, Geraniaceae such as geranium, Liliaceae such as dracena, Moraceae such as ficus, Araceae such as cheeseplant and many others.

[0057] Of particular interest for transformation are plants which are oil crop plants. Oil crop plants are understood as being plants whose oil content is already naturally high and/or which can be used for the industrial production of oils. These plants can have a high oil content and/or else a particular fatty acid composition which is of interest industrially. Preferred plants are those with a lipid content of at least 1% by weight. Oil crops encompass by way of example: *Borago officinalis* (borage); *Camelina* (false flax); *Brassica* species such as *B. campestris*, *B. napus*, *B. rapa*, *B. carinata* (mustard, oilseed rape or turnip rape); *Cannabis sativa* (hemp); *Carthamus tinctorius* (safflower); *Cocos nucifera* (coconut); *Crambe abyssinica* (crambe); *Cuphea* species (*Cuphea* species yield fatty acids of medium chain length, in particular for industrial applications); *Elaeis guinensis* (African oil palm); *Elaeis oleifera* (American oil palm); *Glycine max* (soybean); *Gossypium hirsutum* (American cotton); *Gossypium barbadense* (Egyptian cotton); *Gossypium herbaceum* (Asian cotton); *Helianthus annuus* (sunflower); *Linum usitatissimum* (linseed or flax); *Oenothera biennis* (evening primrose); *Olea europaea* (olive); *Oryza sativa* (rice); *Ricinus communis* (castor);

Sesamum indicum (sesame); *Triticum* species (wheat); *Zea mays* (maize), and various nut species such as, for example, walnut or almond.

[0058] *Camelina* species, commonly known as false flax, are native to Mediterranean regions of Europe and Asia and seem to be particularly adapted to cold semiarid climate zones (steppes and prairies). The species *Camelina sativa* was historically cultivated as an oilseed crop to produce vegetable oil and animal feed. It has been introduced to the high plain regions of Canada and parts of the United States as an industrial oilseed crop. As a result of its high oil content (~35%) of its seeds, its frost tolerance, short production cycle (60-90 days), and insect resistance, it is an interesting target for enhancing photosynthesis and increasing assimilate partitioning to improve its potential as a source for production of biofuels.

[0059] Examples of species from which the bicarbonate transporter gene may be obtained include *Achnanthes orientalis*, *Agmenellum* spp., *Amphiprora hyaline*, *Amphora coffeiformis*, *Amphora coffeiformis* var. *linea*, *Amphora coffeiformis* var. *punctata*, *Amphora coffeiformis* var. *taylori*, *Amphora coffeiformis* var. *tenuis*, *Amphora delicatissima*, *Amphora delicatissima* var. *capitata*, *Amphora* sp., *Anabaena*, *Ankistrodesmus*, *Ankistrodesmus falcatus*, *Boekelovia hooglandii*, *Borodinella* sp., *Botryococcus braunii*, *Botryococcus sudeticus*, *Bracteococcus minor*, *Bracteococcus medionucleatus*, *Carteria*, *Chaetoceros gracilis*, *Chaetoceros muelleri*, *Chaetoceros muelleri* var. *subsalsum*, *Chaetoceros* sp., *Chlamydomas perigranulata*, *Chlorella anitrata*, *Chlorella antarctica*, *Chlorella aureoviridis*, *Chlorella Candida*, *Chlorella capsulate*, *Chlorella desiccata*, *Chlorella ellipsoidea*, *Chlorella emersonii*, *Chlorella fusca*, *Chlorella fusca* var. *vacuolata*, *Chlorella glucotropha*, *Chlorella infusionum*, *Chlorella infusionum* var. *actophila*, *Chlorella infusionum* var. *auxenophila*, *Chlorella kessleri*, *Chlorella lobophora*, *Chlorella luteoviridis*, *Chlorella luteoviridis* var. *aureoviridis*, *Chlorella luteoviridis* var. *lutescens*, *Chlorella miniata*, *Chlorella minutissima*, *Chlorella mutabilis*, *Chlorella nocturna*, *Chlorella ovalis*, *Chlorella parva*, *Chlorella photophila*, *Chlorella pringsheimii*, *Chlorella protothecoides*, *Chlorella protothecoides* var. *acidicola*, *Chlorella regularis*, *Chlorella regularis* var. *minima*, *Chlorella regularis* var. *umbricata*, *Chlorella reisigii*, *Chlorella saccharophila*, *Chlorella saccharophila* var. *ellipsoidea*, *Chlorella salina*, *Chlorella simplex*, *Chlorella sorokiniana*, *Chlorella* sp., *Chlorella sphaerica*, *Chlorella stigmatophora*, *Chlorella vanniellii*, *Chlorella vulgaris*, *Chlorella vulgaris* fo. *tertia*, *Chlorella vulgaris* var. *autotrophica*, *Chlorella vulgaris* var. *viridis*, *Chlorella vulgaris* var. *vulgaris*, *Chlorella vulgaris* var. *vulgaris* fo. *tertia*, *Chlorella vulgaris* var. *vulgaris* fo. *viridis*, *Chlorella xanthella*,

Chlorella zofingiensis, *Chlorella trebouxioides*, *Chlorella vulgaris*, *Chlorococcum infusionum*, *Chlorococcum* sp., *Chlorogonium*, *Chroomonas* sp., *Chrysosphaera* sp., *Cricosphaera* sp., *Crypthecodinium cohnii*, *Cryptomonas* sp., *Cyclotella cryptica*, *Cyclotella meneghiniana*, *Cyclotella* sp., *Chlamydomonas moewusii*, *Chlamydomonas reinhardtii*, *Chlamydomonas* sp. *Dunaliella* sp., *Dunaliella bardawil*, *Dunaliella bioculata*, *Dunaliella granulate*, *Dunaliella maritime*, *Dunaliella minuta*, *Dunaliella parva*, *Dunaliella peircei*, *Dunaliella primolecta*, *Dunaliella salina*, *Dunaliella terricola*, *Dunaliella tertiolecta*, *Dunaliella viridis*, *Dunaliella tertiolecta*, *Eremosphaera viridis*, *Eremosphaera* sp., *Ellipsoidon* sp., *Euglena* spp., *Franceia* sp., *Fragilaria crotonensis*, *Fragilaria* sp., *Gleocapsa* sp., *Gloeothamnion* sp., *Haematococcus pluvialis*, *Hymenomonas* sp., *Isochrysis* aff. *galbana*, *Isochrysis galbana*, *Lepocinclis*, *Micractinium*, *Micractinium*, *Monoraphidium minutum*, *Monoraphidium* sp., *Nannochloris* sp., *Nannochloropsis salina*, *Nannochloropsis* sp., *Navicula acceptata*, *Navicula biskanterae*, *Navicula pseudotenelloides*, *Navicula pelliculosa*, *Navicula saprophila*, *Navicula* sp., *Nephrochloris* sp., *Nephroselmis* sp., *Nitzschia communis*, *Nitzschia alexandrina*, *Nitzschia closterium*, *Nitzschia communis*, *Nitzschia dissipata*, *Nitzschia frustulum*, *Nitzschia hantzschiana*, *Nitzschia inconspicua*, *Nitzschia intermedia*, *Nitzschia microcephala*, *Nitzschia pusilla*, *Nitzschia pusilla elliptica*, *Nitzschia pusilla monoensis*, *Nitzschia quadrangular*, *Nitzschia* sp., *Ochromonas* sp., *Oocystis parva*, *Oocystis pusilla*, *Oocystis* sp., *Oscillatoria limnetica*, *Oscillatoria* sp., *Oscillatoria subbrevis*, *Parachlorella kessleri*, *Pascheria acidophila*, *Pavlova* sp., *Phaeodactylum tricomutum*, *Phagus*, *Phormidium*, *Platymonas* sp., *Pleurochrysis carterae*, *Pleurochrysis dentate*, *Pleurochrysis* sp., *Prototheca wickerhamii*, *Prototheca stagnora*, *Prototheca portoricensis*, *Prototheca moriformis*, *Prototheca zopfii*, *Pseudochlorella aquatica*, *Pyramimonas* sp., *Pyrobotrys*, *Rhodococcus opacus*, *Sarcinoid chrysophyte*, *Scenedesmus armatus*, *Schizochytrium*, *Spirogyra*, *Spirulina platensis*, *Stichococcus* sp., *Synechococcus* sp., *Synechocystis*, *Tagetes erecta*, *Tagetes patula*, *Tetraedron*, *Tetraselmis* sp., *Tetraselmis suecica*, *Thalassiosira weissflogii*, and *Viridiella friderici*.

[0060] In some embodiments, the bicarbonate transporter is from a cyanobacterium and the nucleic acid sequence encoding the cyanobacterial bicarbonate transporter further comprises a sequence encoding a chloroplast envelope targeting peptide operably linked to the bicarbonate transporter coding sequence. Examples of cyanobacterium include *Synechocystis* species, e.g. *Synechocystis* sp. PCC 6803 and *Synechococcus*, e.g., *Synechococcus* PCC7002. A “chloroplast envelope targeting peptide” refers herein to a peptide sequence that can target a chimeric protein including the peptide to the chloroplast

envelope, such as to the chloroplast inner envelope membrane, when the chimeric protein is expressed from the nuclear genome. Examples of suitable chloroplast envelope targeting peptides include transit peptides of precursors of chloroplast envelope membrane proteins, e.g., the transit peptide (aa 1-110) of *Arabidopsis thaliana* atTic20 precursor (Uniprot Q8GZ79, version 52; NP_171986.3); the transit peptide of a chloroplast triose phosphate/phosphate translocator precursor, e.g., the transit peptide (aa 1-72) of the *Pisum sativum* chloroplastic triose phosphate/phosphate translocator precursor (Uniprot P21727, version 73); the transit peptide of *Arabidopsis thaliana* Albino or pale green mutant 1 protein (amino acids 1-51 of GenBank Accession BAB62076.1), or the transit peptides of the *Chlamydomonas reinhardtii* CCP1 precursor or the LCIA precursor.

[0061] The cyanobacterial bicarbonate transporter can be a Na⁺-dependent HCO₃⁻ transporter, e.g., a BicA polypeptide or a SbtA polypeptide as disclosed in WO2015103074.

[0062] In some embodiments, the bicarbonate transporter is from an algae. The algae can be a *Chlamydomonas* species or any of the algae enumerated in Tables 1 and 2 of WO2015103074. The *Chlamydomonas* species can be, e.g. *Chlamydomonas reinhardtii*. The algal bicarbonate transporter can be a CCP1 polypeptide or a LCIA polypeptide. In one embodiment, the bicarbonate transporter protein is homologous to the CCP1 polypeptide or LCIA polypeptide.

[0063] In an embodiment, the bicarbonate transporter comprises the CCP1 gene of *Chlamydomonas reinhardtii* (nucleotide sequence, SEQ ID NO:1, coding sequence of Accession No. XM_001692145.1); polypeptide sequence SEQ ID NO:2, Accession No. XP_001692197.1).

[0064] In an embodiment, the bicarbonate transporter comprises the LCIA protein gene of *Chlamydomonas reinhardtii* (nucleotide sequence, SEQ ID NO:3, coding sequence of Accession No. XM_001691161.1); polypeptide sequence SEQ ID NO:4, Accession No. XP_001691213.1).

[0065] A bicarbonate transporter includes a bicarbonate transporter homologous to SbtA, BicA, CCP1, or LCIA as long as the bicarbonate transporter has bicarbonate transporter activity. “Homolog” is a generic term used in the art to indicate a polynucleotide or polypeptide sequence possessing a high degree of sequence relatedness to a subject sequence. Such relatedness may be quantified by determining the degree of identity and/or similarity between the sequences being compared. Falling within this generic term are the terms “ortholog” meaning a polynucleotide or polypeptide that is the functional equivalent of a polynucleotide or polypeptide in another species, and “paralog” meaning a functionally

similar sequence when considered within the same species. Paralogs present in the same species or orthologs of the bicarbonate transporter gene in other species can readily be identified without undue experimentation, by molecular biological techniques well known in the art. As used herein, SbtA, BicA, CCP1, or LCIA refers to SbtA, BicA, CCP1, or LCIA, respectively, as well as their homologs and orthologs.

[0066] Known coding sequences and/or protein sequences having significant similarity to *Chlamydomonas reinhardtii* CCP1 or *Chlamydomonas reinhardtii* LCIA which are suitable for practicing the disclosed methods to generate transgenic plants with enhanced photosynthesis are disclosed in WO2015103074.

[0067] As used herein, “percent homology” of two amino acid sequences or of two nucleic acid sequences is determined using the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci., U.S.A.* **87**: 2264-2268. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul et al. (1990) *J. Mol. Biol.* **215**: 403-410. BLAST nucleotide searches are performed with the NBLAST program, score=100, word length 12, to obtain nucleotide sequences homologous to a nucleic acid molecule of the invention. BLAST protein searches are performed with the XBLAST program, score=50, word length=3, to obtain amino acid sequences homologous to a reference polypeptide (e.g., SEQ ID NO:2). To obtain gapped alignments for comparison purposes, Gapped BLAST is utilized as described in Altschul et al. (1997) *Nucleic Acids Res.* **25**: 3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters are typically used. (See <http://www.ncbi.nlm.nih.gov>)

[0068] In addition, polynucleotides that are substantially identical to a polynucleotide encoding a SbtA, BicA, CCP1, or LCIA polypeptide are included. By “substantially identical” is meant a polypeptide or polynucleotide having a sequence that is at least about 85%, specifically about 90%, and more specifically about 95% or more identical to the sequence of the reference amino acid or nucleic acid sequence. For polypeptides, the length of the reference polypeptide sequence will generally be at least about 16 amino acids, or specifically at least about 20 amino acids, more specifically at least about 25 amino acids, and most specifically at least about 35 amino acids. For nucleic acids, the length of the reference nucleic acid sequence will generally be at least about 50 nucleotides, specifically at least about 60 nucleotides, more specifically at least about 75 nucleotides, and most specifically at least about 110 nucleotides.

[0069] Typically, homologous sequences can be confirmed by hybridization, wherein hybridization under stringent conditions. Using the stringent hybridization (i.e.,

washing the nucleic acid fragments twice where each wash is at room temperature for 30 minutes with 2X sodium chloride and sodium citrate (SCC buffer; 1.150. mM sodium chloride and 15 mM sodium citrate, pH 7.0) and 0.1% sodium dodecyl sulfate (SDS); followed by washing one time at 50°C for 30 minutes with 2X SCC and 0.1% SDS; and then washing two times where each wash is at room temperature for 10 minutes with 2X SCC), homologous sequences can be identified comprising at most about 25 to about 30% base pair mismatches, or about 15 to about 25% base pair mismatches, or about 5 to about 15% base pair mismatches.

[0070] The term “nucleic acid”, “polynucleotide”, or “oligonucleotide” includes DNA molecules and RNA molecules. A polynucleotide may be single-stranded or double-stranded. A polynucleotide can be obtained by a suitable method known in the art, including isolation from natural sources, chemical synthesis, or enzymatic synthesis. Nucleotides may be referred to by their commonly accepted single-letter codes.

[0071] The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a molecule formed from the linking, in a defined order, of at least two amino acids. The link between one amino acid residue and the next is an amide bond and is sometimes referred to as a peptide bond. A polypeptide can be obtained by a suitable method known in the art, including isolation from natural sources, expression in a recombinant expression system, chemical synthesis, or enzymatic synthesis.

[0072] Polynucleotides encoding SbtA, BicA, CCP1, or LCIA polypeptide sequences allow for the preparation of relatively short DNA (or RNA) sequences having the ability to specifically hybridize to such gene sequences. The short nucleic acid sequences may be used as probes for detecting the presence of complementary sequences in a given sample, or may be used as primers to detect, amplify, or mutate a defined segment of the DNA sequences encoding a SbtA, BicA, CCP1, or LCIA polypeptide. A nucleic acid sequence employed for hybridization studies may be greater than or equal to about 14 nucleotides in length to ensure that the fragment is of sufficient length to form a stable and selective duplex molecule. Such fragments are prepared, for example, by directly synthesizing the fragment by chemical means, by application of nucleic acid reproduction technology, such as PCR technology, or by excising selected nucleic acid fragments from recombinant plasmids containing appropriate inserts and suitable restriction sites.

[0073] The term bicarbonate transporter includes polynucleotides that encode the SbtA, BicA, CCP1, or LCIA polypeptides or full-length proteins that contain substitutions, insertions, or deletions into the polypeptide backbone. Related polypeptides are aligned with

SbtA, BicA, CCP1, or LCIA by assigning degrees of homology to various deletions, substitutions and other modifications. Homology can be determined along the entire polypeptide or polynucleotide, or along subsets of contiguous residues. The percent identity is the percentage of amino acids or nucleotides that are identical when the two sequences are compared. The percent similarity is the percentage of amino acids or nucleotides that are chemically similar when the two sequences are compared. SbtA, BicA, CCP1, or LCIA, and homologous polypeptides are preferably greater than or equal to about 75%, preferably greater than or equal to about 80%, more preferably greater than or equal to about 90% or most preferably greater than or equal to about 95% identical.

[0074] A homologous polypeptide may be produced, for example, by conventional site-directed mutagenesis of polynucleotides (which is one avenue for routinely identifying residues of the molecule that are functionally important or not), by random mutation, by chemical synthesis, or by chemical or enzymatic cleavage of the polypeptides.

[0075] In the case of polypeptide sequences that are less than 100% identical to a reference sequence, the non-identical positions are preferably, but not necessarily, conservative substitutions for the reference sequence. Conservative substitutions typically include substitutions within the following groups: glycine and alanine; valine, isoleucine, and leucine; aspartic acid and glutamic acid; asparagine and glutamine; serine and threonine; lysine and arginine; and phenylalanine and tyrosine.

[0076] Where a particular polypeptide is said to have a specific percent identity to a reference polypeptide of a defined length, the percent identity is relative to the reference peptide. Thus, a peptide that is 50% identical to a reference polypeptide that is 100 amino acids long can be a 50 amino acid polypeptide that is completely identical to a 50 amino acid long portion of the reference polypeptide. It might also be a 100 amino acid long polypeptide that is 50% identical to the reference polypeptide over its entire length. Of course, many other polypeptides will meet the same criteria.

[0077] Reference herein to either the nucleotide or amino acid sequence of SbtA, BicA, CCP1, or LCIA also includes reference to naturally occurring variants of these sequences. Non-naturally occurring variants that differ from the nucleotide or amino acid sequence of SbtA, BicA, CCP1, or LCIA and retain biological function are also included herein. For example, non-naturally occurring variants that differ from SEQ ID NOs:1 or 3 (nucleotide) and 2 or 4 (amino acid) and retain biological function are also included herein. Preferably the variants comprise those polypeptides having conservative amino acid changes, i.e., changes of similarly charged or uncharged amino acids. Genetically encoded amino

acids are generally divided into four families: (1) acidic (aspartate, glutamate); (2) basic (lysine, arginine, histidine); (3) non-polar (alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan); and (4) uncharged polar (glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine). Phenylalanine, tryptophan, and tyrosine are sometimes classified jointly as aromatic amino acids. As each member of a family has similar physical and chemical properties as the other members of the same family, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the binding properties of the resulting molecule. Whether an amino acid change results in a functional polypeptide can readily be determined by assaying the properties of transgenic plants containing the SbtA, BicA, CCP1, or LCIA derivatives.

[0078] Reference to SbtA, BicA, CCP1, or LCIA also refers to polypeptide derivatives of SbtA, BicA, CCP1, or LCIA. As used herein, “polypeptide derivatives” include those polypeptides differing in length from a naturally-occurring SbtA, BicA, CCP1, or LCIA and comprising about five or more amino acids in the same primary order as is found in SbtA, BicA, CCP1, or LCIA. Polypeptides having substantially the same amino acid sequence as ASbtA, BicA, CCP1, or LCIA but possessing minor amino acid substitutions that do not substantially affect the ability of SbtA, BicA, CCP1, or LCIA polypeptide derivatives to interact with SbtA, BicA, CCP1, or LCIA-specific molecules, respectively, such as antibodies, are within the definition of SbtA, BicA, CCP1, or LCIA polypeptide derivatives. Polypeptide derivatives also include glycosylated forms, aggregative conjugates with other molecules and covalent conjugates with unrelated chemical moieties.

[0079] In one embodiment, the bicarbonate transporter (e.g., SbtA, BicA, CCP1, or LCIA genes or their homologs) are expressed in vectors suitable for *in vivo* expression such as, for example, plant expression systems. The bicarbonate transporter polynucleotides are inserted into a recombinant expression vector or vectors.

[0080] The term “expression vector” refers to a plasmid, virus, or other means known in the art that has been manipulated by insertion or incorporation of the bicarbonate transporter genetic sequence. The term “plasmids” generally is designated herein by a lower case p preceded and/or followed by capital letters and/or numbers, in accordance with standard naming conventions that are familiar to those of skill in the art. Plasmids disclosed herein are either commercially available, publicly available on an unrestricted basis, or can be

constructed from available plasmids by routine application of well-known, published procedures. Many plasmids and other cloning and expression vectors are well known and readily available, or those of ordinary skill in the art may readily construct any number of other plasmids suitable for use. These vectors are transformed into a suitable host cell to form a host cell vector system for the production of a polypeptide.

[0081] As used herein, "expression cassette" means a recombinant nucleic acid molecule comprising at least one polynucleotide sequence of interest operably linked with at least a control sequence (e.g., a promoter). The polynucleotide sequence of interest can be, e.g., a heterologous polynucleotide encoding a bicarbonate transporter, a cell wall invertase (CWI), and/or a suppressor of a cell wall invertase inhibitor (CWII). Thus, expression cassettes designed to express a heterologous polynucleotide encoding polypeptides having the activity of a bicarbonate transporter, a heterologous polynucleotide encoding a polypeptide having the enzyme activity of a cell wall invertase and/or a heterologous polynucleotide encoding a suppressor of a cell wall invertase inhibitor are disclosed herein.

[0082] An expression cassette comprising a recombinant nucleic acid molecule may be chimeric, meaning that at least one of its components is heterologous with respect to at least one of its other components. In some embodiments, the expression cassettes comprising the heterologous polynucleotides can comprise one or more regulatory elements in addition to a promoter as described herein (e.g., enhancers, introns, translation leader sequences, translation termination sequences, and polyadenylation signal sequences).

[0083] The term recombinant polynucleotide or nucleic acid refers to a polynucleotide that is made by the combination of two otherwise separated segments of sequence accomplished by the artificial manipulation of isolated segments of polynucleotides by genetic engineering techniques or by chemical synthesis. In so doing, one may join together polynucleotide segments of desired functions to generate a desired combination of functions.

[0084] Furthermore, such polynucleotides can be stacked with any combination of nucleotide sequences to create plants, plant parts and/or plant cells having the desired phenotype. Stacked combinations can be created by any method including, but not limited to, any conventional methodology (e.g., cross breeding for plants), or by genetic transformation. If stacked by genetic transformation, nucleotide sequences encoding additional desired traits can be combined at any time and in any order. For example, a transgenic plant comprising one or more desired traits can be used as the target to introduce further traits by subsequent transformation. The additional nucleotide sequences can be introduced simultaneously in a

co-transformation protocol with a nucleotide sequence, nucleic acid molecule, nucleic acid construct, and/or other composition of the invention, provided by any combination of expression cassettes. For example, if two nucleotide sequences will be introduced, they can be incorporated in separate cassettes (trans) or can be incorporated on the same cassette (cis). Expression of the nucleotide sequences can be driven by a single promoter or by separate promoters, which can be the same or different, or a combination thereof. It is further recognized that nucleotide sequences can be stacked at a desired genomic location using a site-specific recombination system. See, e.g., Int'l Patent Application Publication Nos. WO 99/25821; WO 99/25854; WO 99/25840; WO 99/25855 and WO 99/25853.

[0085] The terms "isolated" or "purified", used interchangeably herein, refers to a nucleic acid, a polypeptide, or other biological moiety that is removed from components with which it is naturally associated. The term "isolated" can refer to a polypeptide that is separate and discrete from the whole organism with which the molecule is found in nature or is present in the substantial absence of other biological macro-molecules of the same type. The term "isolated" with respect to a polynucleotide can refer to a nucleic acid molecule devoid, in whole or part, of sequences normally associated with it in nature; or a sequence, as it exists in nature, but having heterologous sequences in association therewith; or a molecule disassociated from the chromosome. Purity and homogeneity are typically determined using analytical chemistry techniques, for example polyacrylamide gel electrophoresis or high performance liquid chromatography. In some embodiments, the term "purified" means that the nucleic acid or protein is at least 85% pure, specifically at least 90% pure, more specifically at least 95% pure, or yet more specifically at least 99% pure.

[0086] The term transgene refers to a recombinant polynucleotide or nucleic acid that comprises a coding sequence encoding a protein or RNA molecule.

[0087] The bicarbonate transporter polynucleotides are inserted into a vector adapted for expression in a plant, bacterial, yeast, insect, amphibian, or mammalian cell that further comprises the regulatory elements necessary for expression of the nucleic acid molecule in the plant, bacterial, yeast, insect, amphibian, or mammalian cell operatively linked to the nucleic acid molecule encoding a bicarbonate transporter.

[0088] "Operatively linked" or "operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. An expression control sequence operatively linked to a coding sequence is ligated such that expression of the coding sequence is achieved under conditions compatible with the expression control sequences. For instance, a promoter is operatively linked with a

nucleotide sequence if the promoter effects the transcription or expression of the nucleotide sequence. Those skilled in the art will appreciate that the control sequences (e.g., promoter) need not be contiguous with the nucleotide sequence to which it is operably linked, as long as the control sequences function to direct the expression thereof. Thus, for example, intervening untranslated, yet transcribed, sequences can be present between a promoter and a nucleotide sequence, and the promoter can still be considered "operatively linked" to the nucleotide sequence.

[0089] As used herein, the term "expression control sequences" refers to nucleic acid sequences that regulate the expression of a nucleic acid sequence to which it is operatively linked. Expression control sequences are operatively linked to a nucleic acid sequence when the expression control sequences control and regulate the transcription and, as appropriate, translation of the nucleic acid sequence. Thus, expression control sequences can include appropriate promoters, enhancers, transcription terminators, a start codon (i.e., ATG) in front of a protein-encoding gene, splicing signals for introns (if introns are present), translation leader sequences, translation termination sequences, and polyadenylation signal sequences maintenance of the correct reading frame of that gene to permit proper translation of the mRNA, and stop codons. The term "control sequences" is intended to include, at a minimum, components whose presence can influence expression, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences. Expression control sequences can include a promoter. By "promoter" is meant minimal sequence sufficient to direct transcription. Also included are those promoter elements which are sufficient to render promoter-dependent gene expression controllable for cell-type specific, tissue-specific, or inducible by external signals or agents; such elements may be located in the 5' or 3' regions of the gene. Both constitutive and inducible promoters are included. If a promoter is inducible, there are sequences present that mediate regulation of expression so that the associated sequence is transcribed only when an inducer (e.g., light or an exogenous chemical regulator) is available to the plant or plant tissue. An exemplary promoter to provide basal expression and avoid overexpression in transgenic plants is the 35S cauliflower mosaic virus (CaMV) promoter.

[0090] Any promoter useful for initiation of transcription in a cell of a plant can be used in the expression cassettes of the present invention. Promoters can include, for example, constitutive, inducible, temporally regulated, developmentally regulated, chemically regulated, tissue-preferred and/or tissue-specific promoters for use in the preparation of recombinant nucleic acid molecules, i.e., "chimeric genes" or "chimeric polynucleotides." A

promoter can be identified in and isolated from the organism to be transformed and then inserted into the nucleic acid construct to be used in transformation of the organism.

[0091] The choice of promoter will vary depending on the temporal and spatial requirements for expression, and also depending on the host cell to be transformed. Thus, for example, expression of a polynucleotide encoding a heterologous bicarbonate transporter polypeptide can be in any plant, plant part, (e.g., in leaves, in stalks or stems, in ears, in inflorescences (e.g. spikes, panicles, cobs, etc.), in roots, seeds and/or seedlings, and the like), or plant cells (including algae cells). For example, in the case of a multicellular organism such as a plant where expression in a specific tissue or organ is desired, a tissue-specific or tissue preferred promoter can be used (e.g., a root specific/preferred promoter). In contrast, where expression in response to a stimulus is desired a promoter inducible by stimuli or chemicals can be used. Where continuous expression at a relatively constant level is desired throughout the cells or tissues of an organism a constitutive promoter can be chosen.

[0092] Thus, promoters useful with the invention include, but are not limited to, those that drive expression of a nucleotide sequence constitutively, those that drive expression when induced, and those that drive expression in a tissue- or developmentally-specific manner. These various types of promoters are known in the art. Promoters can be identified in and isolated from the plant to be transformed and then inserted into the expression cassette to be used in transformation of the plant.

[0093] Non-limiting examples of a promoter include the promoter of the RubisCo small subunit gene 1 (PrbcS 1), the promoter of the actin gene (Pactin), the promoter of the nitrate reductase gene (Pnr) and the promoter of duplicated carbonic anhydrase gene 1 (Pdcal) (See, Walker et al. *Plant Cell Rep.* 23:727-735 (2005); Li et al. *Gene* 403:132-142 (2007); Li et al. *Mol Biol. Rep.* 37 : 1143 - 1154 (2010)). PrbcS 1 and Pactin are constitutive promoters and Pnr and Pdcal are inducible promoters. Pnr is induced by nitrate and repressed by ammonium (Li et al. *Gene* 403:132-142 (2007)) and Pdcal is induced by salt (Li et al. *Mol Biol. Rep.* 37: 1143-1154 (2010)).

[0094] Examples of constitutive promoters useful for plants include, but are not limited to, cestrum virus promoter (cmp) (U.S. Patent No. 7,166,770), the rice actin 1 promoter (Wang et al. (1992) *Mol. Cell. Biol.* 12:3399-3406; as well as US Patent No. 5,641,876), CaMV 35S promoter (Odell et al. (1985) *Nature* 313:810-812), CaMV 19S promoter (Lawton et al. (1987) *Plant Mol. Biol.* 9:315-324), nos promoter (Ebert et al. (1987) *Proc. Natl. Acad. Sci USA* 84:5745-5749), Adh promoter (Walker et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:6624-6629), sucrose synthase promoter (Yang & Russell (1990) *Proc.*

Natl. Acad. Sci. USA 87:4144-4148), and the ubiquitin promoter. The constitutive promoter derived from ubiquitin accumulates in many cell types. Ubiquitin promoters have been cloned from several plant species for use in transgenic plants, for example, sunflower (Binet et al., 1991. Plant Science 79: 87-94), maize (Christensen et al, 1989. Plant Molec. Biol. 12: 619-632), and arabidopsis (Norris et al. 1993. Plant Molec. Biol. 21 :895-906). The maize ubiquitin promoter (UbiP) has been developed in transgenic monocot systems and its sequence and vectors constructed for monocot transformation are disclosed in the patent publication EP 0 342 926. The ubiquitin promoter is suitable for the expression of the nucleotide sequences of the invention in transgenic plants, especially monocotyledons. Further, the promoter expression cassettes described by McElroy et al. (Mol. Gen. Genet. 231: 150-160 (1991)) can be easily modified for the expression of the nucleotide sequences of the invention and are particularly suitable for use in monocotyledonous hosts.

[0095] In some embodiments, tissue specific/tissue preferred promoters can be used for expression of a heterologous polynucleotide in a plant cell. Tissue specific or preferred expression patterns include, but are not limited to, green tissue specific or preferred, root specific or preferred, stem specific or preferred, and flower specific or preferred. Promoters suitable for expression in green tissue include many that regulate genes involved in photosynthesis and many of these have been cloned from both monocotyledons and dicotyledons. In one embodiment, a promoter useful with the invention is the maize PEPC promoter from the phosphoenol carboxylase gene (Hudspeth & Grula, Plant Molec. Biol. 12:579-589 (1989)). Non-limiting examples of tissue-specific promoters include those associated with genes encoding the seed storage proteins (such as β -conglycinin, cruciferin, napin and phaseolin), zein or oil body proteins (such as oleosin), or proteins involved in fatty acid biosynthesis (including acyl carrier protein, stearyl-ACP desaturase and fatty acid desaturases (fad 2-1)), and other nucleic acids expressed during embryo development (such as Bce4, see, e.g., Kridl et al. (1991) Seed Sci. Res. 1:209-219; as well as EP Patent No. 255378). Tissue-specific or tissue-preferential promoters useful for the expression of the nucleotide sequences of the invention in plants, particularly maize, include but are not limited to those that direct expression in root, pith, leaf or pollen. Such promoters are disclosed, for example, in WO 93/07278, herein incorporated by reference in its entirety. Other non-limiting examples of tissue specific or tissue preferred promoters useful with the invention the cotton rubisco promoter disclosed in US Patent 6,040,504; the rice sucrose synthase promoter disclosed in US Patent 5,604,121; the root specific promoter described by de Framond (FEBS 290:103-106 (1991); EP 0 452 269 to Ciba- Geigy); the stem specific

promoter described in U.S. Patent 5,625,136 (to Ciba-Geigy) and which drives expression of the maize *trpA* gene; and the cestrum yellow leaf curling virus promoter disclosed in WO 01/73087.

[0096] Additional examples of plant tissue-specific/tissue preferred promoters include, but are not limited to, the root hair-specific cis-elements (RHEs) (Kim et al. *The Plant Cell* 18:2958-2970 (2006)), the root-specific promoters RCc3 (Jeong et al. *Plant Physiol.* 153 :185-197 (2010)) and RB7 (U.S. Patent No. 5459252), the lectin promoter (Lindstrom et al. (1990) *Dev. Genet.* 11 :160-167; and Vodkin (1983) *Prog. Clin. Biol. Res.* 138:87-98), corn alcohol dehydrogenase 1 promoter (Dennis et al. (1984) *Nucleic Acids Res.* 12:3983-4000), S-adenosyl-L-methionine synthetase (SAMS) (Vander Mijnsbrugge et al. (1996) *Plant and Cell Physiology*, 37(8):1108-1115), corn light harvesting complex promoter (Bansal et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:3654-3658), com heat shock protein promoter (O'Dell et al. (1985) *EMBO J.* 5:451-458; and Rochester et al. (1986) *EMBO J.* 5:451-458), pea small subunit RuBP carboxylase promoter (Cashmore, "Nuclear genes encoding the small subunit of ribulose-1,5-bisphosphate carboxylase" pp. 29-39 In: *Genetic Engineering of Plants* (Hollaender ed., Plenum Press 1983; and Poulsen et al. (1986) *Mol. Gen. Genet.* 205: 193-200), Ti plasmid mannopine synthase promoter (Langridge et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:3219-3223), Ti plasmid nopaline synthase promoter (Langridge et al. (1989), *supra*), petunia chalcone isomerase promoter (van Tunen et al. (1988) *EMBO J.* 7: 1257-1263), bean glycine rich protein 1 promoter (Keller et al. (1989) *Genes Dev.* 3:1639-1646), truncated CaMV 35S promoter (O'Dell et al. (1985) *Nature* 313:810-812), potato patatin promoter (Wenzler et al. (1989) *Plant Mol. Biol.* 13:347-354), root cell promoter (Yamamoto et al. (1990) *Nucleic Acids Res.* 18:7449), maize zein promoter (Kriz et al. (1987) *Mol. Gen. Genet.* 207:90-98; Langridge et al. (1983) *Cell* 34: 1015-1022; Reina et al. (1990) *Nucleic Acids Res.* 18:6425; Reina et al. (1990) *Nucleic Acids Res.* 18:7449; and Wandelt et al. (1989) *Nucleic Acids Res.* 17:2354), globulin-1 promoter (Belanger et al. (1991) *Genetics* 129:863-872), α -tubulin cab promoter (Sullivan et al. (1989) *Mol. Gen. Genet.* 215:431-440), PEPCase promoter (Hudspeth & Grula (1989) *Plant Mol. Biol.* 12:579-589), R gene complex-associated promoters (Chandler et al. (1989) *Plant Cell* 1 : 1175-1183), and chalcone synthase promoters (Franken et al. (1991) *EMBO J.* 10:2605-2612).

[0097] Particularly useful for seed-specific expression is the pea vicilin promoter (Czako et al. (1992) *Mol. Gen. Genet.* 235:33-40; as well as the seed-specific promoters disclosed in U.S. Patent No. 5,625,136. Useful promoters for expression in mature leaves are

those that are switched at the onset of senescence, such as the SAG promoter from *Arabidopsis* (Gan et al. (1995) *Science* 270:1986-1988).

[0098] In addition, promoters functional in chloroplasts can be used. Non-limiting examples of such promoters include the bacteriophage T3 gene 9 5' UTR and other promoters disclosed in U.S. Patent No. 7,579,516. Other promoters useful with the invention include but are not limited to the S-E9 small subunit RuBP carboxylase promoter and the Kunitz trypsin inhibitor gene promoter (Kti3).

[0099] Chemical inducible promoters useful with plants are known in the art and include, but are not limited to, the maize In2-2 promoter, which is activated by benzenesulfonamide herbicide safeners, the maize GST promoter, which is activated by hydrophobic electrophilic compounds that are used as pre-emergent herbicides, and the tobacco PR-1a promoter, which is activated by salicylic acid {e.g., the PR1a system}, steroid-responsive promoters {see, e.g., the glucocorticoid-inducible promoter in Schena et al. (1991) *Proc. Natl. Acad. Sci. USA* 88, 10421-10425 and McNellis et al. (1998) *Plant J.* 14, 247-257} and tetracycline-inducible and tetracycline-repressible promoters {see, e.g., Gatz et al. (1991) *Mol. Gen. Genet.* 227, 229-237, and U.S. Patent Numbers 5,814,618 and 5,789,156, Lac repressor system promoters, copper-inducible system promoters, salicylate-inducible system promoters {e.g., the PR1a system}, glucocorticoid-inducible promoters (Aoyama et al. (1997) *Plant J.* 11 :605-612), and ecdysone-inducible system promoters.

[00100] Other non-limiting examples of inducible promoters include ABA- and turgor-inducible promoters, the auxin-binding protein gene promoter (Schwob et al. (1993) *Plant J.* 4:423-432), the UDP glucose flavonoid glycosyl-transferase promoter (Ralston et al. (1988) *Genetics* 119: 185-197), the MPI proteinase inhibitor promoter (Cordero et al. (1994) *Plant J.* 6: 141-150), and the glyceraldehyde-3 -phosphate dehydrogenase promoter (Kohler et al. (1995) *Plant Mol. Biol.* 29:1293-1298; Martinez et al. (1989) *J Mol. Biol.* 208:551-565; and Quigley et al. (1989) *J Mol. Biol.* 208:412-421). Also included are the benzene sulphonamide-inducible (US Patent No. 5,364,780) and alcohol-inducible (Int'l Patent Application Publication No s. WO 97/06269 and WO 97/06268) systems and glutathione S-transferase promoters. Likewise, one can use any of the inducible promoters described in Gatz (1996) *Current Opinion Biotechnol.* 7:168-172 and Gatz (1997) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48:89-108. Other chemically inducible promoters useful for directing the expression of the nucleotide sequences of this invention in plants are disclosed in US Patent 5,614,395 herein incorporated by reference in its entirety. Chemical induction of gene expression is also detailed in the published application EP 0 332 104 (to Ciba- Geigy)

and U.S. Patent 5,614,395. In some embodiments, a promoter for chemical induction can be the tobacco PR- la promoter.

[00101] In some particular embodiments, promoters useful with algae include, but are not limited to, the promoter of the RubisCo small subunit gene 1 (PrbcSl), the promoter of the actin gene (Pactin), the Arabidopsis Actin1 promoter (Yong-Qiang An et al. 1996 The Plant Journal 10(1):107-121; Yong-Qiang An et al. 2010 Plant Mol Bio Rep 28:481-490) and the tobacco EntCUP4 promoter (Malik et al. 2002 Theor Appl Genet 105:505-514), the promoter of the nitrate reductase gene (Pnr) and the promoter of duplicated carbonic anhydrase gene 1 (Pdcal) (See, Walker et al. Plant Cell Rep. 23:727-735 (2005); Li et al. Gene 403: 132-142 (2007); Li et al. Mol Biol. Rep. 37: 1143-1154 (2010)), the promoter of the a70-type plastid rRNA gene (Prnr), the promoter of the psbA gene (encoding the photo system-II reaction center protein D1) (PpsbA), the promoter of the psbD gene (encoding the photo system-II reaction center protein D2) (PpsbD), the promoter of the psaA gene (encoding an apoprotein of photosystem I) (PpsaA), the promoter of the ATPase alpha subunit gene (PatpA), and promoter of the RuBisCo large subunit gene (PrbcL), and any combination thereof (See, e.g., De Cosa et al. Nat. Biotechnol. 19:71-74 (2001); Daniell et al. BMC Biotechnol. 9:33 (2009); Muto et al. BMC Biotechnol. 9:26 (2009); Surzycki et al. Biologicals 37: 133-138 (2009)).

[00102] In some embodiments, a promoter useful with the invention can be one or more endogenous promoters of *Camelina sativa* cell wall invertase inhibitor, PCWIII and/or PCWII2. PCWIII (SEQ ID NO:20) can provide tissue specific/tissue preferred expression in the vasculature of various plant tissues. PCWII2 (SEQ ID NO:21) can provide tissue specific/tissue preferred expression in the root tip and stele. Thus, these two promoters can be useful for providing tissue specific/tissue preferred expression in plants generally.

[00103] With respect to a coding sequence, the term “plant-expressible” means that the coding sequence (nucleotide sequence) can be efficiently expressed by plant cells, tissue and/or whole plants. As used herein, a plant-expressible coding sequence has a GC composition consistent with acceptable gene expression in plant cells, a sufficiently low CpG content so that expression of that coding sequence is not restricted by plant cells, and codon usage that is consistent with that of plant genes. Where it is desired that the properties of the plant-expressible gene are identical to those of the naturally occurring gene, the plant-expressible homolog will have a synonymous coding sequence or a substantially synonymous coding sequence. A substantially synonymous coding sequence is one in that there are codons that encode similar amino acids to a comparison sequence, or if the amino acid

substituted is not similar in properties to the one it replaces, that change has no significant effect on enzymatic activity for at least one substrate of that enzyme. As discussed herein, it is well understood that in most cases, there is some flexibility in amino acid sequence such that function is not significantly changed. Conservative changes in amino acid sequence, and the resultant similar protein can be readily tested using procedures such as those disclosed herein. Where it is desired that the plant-expressible gene have different properties, there can be variation in the amino acid sequence as compared to the wild-type gene, and the properties of enhanced photosynthesis can be readily determined as described herein.

[00104] “Plant-expressible transcriptional and translational regulatory sequences” are those that can function in plants, plant tissue and/or plant cells to effect the transcriptional and translational expression of the nucleotide sequences with that they are associated. Included are 5' sequences that qualitatively control gene expression (turn on or off gene expression in response to environmental signals such as light, or in a tissue-specific manner) and quantitative regulatory sequences that advantageously increase the level of downstream gene expression. An example of a sequence motif that serves as a translational control sequence is that of the ribosome binding site sequence. Polyadenylation signals are examples of transcription regulatory sequences positioned downstream of a target sequence. Exemplary flanking sequences include the 3' flanking sequences of the nos gene of the *Agrobacterium tumefaciens* Ti plasmid. The upstream nontranslated sequence of a bacterial merA coding sequence can be utilized to improve expression of other sequences in plants as well.

[00105] The plant-expressible transcription regulatory sequence optionally comprises a constitutive promoter to drive gene expression throughout the whole plant or a majority of plant tissues. In one embodiment, the constitutive promoter drives gene expression at a higher level than the endogenous plant gene promoter. In one embodiment, the constitutive promoter drives gene expression at a level that is at least two-fold higher, specifically at least five-fold higher, and more specifically at least ten-fold higher than the endogenous plant gene promoter. Suitable constitutive promoters include plant virus promoters such as the cauliflower mosaic virus (CaMV) 35S and 19S promoters. An exemplary plant virus promoter is the cauliflower mosaic virus 35S promoter. Suitable constitutive promoters further include promoters for plant genes that are constitutively expressed such as the plant ubiquitin, Rubisco, and actin promoters such as the ACT1 and ACT2 plant actin genes. Exemplary plant gene promoters include the ACT2 promoter from *Arabidopsis* (locus

AT3G18780; SEQ ID. NO:12 of WO2015103074) and the ACT1 promoter from rice (GenBank Accession no. S44221.1; SEQ ID. NO:13 of WO2015103074).

[00106] Where a regulatory element is to be coupled to a constitutive promoter, generally a truncated (or minimal) promoter is used, for example, the truncated 35S promoter of Cauliflower Mosaic Virus. Truncated versions of other constitutive promoters can also be used to provide CAAT and TATA-homologous regions; such promoter sequences can be derived from those of *Agrobacterium tumefaciens* T-DNA genes such as nos, ocs and mas and plant virus genes such as the CaMV 19S gene or the ACT2 gene of *Arabidopsis*. Translational control sequences specifically exemplified herein are the nucleotides between 8 and 13 upstream of the ATG translation start codon for bacterial signals and from nucleotides 1 to 7 upstream of the ATG translation start codon for plants.

[00107] A minimal promoter contains the DNA sequence signals necessary for RNA polymerase binding and initiation of transcription. For RNA polymerase II promoters the promoter is identified by a TATA-homologous sequences motif about 20 to 50 base pairs upstream of the transcription start site and a CAAT-homologous sequence motif about 50 to 120 base pairs upstream of the transcription start site. In plants, the CAAT box may be substituted by the AGGA box (Messing et al., (1983) in Genetic Engineering of Plants, T. Kosuge, C. Meredith and A. Hollaender (eds.), Plenum Press, pp. 211-227). By convention, the nucleotides upstream of the transcription start with increasingly large numbers extending upstream of (in the 5' direction) from the start site. In one embodiment, transcription directed by a minimal promoter is low and does not respond either positively or negatively to environmental or developmental signals in plant tissue. An exemplary minimal promoter suitable for use in plants is the truncated CaMV 35S promoter, that contains the regions from -90 to + 8 of the 35S gene. Where high levels of gene expression are desired, transcription regulatory sequences that upregulate the levels of gene expression may be operatively linked to a minimal promoter is used thereto. Such quantitative regulatory sequences are exemplified by transcription enhancing regulatory sequences such as enhancers.

[00108] In one embodiment, the plant-expressible transcription regulatory sequence comprises a tissue or organ-specific promoter to drive gene expression in selected organs such as roots or shoots and tissues therein. In one embodiment, the organ-specific promoter drives gene expression in below ground tissues such as roots and root hairs. In one embodiment, the organ-specific promoter drives gene expression in above ground tissues such as shoots and leaves. An exemplary leaf-specific promoter is the SRS1 promoter. In

one embodiment, the organ-specific promoter drives gene expression in floral and reproductive tissues.

[00109] The plant-expressible transcription regulatory sequence optionally comprises an inducible promoter to drive gene expression in response to selected stimuli. Suitable inducible promoters include a light inducible promoter such as the SRS1 promoter, and the chlorophyll A/13 binding protein light-inducible transcription regulatory sequences.

[00110] Chemical-regulated promoters can be used to modulate the expression of a gene in an organism through the application of an exogenous chemical regulator. Regulation of the expression of nucleotide sequences of the invention via promoters that are chemically regulated enables the polypeptides of the invention to be synthesized only when, for example, a crop of plants are treated with the inducing chemicals. Depending upon the objective, the promoter may be a chemical-inducible promoter, where application of a chemical induces gene expression, or a chemical-repressible promoter, where application of the chemical represses gene expression

[00111] A heterologous polynucleotide operably linked to a promoter and encoding a suppressor of a cell wall invertase inhibitor and/or a heterologous polynucleotide operably linked to a promoter and encoding a polypeptide having the enzyme activity of a cell wall invertase can be introduced (in any order) into a plant in any combination with one or more additional polynucleotides, including a heterologous polynucleotide encoding a bicarbonate transporter, to increase the number of seeds and/or increase the assimilate partitioning directed into fruits and/or seeds and/or increase the seed size in a plant. Further, an engineered endogenous cell wall invertase inhibitor gene of a plant can be combined with the introduction of one or more heterologous polynucleotides into said plant as described herein, and in any order, i.e., the modification of the endogenous cell wall invertase inhibitor gene can be done first, in between, or after the introduction of one or more heterologous polynucleotides into a plant.

[00112] A nucleotide sequence encoding a signal peptide may be operably linked at the 5'- or 3'- terminus of a heterologous nucleotide sequence or nucleic acid molecule. Signal peptides (and the targeting nucleotide sequences encoding them) are well known in the art and can be found in public databases, many available on the internet, such as those disclosed in WO2014209792. A "signal peptide", "transit peptide", or "targeting peptide" herein refers to a short (for example, 5-30 amino acids long) peptide present at the N-terminus of a newly synthesized protein that determines interaction with the protein transport system, and the destination to which that protein is delivered.

[00113] Targeting to a membrane is similar to targeting to an organelle. Thus, specific sequences on a protein (targeting sequences or motifs) can be recognized by a transporter, which then imports the protein into an organelle or in the case of membrane association, the transporter can guide the protein to and associate it with a membrane. Thus, for example, a specific cysteine residue on a C-terminal motif of a protein can be modified post-translation where an enzyme (prenyltransferases) then attaches a hydrophobic molecule (geranylgeranyl or farnesyl) (See, e.g., Running et al. *Proc Natl Acad Sci USA* 101 : 7815-7820 (2004); Maurer-Stroh et al. *Genome Biology* 4:212 (2003)). This hydrophobic addition guides and associates the protein to a membrane (in case of the cytosol, the membrane would be the plasma membrane or the cytosolic side of the nuclear membrane (Polychronidou et al. *Molecular Biology of the Cell* 21 : 3409-3420 (2010)). More specifically, in representative embodiments, a protein prenyltransferase can catalyze the covalent attachment of a 15 - carbon farnesyl or 20-carbon geranylgeranyl isoprenoid to C-terminal cysteines of selected proteins carrying a CaaX motif where C=cysteine; a=aliphatic amino acid; x=any amino acid. For plants, this motif most often is CVVQ (SEQ ID NO:34). The addition of prenyl groups facilitates membrane association and protein-protein interactions of the prenylated proteins.

[00114] In still other embodiments of the invention, a signal peptide can direct a polypeptide of the invention to more than one organelle (e.g., dual targeting). Thus, in some embodiments, a signal peptide that can target a polypeptide of the invention to more than one organelle is encoded by an amino acid sequence that includes, but is not limited to, the amino acid sequence of SEQ ID NO:35.

[00115] In each of the embodiments herein, the polynucleotide operably linked to a promoter and encoding the bicarbonate transporter and/or the heterologous polynucleotide operably linked to a promoter and encoding a polypeptide having the enzyme activity of a cell wall invertase can be overexpressed in the stably transformed plant.

[00116] In further embodiments, the heterologous polypeptides and heterologous polynucleotides useful with this invention as described herein (e.g., those encoding polypeptides having the activity of a bicarbonate transporter, CWI, CWII, or a suppressor of CWII) can be modified for use in the disclosed compositions and methods. For example, a native or wild type intergenic spacer sequence in a selected polynucleotide can be substituted with another known spacer or a synthetic spacer sequence. In some embodiments, a polynucleotide or gene can be modified to increase or decrease the activity of the encoded polypeptide. Exemplary *Camelina sativa* CWII gene sequences for modification include SEQ ID Nos:5, 7, 9, 10, 12, 14, 32, and 33.

[00117] The heterologous polynucleotide encoding a polypeptide having the enzyme activity of a bicarbonate transporter, the heterologous polynucleotide encoding a suppressor of a cell wall invertase inhibitor and/or the heterologous polynucleotide encoding a polypeptide having the enzyme activity of a cell wall invertase can be on a single expression vector or cassette or on multiple expression vectors or cassettes, and can be introduced into plants singly or introduced more than one at a time using co-transformation methods as known in the art.

[00118] In some embodiments, the heterologous polynucleotide encoding a bicarbonate transporter, the heterologous polynucleotide encoding a suppressor of a cell wall invertase inhibitor and/or the heterologous polynucleotide encoding a polypeptide having the enzyme activity of a cell wall invertase are introduced into the nucleus or nuclear genome. In representative embodiments, the heterologous polynucleotide encoding a polypeptide having the enzyme activity of a bicarbonate transporter is then localized to the chloroplast. In some embodiments, the heterologous polynucleotide encoding a polypeptide having the enzyme activity of a bicarbonate transporter can be localized to both the chloroplast and the plasma membrane. In further embodiments, the heterologous polynucleotide encoding a polypeptide having the enzyme activity of a cell wall invertase can be localized to the cell wall. In some representative embodiments, the heterologous polynucleotide encoding a suppressor of a cell wall invertase inhibitor can be introduced into the nucleus or nuclear genome or the cytosol where the suppressor acts to degrade the cell wall invertase inhibitor transcript.

[00119] In addition to transformation technology, traditional breeding methods as known in the art (e.g., crossing) can be used to assist in introducing into a single plant each of the heterologous polynucleotides encoding the bicarbonate transporter and/or any other polynucleotides of interest described herein (e.g., polynucleotides encoding cell wall invertase and a suppressor of an inhibitor of cell wall invertase) to produce a plant, plant part, and/or plant cell comprising and expressing each of the heterologous polynucleotides as described herein.

[00120] In still further embodiments, the heterologous polynucleotide encoding a bicarbonate transporter and the heterologous polynucleotide encoding a polypeptide having the enzyme activity of a cell wall invertase can be operably linked to a single promoter and/or can be overexpressed in said plant. Thus, any combination of promoters with heterologous nucleotides of the invention useful for producing plants having increased seed number, increased assimilate partitioning directed into fruits and/or seeds (and/or other plant part) and/or increased seed size can be utilized.

[00121] In some embodiments of the invention, an expression vector or cassette can comprise an enhancer sequence. Enhancer sequences can be derived from, for example, any intron from any highly expressed gene. In particular embodiments, an enhancer sequence usable in an expression vector or cassette disclosed herein includes, but is not limited to, the nucleotide sequence of ggagg (e.g., ribosome binding site).

[00122] An expression cassette or vector also can optionally include a transcriptional and/or translational termination region (i.e., termination region) that is functional in plants, yeast, or bacteria. A variety of transcriptional terminators is available for use in expression cassettes or vectors disclosed herein. The termination region may be native to the transcriptional initiation region, may be native to the operably linked nucleotide sequence of interest, may be native to the host cell, or may be derived from another source (i.e., foreign or heterologous to the promoter, the nucleotide sequence of interest, the host cell, or any combination thereof). Non-limiting examples of transcriptional terminators useful for plants can be a CAMV 35S terminator, a tml terminator, a nopaline synthase terminator and/or a pea rbcS E9 terminator, a RubisCo small subunit gene 1 (TrbcSl) terminator, an actin gene (Tactin) terminator, a nitrate reductase gene (Tnr) terminator, and/or aa duplicated carbonic anhydrase gene 1 (TdcAl) terminator.

[00123] Further non-limiting examples of terminators useful with this invention for expression of the heterologous polynucleotides of the invention or other heterologous polynucleotides of interest in algae include a terminator of the psbA gene (TpsbA), a terminator of the psaA gene (encoding an apoprotein of photosystem I) (TpsaA), a terminator of the psbD gene (TpsbD), a RuBisCo large subunit terminator (TrbcL), a terminator of the σ -type plastid rRNA gene (Trrn), and/or a terminator of the ATPase alpha subunit gene (TatpA).

[00124] The choice of vector used for constructing a recombinant DNA molecule depends on the functional properties desired, e.g., replication, protein expression, and the host cell to be transformed. In one embodiment, the vector comprises a prokaryotic replicon, i.e., a DNA sequence having the ability to direct autonomous replication and maintenance of the recombinant DNA molecule extra-chromosomally when introduced into a prokaryotic host cell, such as a bacterial host cell. In addition, the vector may also comprise a gene whose expression confers a selective advantage, such as a drug resistance, to the bacterial host cell when introduced into those transformed cells. Suitable bacterial drug resistance genes are those that confer resistance to ampicillin or tetracycline, among other selective agents. The

neomycin phosphotransferase gene has the advantage that it is expressed in eukaryotic as well as prokaryotic cells.

[00125] Vectors typically include convenient restriction sites for insertion of a recombinant DNA molecule. Suitable vector plasmids include pUC8, pUC9, pBR322, and pBR329 available from BioRad Laboratories (Richmond, Calif.) and pPL, pK and K223 available from Pharmacia (Piscataway, N.J.), and pBLUESCRIPT® and pBS available from Stratagene (La Jolla, Calif.). Suitable vectors include, for example, Lambda phage vectors including the Lambda ZAP vectors available from Stratagene (La Jolla, Calif.). Other exemplary vectors include pCMU. Other appropriate vectors may also be synthesized, according to known methods; for example, vectors pCMU/K^b and pCMUII which are modifications of pCMUIV.

[00126] Suitable expression vectors capable of expressing a recombinant nucleic acid sequence in plant cells and capable of directing stable integration within the host plant cell include vectors derived from the tumor-inducing (Ti) plasmid of *Agrobacterium tumefaciens*, and several other expression vector systems known to function in plants. See for example, Verma et al., No. WO87/00551, incorporated herein by reference. Other suitable expression vectors include gateway cloning-compatible plant destination vectors for expression of proteins in transgenic plants, e.g., the pEarleygate series (Earley et al. The Plant Journal Volume 45, Issue 4, pages 616–629, February 2006).

[00127] Expression cassettes and expression vectors optionally contain a selectable marker, which can be used to select a transformed plant, plant part, and/or host cell. As used herein, "selectable marker" means a nucleotide sequence that when expressed imparts a distinct phenotype to a plant, plant part and/or cell expressing the marker and thus allows such a transformed plant, plant part, and/or cell to be distinguished from that which does not have the marker. Such a nucleotide sequence may encode either a selectable or screenable marker, depending on whether the marker confers a trait that can be selected for by chemical means, such as by using a selective agent (e.g., an antibiotic, herbicide, or the like), or whether the marker is simply a trait that one can identify through observation or testing, such as by screening (e.g., the R-locus trait). Of course, many examples of suitable selectable markers are known in the art and can be used in the expression cassettes described herein. Although such a marker gene may be carried on another polynucleotide sequence co-introduced into the host cell, it is most often contained on the cloning vector. Only those host cells into which the marker gene has been introduced will survive and/or grow under selective conditions. Suitable selection genes encode proteins that (a) confer resistance to

antibiotics or other toxic substances, e.g., ampicillin, neomycin, methotrexate, etc.; (b) complement auxotrophic deficiencies; or (c) supply critical nutrients not available from complex media. The choice of the proper selectable marker will depend, in part, on the host cell.

[00128] Examples of selectable markers include, but are not limited to, a nucleotide sequence encoding *aadA* (i.e., spectinomycin and streptomycin resistance), a nucleotide sequence encoding *neo* (i.e., kanamycin resistance), a nucleotide sequence encoding *aphA6* (i.e., kanamycin resistance), a nucleotide sequence encoding *nptII* (i.e., kanamycin resistance), a nucleotide sequence encoding *bar* (i.e., phosphinothricin resistance), a nucleotide sequence encoding *cat* (i.e., chloramphenicol resistance), a nucleotide sequence encoding *badH* (i.e., betaine aldehyde resistance), a nucleotide sequence encoding *egfp*, (i.e., enhanced green fluorescence protein), a nucleotide sequence encoding *gfp* (i.e., green fluorescent protein), a nucleotide sequence encoding *luc* (i.e., luciferase), a nucleotide sequence encoding *ble* (bleomycin resistance), a nucleotide sequence encoding *ereA* (erythromycin resistance), and any combination thereof.

[00129] Further examples of selectable markers useful with the invention include, but are not limited to, a nucleotide sequence encoding an altered 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase, which confers resistance to glyphosate (Hinchee et al. (1988) *Biotech.* 6:915-922); a nucleotide sequence encoding a nitrilase such as *bxn* from *Klebsiella ozaenae* that confers resistance to bromoxynil (Stalker et al. (1988) *Science* 242:419-423); a nucleotide sequence encoding an altered acetolactate synthase (ALS) that confers resistance to imidazolinone, sulfonyleurea or other ALS-inhibiting chemicals (EP Patent Application No. 154204); a nucleotide sequence encoding a methotrexate-resistant dihydrofolate reductase (DHFR) (Thillet et al. (1988) *J Biol. Chem.* 263:12500-12508); a nucleotide sequence encoding a dalapon dehalogenase that confers resistance to dalapon; a nucleotide sequence encoding a mannose-6-phosphate isomerase (also referred to as phosphomannose isomerase (PMI)) that confers an ability to metabolize mannose (US Patent Nos. 5,767,378 and 5,994,629); a nucleotide sequence encoding an altered anthranilate synthase that confers resistance to 5-methyl tryptophan; and/or a nucleotide sequence encoding *hph* that confers resistance to hygromycin.

[00130] Additional selectable markers include, but are not limited to, a nucleotide sequence encoding β -glucuronidase or *uidA* (GUS) that encodes an enzyme for which various chromogenic substrates are known; an R-locus nucleotide sequence that encodes a product that regulates the production of anthocyanin pigments (red color) in plant tissues (Dellaporta

et al, "Molecular cloning of the maize R-nj allele by transposon-tagging with Ac" 263-282 In: Chromosome Structure and Function: Impact of New Concepts, 18th Stadler Genetics Symposium (Gustafson & Appels eds., Plenum Press 1988)); a nucleotide sequence encoding β -lactamase, an enzyme for which various chromogenic substrates are known {e.g., PAD AC, a chromogenic cephalosporin) (Sutcliffe (1978) Proc. Natl. Acad. Sci. USA 75:3737-3741); a nucleotide sequence encoding xylE that encodes a catechol dioxygenase (Zukowsky et al. (1983) Proc. Natl. Acad. Sci. USA 80:1101-1105); a nucleotide sequence encoding tyrosinase, an enzyme capable of oxidizing tyrosine to DOPA and dopaquinone, which in turn condenses to form melanin (Katz et al. (1983) J Gen. Microbiol. 129:2103-2114); a nucleotide sequence encoding β -galactosidase, an enzyme for which there are chromogenic substrates; a nucleotide sequence encoding luciferase (lux) that allows for bioluminescence detection (Ow et al. (1986) Science 234:856-859); a nucleotide sequence encoding Bla that confers ampicillin resistance; or a nucleotide sequence encoding aequorin which may be employed in calcium-sensitive bioluminescence detection (Prasher et al. (1985) Biochem. Biophys. Res. Comm. 126:1259-1268), and/or any combination thereof. One of skill in the art is capable of choosing a suitable selectable marker for use in an expression cassette of this invention.

[00131] One of the most commonly used markers for the selection of transgenic plants is resistance to glufosinate ammonium, an herbicide that is sold under a variety of trade names including Basta and Finale. Resistance to glufosinate ammonium is conferred by the bacterial bialaphos resistance gene (*BAR*) encoding the enzyme phosphinotricin acetyl transferase (PAT). The major advantage of glufosinate ammonium selection is that it can be performed on plants growing in soil and does not require the use of sterile techniques.

[00132] In one embodiment, the bicarbonate transporter coding sequence is cloned into a vector suitable for expression in *Camelina* under the control of different constitutive promoters including the CaMV 35S promoter and the actin promoters from *Arabidopsis* and rice. In one embodiment, the bicarbonate transporter coding sequence is regulated by an organ or tissue-specific or an inducible promoter. An exemplary tissue-specific promoter is the leaf-specific SRS1 promoter. In one embodiment, the bicarbonate transporter coding sequence is cloned into a plant expression cassette construct or vector comprising a promoter, convenient cloning sites and the nos transcription terminator (NOST). In one embodiment, the bicarbonate transporter is cloned into a plant expression cassette in a pEarlygate plasmid.

[00133] Transformation of a host cell with an expression vector or other DNA is carried out by conventional techniques as are well known to those skilled in the art. By

“transformation” is meant a permanent or transient genetic change induced in a cell following incorporation of new DNA (i.e., DNA exogenous to the cell). Where the cell is a plant cell, a permanent genetic change is generally achieved by introduction of the DNA into the genome of the cell. By “transformed cell” or “host cell” is meant a cell (e.g., prokaryotic or eukaryotic) into which (or into an ancestor of which) has been introduced, by means of recombinant DNA techniques, a DNA molecule encoding a bicarbonate transporter (e.g., a SbtA, BicA, CCP1, or LCIA polypeptide), or fragment thereof.

[00134] Recombinant host cells, in the present context, are those that have been genetically modified to contain a heterologous DNA molecule. The DNA can be introduced by a means that is appropriate for the particular type of cell, including without limitation, transfection, transformation, lipofection, or electroporation.

[00135] A “transgenic plant” is one that has been genetically modified to contain and express recombinant DNA sequences, either as regulatory RNA molecules or as proteins. As specifically exemplified herein, a transgenic plant is genetically modified to contain and express a recombinant DNA sequence operatively linked to and under the regulatory control of transcriptional control sequences that function in plant cells or tissue or in whole plants. As used herein, a transgenic plant also encompasses progeny of the initial transgenic plant where those progeny contain and are capable of expressing the recombinant coding sequence under the regulatory control of the plant-expressible transcription control sequences described herein. Seeds containing transgenic embryos are encompassed within this definition.

[00136] Individual plants within a population of transgenic plants that express a recombinant gene may have different levels of gene expression. The variable gene expression is due to multiple factors including multiple copies of the recombinant gene, chromatin effects, and gene suppression. Accordingly, a phenotype of the transgenic plant may be measured as a percentage of individual plants within a population. In one embodiment, greater than or equal to about 25% of the transgenic plants express the phenotype. Specifically, greater than or equal to about 50% of the transgenic plants express the phenotype. More specifically, greater than or equal to about 75% of the transgenic plants express the phenotype. The phenotype is increased CO₂ assimilation, increased assimilate partitioning, increased growth, increased seed yield, reduced transpiration rate, increased water use efficiency, and/or increased nitrogen use efficiency.

[00137] The transgenic plant has been transformed with a recombinant polynucleotide or nucleic acid molecule comprising a protein or functional nucleic acid coding sequence operatively linked to a plant-expressible transcription regulatory sequence.

[00138] A recombinant DNA construct including a plant-expressible gene or other DNA of interest is inserted into the genome of a plant by a suitable method. Suitable methods include, for example, *Agrobacterium tumefaciens*-mediated DNA transfer, direct DNA transfer, liposome-mediated DNA transfer, electroporation, co-cultivation, diffusion, particle bombardment, microinjection, gene gun, calcium phosphate coprecipitation, viral vectors, and other techniques. Suitable plant transformation vectors include those derived from a Ti plasmid of *Agrobacterium tumefaciens*. In addition to plant transformation vectors derived from the Ti or root-inducing (Ri) plasmids of *Agrobacterium*, alternative methods can be used to insert DNA constructs into plant cells. A transgenic plant can be produced by selection of transformed seeds or by selection of transformed plant cells and subsequent regeneration.

[00139] In one embodiment, the coding sequence of interest, for example a bicarbonate transporter, is subcloned under the control of the CaMV 35S promoter and the 3' OCS terminator into the plant expression T-DNA binary vector pEarleyGate 100. This coding sequence and promoter were previously shown to be strongly transcriptionally expressed in most plant tissues. *Camelina sativa* is transformed using vacuum infiltration technology, and the T1 generation seeds are screened for BASTA resistance. Transgenic plants transformed with the heterologous polynucleotide are produced. In one embodiment, the plant also expresses one or more additional heterologous coding sequences or has been genetically engineered to have a modified CWI gene with increased expression and/or a modified CWII gene with decreased expression.

[00140] In one embodiment, the transgenic plants are grown (e.g., on soil) and harvested. In one embodiment, above ground tissue is harvested separately from below ground tissue. Suitable above ground tissues include shoots, stems, leaves, flowers, grain, and seed. Exemplary below ground tissues include roots and root hairs. In one embodiment, whole plants are harvested and the above ground tissue is subsequently separated from the below ground tissue.

[00141] The terms "increase," "increasing," "increased," "enhance," "enhanced," "enhancing," and "enhancement" (and grammatical variations thereof), as used herein, describe an elevation in, for example, seed number, assimilate partitioning in to a seed or fruit (and/or any other plant part) and/or increased seed size (e.g., volume, weight, and the like) in a plant, plant part or plant cell. This increase can be observed by comparing the increase in the plant, plant part or plant cell transformed with, for example, one or more heterologous polynucleotides encoding a sequence of interest to the appropriate control (e.g., the same

plant, plant part, and/or plant cell lacking (i.e., not transformed with) the one or more heterologous polynucleotides). Thus, as used herein, the terms "increase," "increasing," "increased," "enhance," "enhanced," "enhancing," and "enhancement" (and grammatical variations thereof), and similar terms indicate an elevation of at least about, e.g., 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500% or more, or any range therein, as compared to a control.

[00142] As used herein, the terms "reduce," "reduced," "reducing," "reduction," "diminish," "suppress," and "decrease" (and grammatical variations thereof), describe, for example, a decrease in cell wall invertase inhibitor expression or production in a plant, plant cell and/or plant part as compared to a control as described herein. Thus, as used herein, the terms "reduce," "reduces," "reduced," "reduction," "diminish," "suppress," and "decrease" and similar terms mean a decrease of at least about, e.g., 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%), and the like, or any range therein, as compared to a control (e.g., a plant, plant part and/or plant cell that does not comprise the heterologous polynucleotides).

[00143] The term "suppressor" as used herein, means a molecule (e.g., a polynucleotide or polypeptide) that when incorporated into a plant, plant part, or plant cell can "reduce," "diminish," "suppress," and "decrease" the activity of another molecule (e.g., a polynucleotide or polypeptide) by at least about, e.g., 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%), and the like, or any range therein, as compared to a control (e.g., a plant, plant part and/or plant cell that does not comprise said suppressor). Thus, a heterologous polynucleotide encoding a suppressor of a cell wall invertase inhibitor (CWII) can comprise a polypeptide that suppresses CWII or it can encode a functional nucleic acid (e.g., RNAi) that suppresses CWII.

[00144] As used herein, the terms "express," "expresses," "expressed" or "expression," and the like, with respect to a nucleotide sequence (e.g., RNA or DNA) indicates that the nucleotide sequence is transcribed and, optionally, translated. Thus, a

nucleotide sequence may express a polypeptide of interest or a functional untranslated RNA. A "functional" RNA includes any untranslated RNA that has a biological function in a cell, e.g., regulation of gene expression. Such functional RNAs include but are not limited to RNAi (e.g., siRNA, shRNA, antisense RNA), miRNA, ribozymes, RNA aptamers, and the like.

[00145] As used herein, the term "overexpression" means increased expression over that in the control. In some embodiments, "overexpression" can include expression of a heterologous polynucleotide not normally expressed in an organism. In other embodiments, overexpression can include heterologous expression of an endogenous polynucleotide comprised in a heterologous expression cassette such that the amount of the endogenous polypeptide produced as a result of the endogenous polynucleotide comprised in the heterologous expression cassette is greater than is produced in the organism not transformed with said heterologous expression cassette. Thus, overexpression of a polynucleotide or polypeptide means an elevation of expression of at least about, e.g., 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500% or more, or any range therein, as compared to a control (e.g., a plant, plant part and/or plant cell that does not comprise the heterologous polynucleotides disclosed herein).

[00146] As used herein, "having the enzyme activity of" refers to a polypeptide having one or more enzymatic activities of said polypeptide. Thus, a polypeptide having the enzyme activities in accordance with this invention have at least about, e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500% or more of one or more of the enzyme activities of said polypeptide.

[00147] In any of the embodiments described herein, one or more of said polynucleotides can be introduced into a plant, plant part, and/or plant part. Thus, one or more polynucleotides encoding a particular polypeptide or functional nucleic acid as described herein can be introduced into a plant, and/or one or more polynucleotides encoding different polypeptides and/or different functional nucleic acids as described herein can be introduced into a plant in any combination.

[00148] In further aspects of the invention, a method for producing a plant having increased photosynthesis (CO₂ assimilation) and increased assimilate (e.g., sucrose) partitioning directed into fruits and/or seeds of a plant and increased seed size is disclosed.

[00149] The method comprises transforming a plant cell with a first polynucleotide operatively linked to a promoter and encoding a heterologous bicarbonate transporter

polypeptide; and modifying the plant's endogenous cell wall invertase inhibitor gene to reduce cell wall invertase inhibitor activity of the modified gene, expressing in the plant a suppressor of an inhibitor of cell wall invertase (CWII), and/or expressing or overexpressing a cell wall invertase (CWI) in the plant.

[00150] In an embodiment, the method comprises transforming a plant cell with a first polynucleotide operably linked to a promoter and encoding a heterologous bicarbonate transporter polypeptide; and performing at least one of the following steps: transforming the plant cell with at least one of a second polynucleotide operably linked to a promoter and encoding a heterologous cell wall invertase (CWI), and a third polynucleotide operably linked to a promoter and encoding a suppressor of an endogenous cell wall invertase inhibitor (CWII); modifying an endogenous cell wall invertase inhibitor (CWII) gene of the plant to reduce expression of CWII activity in the plant compared to expression of the endogenous CWII gene; and modifying an endogenous cell wall invertase (CWI) gene of the plant to increase expression of CWI activity in the plant compared to expression of the endogenous CWI gene. The introduction can result in a stably transformed plant cell; and regenerating a stably transformed plant from the plant cell. The method can further comprise growing a plant from the plant cell; and/or selecting seeds from a plant in which a trait is enhanced in comparison with a corresponding control plant, e.g., a plant that is not transformed with the first, second, or third polynucleotides; and in which the CWI and CWII genes are unmodified. The enhanced trait is preferably increased photosynthesis (CO_2 assimilation), increased vegetative growth rate, increased biomass yield, increased seed yield, increased seed weight, or increased drought tolerance

[00151] As used herein, "modifying" or "engineering" the plant's endogenous cell wall invertase inhibitor gene to reduce the cell wall invertase inhibitor activity of the modified or engineered gene includes not only the production of a cell wall invertase inhibitor polypeptide having reduced cell wall invertase inhibitor activity but also includes modification or engineering of the cell wall invertase inhibitor gene such that expression of the cell wall invertase inhibitor polypeptide is reduced. Exemplary *Camelina sativa* CWII gene sequences for modification or engineering include SEQ ID Nos:5, 7, 9, 10, 12, 14, 32, and 33.

[00152] The suppressor can be an antisense RNA complementary to the messenger RNA (mRNA) of the endogenous CWII. When the transgenic plant is a *Camelina sativa*, the antisense RNA preferably comprises SEQ ID NO:36 or 37. The suppressor can be an RNAi nucleic acid that reduces expression of the CWII mRNA. When the transgenic plant is a

Camelina sativa, the suppressor preferably comprises SEQ ID NO:38 (S1), 39(S2), or 40 (S3) and the suppressor gene sequence preferably comprises SEQ ID NO:16 (P1S1), 1217P2S2), 18 (P1S3), or 19 (P2S3).

[00153] Methods for developing antisense silencing constructs or inhibitors generally are well known in the art. Thus, for example, for the purpose of silencing an inhibitor of cell wall invertase (CWII) of interest, the nucleotide sequence of the CWII of interest can be identified by sequence homology to known CWIIs using techniques that are standard in the art (See, e.g., Jin et al. *Plant Cell* 21 :2072-2089 (2009)). Based on the nucleotide sequence of the CWII of interest, antisense nucleotide sequences can be prepared. Thus, for example, a CWII from *Camelina sativa* can be used to prepare RNAi for silencing a camelina cell wall invertase inhibitor (e.g., S SEQ ID Nos:5, 7, 9, 10, 12, 14, 32, and 33). Once a cell wall invertase inhibitor has been identified homologous nucleotide sequences of CWII from a plant of interest can be readily identified using methods known in the art for identifying homologous nucleotide sequences (e.g., Hothorn et al. *Proc Natl Acad Sci USA*. 107(40):17427-32 (2010)).

[00154] In other embodiments, the activity of one or more cell wall invertase inhibitors can be repressed by knocking out the endogenous CWII genes using methods known in the art. Thus, as an alternative to silencing endogenous CWII through the introduction of a heterologous nucleotide sequence encoding a functional nucleic acid (e.g., RNAi, antisense, amiRNA), endogenous CWII of a plant can be modified to be non-functional (i.e., knocked-out) or to have reduced activity using known methods, for example, Zinc finger nuclease (ZFN) technology (see, e.g., Umov et al. *Genome editing with engineered zinc finger nucleases*. *Nature Reviews* 11 :636-646 (2010)); Transcription Activator-Like Effector Nuclease (TALEN) technology (see, e.g., Miller, J. C. et al. A TALE nuclease architecture for efficient genome editing. *Nat. Biotechnol* 29, 143-148 (2011); and Christian, M. et al. Targeting DNA double-strand breaks with TAL effector nucleases. *Genetics* 186, 757-761 (2010)); the CRISPR/Cas system (see, e.g., Jiang, W., Bikard, D., Cox, D., Zhang, F. & Marraffini, L.A. RNA-guided editing of bacterial genomes using CRISPR-Cas systems. *Nat. Biotechnol.* 31, 233-239 (2013)); and engineered meganucleases technology (see, e.g., Antunes et al. Targeted DNA excision in *Arabidopsis* by a re-engineered homing endonuclease. *BMC Biotechnology* 12:86 (2012)). As would be understood by the skilled artisan, such methods can be readily applied to the polynucleotides/genes described herein, including, but not limited to, polynucleotides/genes encoding endogenous cell wall invertase and polynucleotides/genes encoding endogenous cell wall invertase inhibitor to alter the

activity of the encoded peptide (i.e., overexpress endogenous cell wall invertase and reduce the activity of endogenous cell wall invertase inhibitor).

[00155] Accordingly, in some embodiments methods are provided for producing a transgenic plant by suppressing the plant's endogenous cell wall invertase inhibitor using, for example, RNAi technology, or by modifying or engineering an endogenous cell wall invertase inhibitor gene by, for example, genome editing or mutation, so that the activity of the endogenous cell wall invertase inhibitor is reduced, or eliminated.

[00156] In other embodiments, methods are provided for producing a transgenic plant by expressing a heterologous cell wall invertase and/or overexpressing a plant's endogenous cell wall invertase, or any combination of overexpression of a plant's endogenous cell wall invertase, expression of a heterologous cell wall invertase, and suppression of a cell wall invertase inhibitor, and/or modification or engineering of the endogenous cell wall invertase inhibitor gene to reduce the cell wall invertase inhibitor activity of said gene. In representative embodiments, an endogenous cell wall invertase gene can be modified to overexpress in a plant. In further representative embodiments, an endogenous cell wall invertase inhibitor gene can be modified to reduce the cell wall invertase inhibitor activity of the polypeptide encoded by said gene.

[00157] Thus, in some embodiments, the present invention provides a method for producing a plant comprising: introducing into a plant cell a heterologous polynucleotide operably linked to a promoter and encoding a suppressor of a cell wall invertase inhibitor (CWII); and regenerating a stably transformed plant from the plant cell.

[00158] In some embodiments, the suppressor of the inhibitor of cell wall invertase can be an RNAi. An exemplary RNAi suppressor of cell wall invertase inhibitor can be a sequence-specific inverted repeat (sense-intron-antisense). In representative embodiments, an RNAi useful with this invention for inhibition of cell wall invertase can comprise, consist essentially of or consist of the nucleotide sequence of SEQ ID NO:38, SEQ ID NO:39, and/or SEQ ID NO:40 or a nucleotide sequence having substantial identity to said sequences SEQ ID NO:38, SEQ ID NO:39, and/or SEQ ID NO:40, any fragment of SEQ ID NO:38, SEQ ID NO:39, and/or SEQ ID NO:40 capable of inhibiting cell wall invertase (e.g., a fragment comprising 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000 nucleotides, and the like and any range therein of SEQ ID NO:38, SEQ ID NO:39, and/or SEQ ID NO:40).

[00159] In particular embodiments, a polynucleotide encoding a suppressor of a cell wall invertase inhibitor (e.g., CWII RNAi) can be operably linked to endogenous camelina promoters of the cell wall invertase inhibitors (e.g., SEQ ID NO:20, SEQ ID NO:21). Exemplary CWII RNAi gene constructs can comprise, consist essentially of or consist of the nucleotide sequence of SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, and/or SEQ ID NO:19 or a nucleotide sequence having substantial identity to the sequences SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, and/or SEQ ID NO:19.

[00160] Also provided are methods for producing a plant comprising introducing a heterologous nucleotide sequence operably linked to a promoter and encoding a polypeptide having the enzyme activity of a cell wall invertase.

[00161] In representative embodiments, a heterologous polynucleotide encoding a polypeptide having the enzyme activity of a cell wall invertase can comprise, consist essentially of, or consist of a nucleotide sequence of SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28 and/or SEQ ID NO:30, or a nucleotide sequence having substantial identity to said nucleotide sequences of SEQ ID NO: 22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28 and/or SEQ ID NO:30,. In other embodiments, an amino acid sequence of a cell wall invertase can optionally comprise, consist essentially of or consist of the amino acid sequence of SEQ ID NO:23, SEQ ID NO:25; SEQ ID NO:27, SEQ ID NO:29, and/or SEQ ID NO: 31, or an amino acid sequence having substantial identity to said nucleotide sequences of the amino acid sequence of SEQ ID NO: 23, SEQ ID NO:25; SEQ ID NO:27, SEQ ID NO:29, and/or SEQ ID NO: 31.

[00162] Any method of modifying an endogenous nucleotide sequence or gene in a cell can be used to modify an endogenous cell wall invertase inhibitor gene in a plant cell to produce a plant cell having an endogenous cell wall invertase inhibitor gene encoding a polypeptide having reduced or no cell wall invertase inhibitor activity as described herein. In representative embodiments, the endogenous cell wall invertase inhibitor is modified using the CRISPR-Cas system. In some embodiments, the activity of the modified endogenous cell wall invertase inhibitor in a plant cell is reduced by at least about 10% to about 100%. Thus, in some embodiments, the activity of the modified endogenous cell wall invertase inhibitor in a plant cell is reduced by about 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, and the like, and any value or range therein.

[00163] Thus, in some embodiments, a method for producing a plant is provided, comprising introducing into a plant cell a heterologous polynucleotide operably linked to a promoter and encoding a polypeptide having the enzyme activity of a cell wall invertase (CWI). In particular embodiments, a CWI polynucleotide can be operably linked to one or more of the promoters of the cell wall invertase inhibitors (e.g., SEQ ID NO:20, SEQ ID NO:21) from camelina. In the alternative or in addition to introducing a heterologous polynucleotide encoding a cell wall invertase, an endogenous cell wall invertase gene of a plant can be modified to be overexpressed.

[00164] The compositions and methods disclosed herein aim to improve plant growth and yields by combining the expression of heterologous proteins of the CO₂ concentrating mechanisms from cyanobacteria or the green algae, e.g., *Chlamydomonas reinhardtii*, to enhance photosynthesis, with a reduction in the expression of CWII or with an increase in expression of CWI to enhance fixed carbon allocation into vegetative and seed tissues.

[00165] The compositions and methods disclosed herein are further illustrated by the following non-limiting examples, which are merely illustrative and are not intended to limit the scope hereof. Any variations in the exemplified compositions and methods that occur to the skilled artisan are intended to fall within the scope of the present invention.

EXAMPLES

Example 1. Generation of transgenic plants having leaf specific expression of two bicarbonate transporters from *Chlamydomonas reinhardtii*, CCP1 and LCIA.

[00166] In this example transgenic plants are generated containing CCP1, LCIA, or stacked CCP1 and LCIA genes from *Chlamydomonas reinhardtii* optimized for leaf specific expression.

[00167] Methods used are analogous to those disclosed previously in WO2015103074

[00168] CCP1 fused to the cMyc epitope tag was inserted into a binary plant transformation vector based on pEarleyGate 100 (pEG100) containing a modified 35S cauliflower mosaic virus (CaMV) promoter and transformed into Camelina plants by Agrobacterium-mediated, floral dip transformation based on established methods (Lu & Kang, 2008, Plant Cell Rep, 27, 273-278). T1 transformants were screened using BASTA selection and the genotypes confirmed by PCR. Homozygous T3 lines for the Camelina^{CCP1} lines expressing the individual CCP1 transporter from *Chlamydomonas* were selected.

[00169] Constructs expressing LCIA from *Chlamydomonas* analogous to those described above for CCP1 expression were also created in pEG100 and transformed into *Camelina sativa* by *Agrobacterium*-mediated, floral dip transformation. T1 transformants were screened using BASTA selection and the genotypes confirmed by PCR. Homozygous T3 lines for the *Camelina*^{LCIA} lines expressing the individual LCIA gene from *Chlamydomonas* are selected and subsequently analyzed for various functions.

[00170] In addition to the single gene expression constructs, “stacked” expression constructs were generated in which multiple bicarbonate transporter genes were expressed. Construction was analogous to that described above for the individual *chlamydomonas* transporters.

[00171] The, stacked gene constructs expressing both CCP1 and LCIA from *Chlamydomonas* were subsequently transformed into *Camelina sativa*. The T3 plant generation from each stacked construct has been isolated and tested

[00172] Figure 1 presents the vector and gene constructs for stacked gene expression of LCIA and CCP1 in *Camelina sativa*. Figure 2 shows two representative T1 plants from the transformation and the genotype confirmation that both genes are present in the transgene insertion. Figure 2C also shows representative RT-PCR results from one LCIA-CCP1 stacked transformant, confirming expression of both transgenes.

Example 2. Generation of LCIA-CCP1- 27 and -34 transgenic lines transformed with the four RNAi silencing constructs targeting the cell wall invertase inhibitor (CWII) 1 and 2 genes.

[00173] We selected two independent T3 homozygous LCIA-CCP1 transgenic lines, LCIA- CCP1- 27 and LCIA- CCP1-34, for transformation with RNA interference constructs, which target the expression of two major families of cell wall invertase inhibitors (CWII1 and CWII2). The RNAi constructs used were P1S1 (SEQ ID NO:16), P1S3 (SEQ ID NO:18), P2S2 (SEQ ID NO:17), and P2S3 (SEQ ID NO:19). Table 1 presents a summary of the transformation constructs, target CWII genes and genetic background of the *Camelina* lines used to target expression of the CWII1 and CWII2 families. Schematic diagrams of the pEG301-P1-IHP1, pEG301-P1-IHP3, pEG301-P2-IHP2, and pEG301-P2-IHP3 are presented in Figures 3A-3D.

Table 1. Summary of transformation strategy for introducing CWII RNAi constructs into LCIA-CCP1 and wild type Camelina

Transformation vector	CWii RNAi gene construct	Genetic background transformed	CWii gene family	Target genes	Screening method
pEG301-P1-IHP1	P1S1	WT	CWii1	Csa03g051630;	Bar ^R
		LCIA-CCP1-27		Csa17g075360;	DsRed/Bar ^R
		LCIA-CCP1-34		Csa14g051860	DsRed/Bar ^R
pEG301-P1-IHP3	P1S3	WT	CWii1 and CWii2	Csa03g051630;	Bar ^R
		LCIA-CCP1-27		Csa17g075360;	DsRed/Bar ^R
		LCIA-CCP1-34		Csa14g051860;	DsRed/Bar ^R
pEG301-P2-IHP2	P2S2	WT	CWii2	Csa02g074170;	Bar ^R
		LCIA-CCP1-27		Csa18g038260;	DsRed/Bar ^R
		LCIA-CCP1-34		Csa11g101740	DsRed/Bar ^R
pEG301-P2-IHP3	P2S3	WT	CWii1 and CWii2	Csa03g051630;	Bar ^R
		LCIA-CCP1-27		Csa17g075360;	DsRed/Bar ^R
		LCIA-CCP1-34		Csa14g051860;	DsRed/Bar ^R
		WT		Csa02g074170;	Bar ^R
				Csa18g038260;	DsRed/Bar ^R
				Csa11g101740	DsRed/Bar ^R

[00174] We identified T1 transformants of the LCIA-CCP1-27 and -34 lines with the four CWII constructs shown in Figures 3A-3D. All plants exhibit DsRed (linked to LCIA-CCP1) and BASTA resistance (linked to CWII). PCR confirmation of a representative set of T1 transformants is shown in figure 4. Figure 4 shows the PCR confirmation of representative LCIA-CCP1 plants transformed with pEG-301-P2-IHP3 (P2-IHP3), pEG-301-P2-IHP2 (P2-IHP2), pEG-301-P1-IHP1 (P1-IHP1), and pEG-301-P1-IHP3 (P1-IHP3) vectors. Lanes 1 and 8 in the top panel and 1 and 12 in the bottom panel contain positive controls using amplification of the purified vector. The positions of the expected PCR products are indicated by arrows.

Example 3. Assessment of growth, yield, and photosynthesis parameters in P2S2xLCIA-CCP1 lines.

[00175] We analyzed six T3 homozygous lines for the P2S2xLCIA-CCP1 transformants by assessing expression of CWII1 and 2, growth rate, photosynthesis and seed yields relative to the LCIA-CCP1 and wild type (WT) controls. P2S2 expresses RNAi, which targets the CWII2 gene. The data for the expression of CWII2 as measured by

quantitative Real Time-PCR analysis of CWI2 mRNA levels in the T3 lines is shown in Figure 5. In these measurements, Actin2 was used as an endogenous reference. Relative quantification was carried out using the comparative Ct method. Values presented in Figure 5 are mean $\pm$ SE (n=3). Tukey-Kramer test, $P > 0.05$. 15 DAS plants. WT and LCIA-CCP1-34-9-0 data were triplicate samples from each of three individual plants. All P2S2 data were triplicate samples from single individuals.

[00176] We selected two of the lines exhibiting maximum reduction in CWI2 expression (P2S2xLCIA-CCP1-34-2-1 and P2S2xLCIA-CCP1-34-1-6) for analysis in growth chambers under conditions that mimic field conditions. Five plants from each line were compared to wild type and LCIA-CCP1 parental control plants. Figure 6A demonstrates that the P2S2xLCIA-CCP1 lines exhibited a significantly increased growth rate within the first 27 days relative to wild type plants. The growth rate of LCIA-CCP1 plants was intermediate between the P2S2 transformants and wild type plants. The height of the wild type and LCIA-CCP1 plants eventually equaled the P2S2 transformants as the plants matured (41 days; figure 6C).

[00177] The expression of CWI2 in the LCIA-CCP1 parental line was ~35% higher than the wild type control, suggesting that the gene is moderately upregulated in plants expressing LCIA+CCP1. The P2S2 transformants exhibited a range of CWI2 expression levels. The maximum suppression of CWI2 expression was observed in P2S2xLCIA-CCP1-34-1-6(3). This line exhibited a 60% reduction in CWI2 expression relative to the LCIA-CCP1 control (figure 5).

[00178] Photosynthesis as measured by CO₂ assimilation in the P2S2xLCIA-CCP1 lines was indistinguishable from the LCIA-CCP1 parental control and ~18% higher than the wild type control (figure 7). These data indicate that RNAi suppression of CWI2 does not negatively impact the increase in photosynthetic rates observed in the LCIA-CCP1 plants.

[00179] We measured the seed yields from the P2S2xLCIA-CCP1-34-2-1 and P2S2xLCIA-CCP1-34-1-6 lines. Consistent with previous observations, the LCIA-CCP1-34 parental control showed an increase in yield (grams of seed per plant) of 17% above the wild type control. P2S2xLCIA-CCP1-34-2-1 and P2S2xLCIA-CCP1-34-1-6 showed increases of 20% and 35% compared to wild type plants (Figure 8). These data suggest that moderate down-regulation of CWI2 in LCIA-CCP1 plants results in a moderate increase in seed yields relative to the increase observed in LCIA-CCP1 plants alone.

[00180] We also examined the seed weight in the P2S2xLCIA-CCP1-34-2-1 and P2S2xLCIA-CCP1-34-1-6 lines (Figure 9). As previously observed the LCIA-CCP1 plants

showed a decrease in seed weight (weight/100 seeds) relative to wild type plants. Seed weights in the P2S2xLCIA-CCP1-34-2-1 and P2S2xLCIA-CCP1-34-1-6 lines were 16% below wild type plants and were not statistically different from those observed in the LCIA-CCP1 parental control.

[00181] We conclude that RNAi down-regulation of CWII2 in the LCIA-CCP1 background results in significant increases in vegetative growth rate relative to the LCIA-CCP1 parental control and wild type plants, but does not have a significant impact on increasing seed yields or seed weights compared to LCIA-CCP1 plants alone. This is consistent with the observation that CWII2 is primarily expressed in leaf tissues during vegetative growth.

[00182] CWII1 is expressed more highly in floral tissues and developing seeds. Consequently, we investigated the levels of CWII1 in the P2S2 plants to determine how this gene responds to the RNAi expression. Interestingly, the levels of CWII1 expression increase by ~3-fold in the P2S2xLCIA-CCP1-34-2-1 and P2S2xLCIA-CCP1-34-1-6 lines (Figure 10). We hypothesize that the increase in CWII1 expression could limit the impact of down-regulating CWII2 and thereby limit carbon allocation to seed tissue. These results suggest that limiting the expression of CWII1 and 2 in combination (e.g. using the P1S3 construct) should be more effective in enhancing yields in the LCIA-CCP1 plants.

Example 4. Modification of the CWII via the CRISPR-Cas system

[00183] An alternative approach to suppressing the cell wall invertase inhibitor is to use genome editing. In the present example, the activity of the CWII is reduced in *Camelina* using the CRISPR-Cas system.

[00184] *Camelina* (WT or plants transgenic for LCIA or CCP1) are transformed (by any method already described for other transgenes) with a nucleotide sequence encoding a CRISPR-associated protein 9 (Cas9) gene under the control of a strong constitutive promoter and at least one single guide RNA (sgRNA) molecule, which comprises a portion of the CWII target gene and which is under a similarly strong constitutive promoter. The Cas9 and the sgRNA is either on the same construct or on a separate construct, to be transformed into plants simultaneously or consecutively (for example, transgenic plants recovered from one transformation can be transformed with the other construct).

[00185] Cas9 interacts with a guide RNA molecule to create double-stranded breaks in genomic DNA at the site of homology to the guide RNA (e.g., CWII). Repair is done by the cell through non-homologous end joining and causes indels that shift the reading frame of

a coding sequence thus resulting in an inactive target protein. The Cas9 transgene is expressed with a nuclear targeting peptide.

[00186] The sgRNA is designed to be 19-22 nt long with full sequence homology to a region of interest within the target gene, CWII. The target sequence must be followed by a protospacer adjacent motif (PAM) and thus is selected with this in mind. The sgRNA can be used individually or multiplexed to achieve multiple edits within a single gene or multiple genes. The sequence is then queried against any available genomic database to screen for ' homology that could result in off-target mutations. The optimal sequences are perfect matches to their targets and have no homology in other sites in the genome.

EXAMPLE 5. Transforming various crops with the vectors

Agrobacterium-mediated transformation of maize

[00187] The vectors provided in the invention can be used for Agrobacterium-mediated transformation of maize following a previously described procedure (Frame et al., 2006, Agrobacterium Protocols Wang K., ed., Vol. 1, pp 185-199, Humana Press).

[00188] *Plant material:* Plants grown in a greenhouse are used as an explant source. Ears are harvested 9-13 d after pollination and surface sterilized with 80% ethanol.

[00189] *Explant isolation, infection and co-cultivation:* Immature zygotic embryos (1.2-2.0 mm) are aseptically dissected from individual kernels and incubated in *A. tumefaciens* strain EHA101 culture (grown in 5 ml N6 medium supplemented with 100 μ M acetosyringone for stimulation of the bacterial vir genes for 2-5 h prior to transformation) at room temperature for 5 min. The infected embryos are transferred scutellum side up on to a co-cultivation medium (N6 agar-solidified medium containing 300 mg/l cysteine, 5 μ M silver nitrate and 100 μ M acetosyringone) and incubated at 20°C, in the dark for 3 d. Embryos are transferred to N6 resting medium containing 100 mg/l cefotaxime, 100 mg/l vancomycin and 5 μ M silver nitrate and incubated at 28°C, in the dark for 7 d.

[00190] *Callus selection:* All embryos are transferred on to the first selection medium (the resting medium described above supplemented with 1.5 mg/l bialaphos) and incubated at 28°C, in the dark for 2 weeks followed by subculture on a selection medium containing 3 mg/l bialaphos. Proliferating pieces of callus are propagated and maintained by subculture on the same medium every 2 weeks.

[00191] *Plant regeneration and selection:* Bialaphos-resistant embryogenic callus lines are transferred on to regeneration medium I (MS basal medium supplemented with 60 g/l sucrose, 1.5 mg/l bialaphos and 100 mg/l cefotaxime and solidified with 3 g/l Gelrite) and incubated at 25°C, in the dark for 2 to 3 weeks. Mature embryos formed during this period are

transferred on to regeneration medium II (the same as regeneration medium I with 3 mg/l bialaphos) for germination in the light (25°C, 80-100 $\mu\text{E}/\text{m}^2/\text{s}$ light intensity, 16/8-h photoperiod). Regenerated plants are ready for transfer to soil within 10-14 days.

Agrobacterium-mediated transformation of sorghum

[00192] The vectors provided in the invention can be used for sorghum transformation following a previously described procedure (Zhao, 2006, *Agrobacterium Protocols* Wang K., ed., Vol. 1, pp 233-244, Humana Press).

[00193] *Plant material:* Plants grown under greenhouse, growth chamber or field conditions are used as an explant source. Immature panicles are harvested 9-12 d post pollination and individual kernels are surface sterilized with 50% bleach for 30 min followed by three washes with sterile distilled water.

[00194] *Explant isolation, infection and co-cultivation:* Immature zygotic embryos (1-1.5 mm) are aseptically dissected from individual kernels and incubated in *A. tumefaciens* strain LBA4404 suspension in PHI-I liquid medium (MS basal medium supplemented with 1 g/l casamino acids, 1.5 mg/l 2,4-D, 68.5 g/l sucrose, 36 g/l glucose and 100 μM acetosyringone) at room temperature for 5 min. The infected embryos are transferred with embryonic axis down on to a co-cultivation PHI-T medium (agar-solidified modified PHI-I medium containing 2.0 mg/l 2,4-D, 20 g/l sucrose, 10 g/l glucose, 0.5 g/l MES, 0.7 g/l proline, 10 mg/l ascorbic acid and 100 μM acetosyringone) and incubated at 25°C, in the dark for 3 d. For resting, embryos are transferred to the same medium (without acetosyringone) supplemented with 100 mg/l carbenicillin and incubated at 28°C, in the dark for 4 d.

[00195] *Callus selection:* Embryos are transferred on to the first selection medium PHI-U (PHI-T medium described above supplemented with 1.5 mg/l 2,4-D, 100 mg/l carbenicillin and 5 mg/l PPT without glucose and acetosyringone) and incubated at 28°C, in the dark for 2 weeks followed by subculture on a selection medium containing 10 mg/l PPT. Proliferating pieces of callus are propagated and maintained by subculture on the same medium every 2 weeks for the remainder of the callus selection process of 10 weeks.

[00196] *Plant regeneration and selection:* Herbicide-resistant callus is transferred on to regeneration medium I (PHI-U medium supplemented with 0.5 mg/l kinetin) and incubated at 28°C, in the dark for 2 to 3 weeks for callus growth and embryo development. Cultures are transferred on to regeneration medium II (MS basal medium with 0.5 mg/l zeatin, 700 mg/l proline, 60 g/l sucrose and 100 mg/l carbenicillin) for shoot formation (28°C, in the dark). After 2-3 weeks, shoots are transferred on to a rooting medium (regeneration II medium supplemented with 20 g/l sucrose, 0.5 mg/l NAA and 0.5 mg/l IBA) and grown at

25°C, 270 $\mu\text{E}/\text{m}^2/\text{s}$ light intensity with a 16/8-h photoperiod. When the regenerated plants are 8-10 cm tall, they can be transferred to soil and grown under greenhouse conditions.

***Agrobacterium*-mediated transformation of rice**

[00197] The vectors provided in the invention can be used for *Agrobacterium*-mediated transformation of rice following a previously described procedure (Herve and Kayano, 2006, *Agrobacterium Protocols* Wang K., ed., Vol. 1, pp 213-222, Humana Press).

[00198] *Plant material*: Mature seeds from japonica rice varieties grown in a greenhouse are used as an explant source.

[00199] *Culture transformation and selection*: Dehusked seeds are surface sterilized with 70% ethanol for 1 min and 3% sodium hypochlorite for 30 min followed by six washes with sterile distilled water. Seeds are plated embryo side up on an induction medium (Gelrite-solidified N6 basal medium supplemented with 300 mg/l casamino acids, 2.88 g/l proline, 30 g/l sucrose and 2 mg/l 2,4-D) and incubated at 32°C, under continuous light for 5 d. Germinated seeds with swelling of the scutellum are infected with *A. tumefaciens* strain LBA4404 (culture from 3-d-old plates resuspended in N6 medium supplemented with 100 μM acetosyringone, 68.5 g/l sucrose and 36 g/l glucose) at room temperature for 2 min followed by transfer on to a co-cultivation medium (N6 Gelrite-solidified medium containing 300 mg/l casamino acids, 30 g/l sucrose, 10 g/l glucose, 2 mg/l 2,4-D and 100 μM acetosyringone) and incubation at 25°C, in the dark for 3 d.

[00200] For selection of transformed embryogenic tissues, whole seedlings washed with 250 mg/l cephotaxine are transferred on to N6 agar-solidified medium containing 300 mg/l casamino acids, 2.88 g/l proline, 30 g/l sucrose, 2 mg/l 2,4-D, 100 mg/l cefotaxime, 100 mg/l vancomycin and 35 mg/l G418 disulfate). Cultures are incubated at 32°C, under continuous light for 2-3 weeks.

[00201] *Plant regeneration and selection*: Resistant proliferating calluses are transferred on to agar-solidified N6 medium containing 300 mg/l casamino acids, 500 mg/l proline, 30 g/l sucrose, 1 mg/l NAA, 5 mg/l ABA, 2 mg/l kinetin, 100 mg/l cefotaxime, 100 mg/l vancomycin and 20 mg/l G418 disulfate. After one week of growth at 32°C, under continuous light, the surviving calluses are transferred on to MS medium (solidified with 10 g/l agarose) supplemented with 2 g/l casamino acids, 30 g/l sucrose, 30 g/l sorbitol, 0.02 mg/l NAA, 2 mg/l kinetin, 100 mg/l cefotaxime, 100 mg/l vancomycin and 20 mg/l G418 disulfate and incubated under the same conditions for another week followed by a transfer on to the same medium with 7 g/l agarose. After 2 weeks, the emerging shoots are transferred on to Gelrite-solidified MS hormone-free medium containing 30 g/l sucrose and grown under

continuous light for 1-2 weeks to promote shoot and root development. When the regenerated plants are 8-10 cm tall, they can be transferred to soil and grown under greenhouse conditions. After about 10-16 weeks, transgenic seeds are harvested.

[00202] Indica rice varieties are transformed with *Agrobacterium* following a similar procedure (Datta and Datta, 2006, *Agrobacterium Protocols* Wang K., ed., Vol. 1, pp 201-212, Humana Press).

Microprojectile bombardment-mediated transformation of sugarcane

[00203] An expression cassette containing a transcription factor gene can be co-introduced with a cassette of a marker gene (e. g., *npt*) into sugarcane via biolistics following a previously described protocol (Taparia et al., 2012, *In Vitro Cell. Dev. Biol.* 48: 15-22))

[00204] *Plant material:* Greenhouse-grown plants with 6-8 visible nodes are used as an explant source. Tops are collected and surface sterilized with 70% ethanol. The outermost leaves are removed under aseptic conditions and immature leaf whorl cross sections (about 2 mm) are cut from the region 1-10 cm above the apical node.

[00205] *Culture initiation, transformation and selection:* The isolated leaf sections are cultured on MS basal media supplemented with 20 g/l sucrose, 1.86 mg/l p-chlorophenoxyacetic acid (CPA), 1.86 mg/l NAA and 0.09 mg/l BA at 28° C, under 30 $\mu\text{mol/m}^2/\text{s}$ light intensity and a 16/8-h photoperiod for 7 d. Embryogenic cultures are subcultured to fresh medium and used for transformation.

[00206] For microprojectile bombardment, leaf disks are plated on the culture initiation medium supplemented with 0.4 M sorbitol 4 hours before gene transfer. Plasmid DNA (200 ng) containing the expression cassettes of a TF and a marker gene is precipitated onto 1.8 mg gold particles (0.6 μm) following a previously described procedure (Altpeter and Sandhu, 2010, *Genetic transformation – biolistics*, Davey & Anthony eds., pp 217-237, Wiley, Hoboken). The DNA (10 ng per shot) is delivered to the explants by a PDS-1000 Biolistic particle delivery system (Biorad) using 1100-psi rupture disk, 26.5 mmHg chamber vacuum and a shelf distance of 6 cm. pressure). The bombarded explants are transferred to the culture initiation medium described above and incubated for 4 days.

[00207] For selection, cultures are transferred on to the initiation medium supplemented with 30 mg/l geneticin and incubated for 10 d followed by another selection cycle under the same conditions.

[00208] *Plant regeneration and selection:* Cultures are transferred on to the selection medium described above without CPA and grown at 28° C, under 100 $\mu\text{mol/m}^2/\text{s}$

light intensity with a 16/8-h photoperiod. Leaf disks with small shoots (about 0.5 cm) are plated on a hormone-free medium with 30 mg/l geneticin for shoot growth and root development. Prior to transfer to soil, roots of regenerated plants can be dipped into a commercially available root promoting powder.

Transformation of wheat by microprojectile bombardment

[00209] The gene constructs provided in the invention can be used for wheat transformation by microprojectile bombardment following a previously described protocol (Weeks et al., 1993, *Plant Physiol.* 102: 1077-1084).

[00210] *Plant material:* Plants from the spring wheat cultivar Bobwhite are grown at 18-20°C day and 14-16°C night temperatures under a 16 h photoperiod. Spikes are collected 10-12 weeks after sowing (12-16 days post anthesis). Individual caryopses at the early-medium milk stage are sterilized with 70% ethanol for 5 min and 20% sodium hypochlorite for 15 min followed by three washes with sterile water.

[00211] *Culture initiation, transformation and selection:* Immature zygotic embryos (0.5-1.5 mm) are dissected under aseptic conditions, placed scutellum side up on a culture induction medium (Phytigel-solidified MS medium containing 20 g/l sucrose and 1.5 mg/l 2,4-D) and incubated at 27°C, in the light (43 $\mu\text{mol}/\text{m}^2/\text{s}$) for 3-5 d.

[00212] For microprojectile bombardment, embryo-derived calluses are plated on the culture initiation medium supplemented with 0.4 M sorbitol 4 hours before gene transfer. Plasmid DNA containing the expression cassettes of a TF and the marker gene *bar* is precipitated onto 0.6- μm gold particles and delivered to the explants as described for sugarcane.

[00213] The bombarded explants are transferred to callus selection medium (the culture initiation medium described above containing 1-2 mg/l bialaphos) and subcultured every 2 weeks.

[00214] *Plant regeneration and selection:* After one-two selection cycles, cultures are transferred on to MS regeneration medium supplemented with 0.5 mg/l dicamba and 2 mg/l bialaphos. For root formation, the resulting bialaphos-resistant shoots are transferred to hormone-free half-strength MS medium. Plants with well-developed roots are transferred to soil and acclimated to lower humidity at 21°C with a 16-h photoperiod (300 $\mu\text{mol}/\text{m}^2/\text{s}$) for about 2 weeks prior to transfer to a greenhouse.

Agrobacterium-mediated transformation of Brassica napus

[00215] *Plant material:* Mature seeds are surface sterilized in 10% commercial bleach for 30 min with gentle shaking and washed three times with sterile distilled water.

[00216] *Culture initiation and transformation:* Seeds are plated on germination medium (MS basal medium supplemented with 30 g/l sucrose) and incubated at 24°C with a 16-h photoperiod at a light intensity of 60-80 $\mu\text{E}/\text{m}^2/\text{s}$ for 4-5 d. For transformation, cotyledons with ~2 mm of the petiole at the base are excised from the resulting seedlings, immersed in *Agrobacterium tumefaciens* strain EHA101 suspension (grown from a single colony in 5 ml of minimal medium supplemented with appropriate antibiotics at 28°C for 48 h) for 1 s and immediately embedded to a depth of ~ 2 mm in a co-cultivation medium (MS basal medium with 30 g/l sucrose and 20 μM benzyladenine). The inoculated cotyledons are incubated under the same growth conditions for 48 h.

[00217] *Plant regeneration and selection:* After co-cultivation, cotyledons are transferred on to a regeneration medium comprising MS medium supplemented with 30 g/l sucrose and 20 μM benzyladenine, 300 mg/l timentinin and 20 mg/l kanamycin sulfate. After 2-3 weeks, regenerated shoots are cut and maintained on MS medium for shoot elongation containing 30 g/l sucrose, 300 mg/l timentin, and 20 mg/l kanamycin sulfate. The elongated shoots are transferred to a rooting medium comprising MS basal medium supplemented with 30 g/l sucrose, 2 mg/l indole butyric acid (IBA) and 500 mg/L carbenicillin. After root formation, plants are transferred to soil and grown to seed maturity under growth chamber or greenhouse conditions.

Agrobacterium-mediated transformation of soybean

[00218] The soybean orthologs of the switchgrass transcription factor genes identified in the invention (Fig. 4) are assembled in binary vectors (Table 9) and used for *Agrobacterium*-mediated transformation of soybean following a previously described procedure (Ko et al., 2006, *Agrobacterium Protocols* Wang K., ed., Vol. 1, pp 397-405, Humana Press).

[00219] *Plant material:* Immature seeds from soybean plants grown under greenhouse or field conditions are used as an explant source. Young pods are harvested and surface sterilized with 70% 2-propanol for 30 sec and 25% Clorox for 20 min followed by three washes with sterile distilled water.

[00220] *Culture transformation and selection:* Under aseptic conditions, immature seeds are removed from the pods and the cotyledons are separated from the seed coat followed by incubation in *A. tumefaciens* culture (grown from a single colony at 28°C, overnight) in co-cultivation medium (MS salts and B5 vitamins) supplemented with 30 g/l sucrose, 40 mg/l 2,4-D and 40 mg/l acetosyringone for 60 min. Infected explants are plated

abaxial side up on agar-solidified co-cultivation medium and incubated at 25°C, in the dark for 4 d.

[00221] For selection of transformed tissues, cotyledons washed with 500 mg/l cephotaxine are placed abaxial side up on a medium for induction of somatic embryo formation (Gelrite-solidified MS medium medium containing 30 g/l sucrose, 40 mg/l 2,4-D, 500 mg/l cefotaxime, and 10 mg/l hygromycin) and incubated at 25°C, under a 23-h photoperiod (10-20 $\mu\text{E}/\text{m}^2/\text{s}$) for 2 weeks. After another two weeks of growth under the same conditions in the presence of 25 mg/l hygromycin, the antibiotic-resistant somatic embryos are transferred on MS medium for embryo maturation supplemented with 60 g/l maltose, 500 mg/l cefotaxime, and 10 mg/l hygromycin and grown under the same conditions for 8 weeks with 2-week subculture intervals.

[00222] *Plant regeneration and selection:* The resulting cotyledonary stage embryos are desiccated at 25°C, under a 23-h photoperiod (60-80 $\mu\text{E}/\text{m}^2/\text{s}$) for 5-7 d followed by culture on MS regeneration medium containing 30 g/l sucrose and 500 mg/l cefotaxime for 4-6 weeks for shoot and root development. When the plants are 5-10 cm tall, they are transferred to soil and grown in a greenhouse after acclimatization for 7 d.

[00223] The compositions and methods disclosed herein include at least the following embodiments.

[00224] Embodiment 1. A transgenic plant comprising a first polynucleotide operably linked to a promoter and encoding a heterologous bicarbonate transporter polypeptide; and at least one of a second polynucleotide comprising an engineered endogenous cell wall invertase inhibitor (CWII) gene, wherein said engineered CWII gene has reduced expression of CWII activity compared to the endogenous CWII gene, a third polynucleotide operably linked to a promoter and encoding a heterologous cell wall invertase (CWI), a fourth polynucleotide operably linked to a promoter and encoding a suppressor of an endogenous cell wall invertase inhibitor (CWII), and a fifth polynucleotide comprising an engineered endogenous cell wall invertase (CWI) gene, wherein said engineered endogenous CWI gene results in increased expression of CWI activity compared to the endogenous CWI gene.

[00225] Embodiment 2. The transgenic plant of embodiment 1, wherein the bicarbonate transporter is from an algae.

[00226] Embodiment 3. The transgenic plant of embodiment 2, wherein the algae is a *Chlamydomonas* species.

[00227] Embodiment 4. The transgenic plant of embodiment 3, wherein the bicarbonate transporter is a CCP1 polypeptide, a CCP2 polypeptide, or an LCIA polypeptide.

[00228] Embodiment 5. The transgenic plant of embodiment 4, wherein the CCP1 polypeptide comprises the amino acid sequence of SEQ ID NO:2, or an amino acid sequence 80% homologous to SEQ ID NO:2.

[00229] Embodiment 6. The transgenic plant of embodiment 4, wherein the LCIA polypeptide comprises the amino acid sequence of SEQ ID NO:4, or an amino acid sequence 80% homologous to SEQ ID NO:4.

[00230] Embodiment 7. The transgenic plant of embodiment 1, wherein the bicarbonate transporter is from a cyanobacterium.

[00231] Embodiment 8. The transgenic plant of any one of embodiments 1-7, comprising at least two heterologous bicarbonate transporters.

[00232] Embodiment 9. The transgenic plant of any one of embodiments 1-8, wherein the bicarbonate transporter localizes to a chloroplast envelope membrane or a mitochondrial inner membrane.

[00233] Embodiment 10. The transgenic plant of any one of embodiments 1-9, which is an oil crop plant selected from the group consisting of *Borago officinalis*, *Brassica campestris*, *Brassica napus*, *Brassica rapa*, *Camelina* species, *Cannabis sativa*, *Carthamus tinctorius*, *Cocos nucifera*, *Crambe abyssinica*, *Cuphea* species, *Elaeis guinensis*, *Elaeis oleifera*, *Glycine max*, *Gossypium hirsutum*, *Gossypium barbadense*, *Gossypium herbaceum*, *Helianthus annuus*, *Linum usitatissimum*, *Oenothera biennis*, *Olea europaea*, *Oryza sativa*, *Ricinus communis*, *Sesamum indicum*, *Triticum* species, *Zea mays*, walnut and almond.

[00234] Embodiment 11. The transgenic plant of any one of embodiments 1-10, wherein the plant is *Camelina sativa*.

[00235] Embodiment 12. The transgenic plant of any one of embodiments 1-11, wherein the transgenic plant has a CO₂ assimilation rate, vegetative growth rate, seed yield, or seed weight at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, or at least 40% higher than a plant of the same species not transformed with the first polynucleotide; and at least one of the second polynucleotide, the third heterologous polynucleotide, and the fourth heterologous polynucleotide.

[00236] Embodiment 13. The transgenic plant of any one of embodiments 1-12, wherein the suppressor is an antisense RNA complementary to the messenger RNA (mRNA) of the endogenous CWI.

[00237] Embodiment 14. The transgenic plant of embodiment 13, wherein the antisense RNA comprises SEQ ID NO: 36 or 37.

[00238] Embodiment 15. The transgenic plant of any one of embodiments 1-12, wherein the suppressor is a RNA interference (RNAi) nucleic acid that reduces expression of the CWII mRNA.

[00239] Embodiment 16. The transgenic plant of embodiment 15, wherein the CWII is a *Camelina sativa* CWII and the fourth polynucleotide comprises SEQ ID NO:38, SEQ ID NO:39, or SEQ ID NO:40.

[00240] Embodiment 17. The transgenic plant of any one of embodiments 1-12, comprising the second polynucleotide, wherein the engineered endogenous CWII gene is engineered by genome editing.

[00241] Embodiment 18. The transgenic plant of embodiment 17, wherein the genome editing comprises: transforming the plant with a nucleotide sequence encoding CRISPR-associated protein 9 (Cas9) under the control of a promoter and with a nucleotide sequence encoding a single guide RNA (sgRNA) under the control of a promoter, wherein the sgRNA comprises 19 to 22 nucleotides and is fully homologous to a region of the CWII gene to be modified.

[00242] Embodiment 19. The transgenic plant of any one of embodiments 1-12, comprising the second polynucleotide, wherein the engineered endogenous CWII gene is engineered by mutation.

[00243] Embodiment 20. The transgenic plant of any one of embodiments 1-19, which is a seed.

[00244] Embodiment 21. The transgenic plant of any one of embodiments 1-19, which is a plant cell.

[00245] Embodiment 22. A method of producing a transgenic plant having an improved trait, the method comprising transforming a plant cell with a first polynucleotide operably linked to a promoter and encoding a heterologous bicarbonate transporter polypeptide; and performing at least one of the following steps: transforming the plant cell with at least one of a second polynucleotide operably linked to a promoter and encoding a heterologous cell wall invertase (CWI), and a third polynucleotide operably linked to a promoter and encoding a suppressor of an endogenous cell wall invertase inhibitor (CWII); modifying an endogenous cell wall invertase inhibitor (CWII) gene of the plant to reduce expression of CWII activity in the plant compared to expression of the endogenous CWII

gene; and modifying an endogenous cell wall invertase (CWI) gene of the plant to increase expression of CWI activity in the plant compared to expression of the endogenous CWI gene.

[00246] Embodiment 23. The method of embodiment 22, further comprising growing a plant from the plant cell; and selecting seeds from a plant in which a trait is enhanced in comparison with a corresponding plant that is not transformed with any of the first, second, or third polynucleotides; and does not contain a modified endogenous cell wall invertase inhibitor (CWII) gene of the plant to reduce expression of CWII activity in the plant compared to expression of the endogenous CWII gene; or a modified endogenous cell wall invertase (CWI) gene of the plant to increase expression of CWI activity in the plant compared to expression of the endogenous CWI gene.

[00247] Embodiment 24. The method of embodiment 22 or 23, wherein the plant is an oil crop plant selected from the group consisting of *Borago officinalis*, *Brassica campestris*, *Brassica napus*, *Brassica rapa*, *Camelina* species, *Cannabis sativa*, *Carthamus tinctorius*, *Cocos nucifera*, *Crambe abyssinica*, *Cuphea* species, *Elaeis guinensis*, *Elaeis oleifera*, *Glycine max*, *Gossypium hirsutum*, *Gossypium barbadense*, *Gossypium herbaceum*, *Helianthus annuus*, *Linum usitatissimum*, *Oenothera biennis*, *Olea europaea*, *Oryza sativa*, *Ricinus communis*, *Sesamum indicum*, *Triticum* species, *Zea mays*, walnut and almond.

[00248] Embodiment 25. The method of embodiment 24, wherein the plant is a *Camelina*.

[00249] Embodiment 26. The method of any one of embodiments 22-25, wherein the enhanced trait is increased photosynthesis, increased vegetative growth rate, increased biomass yield, increased seed yield, increased seed weight, or increased drought tolerance.

[00250] Embodiment 27. The method of any one of embodiments 22-26, wherein the suppressor is an antisense RNA complementary to the messenger RNA (mRNA) of the endogenous CWII.

[00251] Embodiment 28. The method of embodiment 27, wherein the antisense RNA comprises SEQ ID NO: 36 or 37.

[00252] Embodiment 29. The method of any one of embodiments 22-26, wherein the suppressor is a RNA interference (RNAi) nucleic acid that reduces expression of the CWII mRNA.

[00253] Embodiment 30. The method of embodiment 29, wherein the CWII is a *Camelina sativa* CWII and the third polynucleotide comprises SEQ ID NO:38, SEQ ID NO:39, or SEQ ID NO:40.

Table 2. Summary of Sequences in Sequence Listing

SEQ ID NO	Organism	Description
1	<i>Chlamydomonas reinhardtii</i>	CCP1 cDNA
2	<i>Chlamydomonas reinhardtii</i>	CCP1 polypeptide
3	<i>Chlamydomonas reinhardtii</i>	LCIA cDNA
4	<i>Chlamydomonas reinhardtii</i>	LCIA polypeptide
5	<i>Camelina sativa</i>	CWII1 gene Csa03g051630
6	<i>Camelina sativa</i>	CWII1 gene Csa03g051630 polypeptide
7	<i>Camelina sativa</i>	CWII1 gene Csa14g051860
8	<i>Camelina sativa</i>	CWII1 gene Csa14g051860 polypeptide
9	<i>Camelina sativa</i>	CWII1 gene Csa17g075360
10	<i>Camelina sativa</i>	CWII2 gene Csa02g074171
11	<i>Camelina sativa</i>	CWII2 gene Csa02g074171 polypeptide
12	<i>Camelina sativa</i>	CWII2 gene Csa11g101740
13	<i>Camelina sativa</i>	CWII2 gene Csa11g101740 polypeptide
14	<i>Camelina sativa</i>	CWII2 gene Csa18g038260
15	<i>Camelina sativa</i>	CWII2 gene Csa18g038260 polypeptide
16	Artificial Sequence	P1S1
17	Artificial Sequence	P2S2
18	Artificial Sequence	P1S3
19	Artificial Sequence	P2S3
20	<i>Camelina sativa</i>	Promoter CWII1
21	<i>Camelina sativa</i>	Promoter CWII2
22	<i>Lycopersicon pennellii</i>	CWI cDNA
23	<i>Lycopersicon pennellii</i>	CWI polypeptide
24	<i>Oryza sativa</i>	CWI cDNA
25	<i>Oryza sativa</i>	CWI polypeptide
26	<i>Camelina sativa</i>	CWI cDNA
27	<i>Camelina sativa</i>	CWI polypeptide
28	<i>Camelina sativa</i>	CWI cDNA
29	<i>Camelina sativa</i>	CWI polypeptide
30	<i>Camelina sativa</i>	CWI cDNA
31	<i>Camelina sativa</i>	CWI polypeptide
32	<i>Camelina sativa</i>	CWII1 gene
33	<i>Camelina sativa</i>	CWII2 gene
34	<i>Arabidopsis thaliana</i>	Prenyltransferase modification motif-CVVQ
35	<i>Arabidopsis thaliana</i>	signal peptide
36	Artificial Sequence	CWII1 antisense construct
37	Artificial Sequence	CWII2 antisense construct
38	Artificial Sequence	CWII1 RNAi sequence
39	Artificial Sequence	CWII2 RNAi sequence
40	Artificial Sequence	CWII1-CWII2 RNAi sequence

[00254] In general, the invention may alternately comprise, consist of, or consist essentially of, any appropriate components herein disclosed. The invention may additionally, or alternatively, be formulated so as to be devoid, or substantially free, of any components,

materials, ingredients, adjuvants or species used in the prior art compositions or that are otherwise not necessary to the achievement of the function and/or objectives of the present invention. The endpoints of all ranges directed to the same component or property are inclusive and independently combinable (e.g., ranges of “less than or equal to 25 wt%, or 5 wt% to 20 wt%,” is inclusive of the endpoints and all intermediate values of the ranges of “5 wt% to 25 wt%,” etc.). Every minimum numerical limitation given throughout this specification will include every higher numerical limitation, as if such higher numerical limitations were expressly written herein. Every numerical range given throughout this specification will include every narrower numerical range that falls within such broader numerical range, as if such narrower numerical ranges were all expressly written herein. Disclosure of a narrower range or more specific group in addition to a broader range is not a disclaimer of the broader range or larger group. “Combination” is inclusive of blends, mixtures, alloys, reaction products, and the like. Furthermore, the terms “first,” “second,” and the like, herein do not denote any order, quantity, or importance, but rather are used to denote one element from another. The terms “a” and “an” and “the” herein do not denote a limitation of quantity, and are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. “Or” means “and/or.” The suffix “(s)” as used herein is intended to include both the singular and the plural of the term that it modifies, thereby including one or more of that term (e.g., the film(s) includes one or more films). Reference throughout the specification to “one embodiment”, “another embodiment”, “an embodiment”, and so forth, means that a particular element (e.g., feature, structure, and/or characteristic) described in connection with the embodiment is included in at least one embodiment described herein, and may or may not be present in other embodiments. In addition, it is to be understood that the described elements may be combined in any suitable manner in the various embodiments.

[00255] The modifier “about” used in connection with a quantity is inclusive of the stated value and has the meaning dictated by the context (e.g., includes the degree of error associated with measurement of the particular quantity). The notation “ $\pm 10\%$ ” means that the indicated measurement can be from an amount that is minus 10% to an amount that is plus 10% of the stated value. The terms “front”, “back”, “bottom”, and/or “top” are used herein, unless otherwise noted, merely for convenience of description, and are not limited to any one position or spatial orientation. “Optional” or “optionally” means that the subsequently described event or circumstance can or cannot occur, and that the description includes instances where the event occurs and instances where it does not. Unless defined

otherwise, technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs.

[00256] All cited patents, patent applications, and other references are incorporated herein by reference in their entirety. However, if a term in the present application contradicts or conflicts with a term in the incorporated reference, the term from the present application takes precedence over the conflicting term from the incorporated reference

[00257] While particular embodiments have been described, alternatives, modifications, variations, improvements, and substantial equivalents that are or may be presently unforeseen may arise to applicants or others skilled in the art. Accordingly, the appended claims as filed and as they may be amended are intended to embrace all such alternatives, modifications variations, improvements, and substantial equivalents.

CLAIMS

1. A transgenic plant comprising
a first polynucleotide operably linked to a promoter and encoding a heterologous bicarbonate transporter polypeptide; and
at least one of
a second polynucleotide comprising an engineered endogenous cell wall invertase inhibitor (CWII) gene, wherein said engineered CWII gene has reduced expression of CWII activity compared to the endogenous CWII gene,
a third polynucleotide operably linked to a promoter and encoding a heterologous cell wall invertase (CWI),
a fourth polynucleotide operably linked to a promoter and encoding a suppressor of an endogenous cell wall invertase inhibitor (CWII), and
a fifth polynucleotide comprising an engineered endogenous cell wall invertase (CWI) gene, wherein said engineered endogenous CWI gene results in increased expression of CWI activity compared to the endogenous CWI gene.
2. The transgenic plant of claim 1, wherein the bicarbonate transporter is from an algae.
3. The transgenic plant of claim 2, wherein the algae is a *Chlamydomonas* species.
4. The transgenic plant of claim 3, wherein the bicarbonate transporter is a CCP1 polypeptide, a CCP2 polypeptide, or an LCIA polypeptide.
5. The transgenic plant of claim 4, wherein the CCP1 polypeptide comprises the amino acid sequence of SEQ ID NO:2, or an amino acid sequence 80% homologous to SEQ ID NO:2.
6. The transgenic plant of claim 4, wherein the LCIA polypeptide comprises the amino acid sequence of SEQ ID NO:4, or an amino acid sequence 80% homologous to SEQ ID NO:4.
7. The transgenic plant of claim 1, wherein the bicarbonate transporter is from a cyanobacterium.
8. The transgenic plant of any one of claims 1-7, comprising at least two heterologous bicarbonate transporters.
9. The transgenic plant of any one of claims 1-8, wherein the bicarbonate transporter localizes to a chloroplast envelope membrane or a mitochondrial inner membrane.
10. The transgenic plant of any one of claims 1-9, which is an oil crop plant selected from the group consisting of *Borago officinalis*, *Brassica campestris*, *Brassica napus*,

Brassica rapa, Camelina species, Cannabis sativa, Carthamus tinctorius, Cocos nucifera, Crambe abyssinica, Cuphea species, Elaeis guinensis, Elaeis oleifera, Glycine max, Gossypium hirsutum, Gossypium barbadense, Gossypium herbaceum, Helianthus annuus, Linum usitatissimum, Oenothera biennis, Olea europaea, Oryza sativa, Ricinus communis, Sesamum indicum, Triticum species, Zea mays, walnut and almond.

11. The transgenic plant of any one of claims 1-10, wherein the plant is *Camelina sativa*.

12. The transgenic plant of any one of claims 1-11, wherein the transgenic plant has a CO₂ assimilation rate, vegetative growth rate, seed yield, or seed weight at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, or at least 40% higher than a plant of the same species not transformed with the first polynucleotide; and at least one of the second polynucleotide, the third heterologous polynucleotide, and the fourth heterologous polynucleotide.

13. The transgenic plant of any one of claims 1-12, wherein the suppressor is an antisense RNA complementary to the messenger RNA (mRNA) of the endogenous CWII.

14. The transgenic plant of claim 13, wherein the antisense RNA comprises SEQ ID NO: 36 or 37.

15. The transgenic plant of any one of claims 1-12, wherein the suppressor is a RNA interference (RNAi) nucleic acid that reduces expression of the CWII mRNA.

16. The transgenic plant of claim 15, wherein the CWII is a *Camelina sativa* CWII and the fourth polynucleotide comprises SEQ ID NO:38, SEQ ID NO:39, or SEQ ID NO:40.

17. The transgenic plant of any one of claims 1-12, comprising the second polynucleotide, wherein the engineered endogenous CWII gene is engineered by genome editing.

18. The transgenic plant of claim 17, wherein the genome editing comprises: transforming the plant with a nucleotide sequence encoding CRISPR-associated protein 9 (Cas9) under the control of a promoter and with a nucleotide sequence encoding a single guide RNA (sgRNA) under the control of a promoter, wherein the sgRNA comprises 19 to 22 nucleotides and is fully homologous to a region of the CWII gene to be modified.

19. The transgenic plant of any one of claims 1-12, comprising the second polynucleotide, wherein the engineered endogenous CWII gene is engineered by mutation.

20. The transgenic plant of any one of claims 1-19, which is a seed.

21. The transgenic plant of any one of claims 1-19, which is a plant cell.

22. A method of producing a transgenic plant having an improved trait, the method comprising

transforming a plant cell with a first polynucleotide operably linked to a promoter and encoding a heterologous bicarbonate transporter polypeptide; and

performing at least one of the following steps:

transforming the plant cell with at least one of

a second polynucleotide operably linked to a promoter and encoding a heterologous cell wall invertase (CWI), and

a third polynucleotide operably linked to a promoter and encoding a suppressor of an endogenous cell wall invertase inhibitor (CWII);

modifying an endogenous cell wall invertase inhibitor (CWII) gene of the plant to reduce expression of CWII activity in the plant compared to expression of the endogenous CWII gene; and

modifying an endogenous cell wall invertase (CWI) gene of the plant to increase expression of CWI activity in the plant compared to expression of the endogenous CWI gene.

23. The method of claim 22, further comprising

growing a plant from the plant cell; and

selecting seeds from a plant in which a trait is enhanced in comparison with a corresponding plant that is not transformed with any of the first, second, or third polynucleotides; and does not contain a modified endogenous CWII gene; or a modified endogenous CWI gene.

24. The method of claim 22 or 23, wherein the plant is an oil crop plant selected from the group consisting of *Borago officinalis*, *Brassica campestris*, *Brassica napus*, *Brassica rapa*, *Camelina* species, *Cannabis sativa*, *Carthamus tinctorius*, *Cocos nucifera*, *Crambe abyssinica*, *Cuphea* species, *Elaeis guinensis*, *Elaeis oleifera*, *Glycine max*, *Gossypium hirsutum*, *Gossypium barbadense*, *Gossypium herbaceum*, *Helianthus annuus*, *Linum usitatissimum*, *Oenothera biennis*, *Olea europaea*, *Oryza sativa*, *Ricinus communis*, *Sesamum indicum*, *Triticum* species, *Zea mays*, walnut and almond.

25. The method of claim 24, wherein the plant is a *Camelina*.

26. The method of any one of claims 22-25, wherein the enhanced trait is increased photosynthesis, increased vegetative growth rate, increased biomass yield, increased seed yield, increased seed weight, or increased drought tolerance.

27. The method of any one of claims 22-26, wherein the suppressor is an antisense RNA complementary to the messenger RNA (mRNA) of the endogenous CWII.

28. The method of claim 27, wherein the antisense RNA comprises SEQ ID NO: 36 or 37.

29. The method of any one of claims 22-26, wherein the suppressor is a RNA interference (RNAi) nucleic acid that reduces expression of the CWII mRNA.

30. The method of claim 29, wherein the CWII is a *Camelina sativa* CWII and the third polynucleotide comprises SEQ ID NO:38, SEQ ID NO:39, or SEQ ID NO:40.

FIG. 1

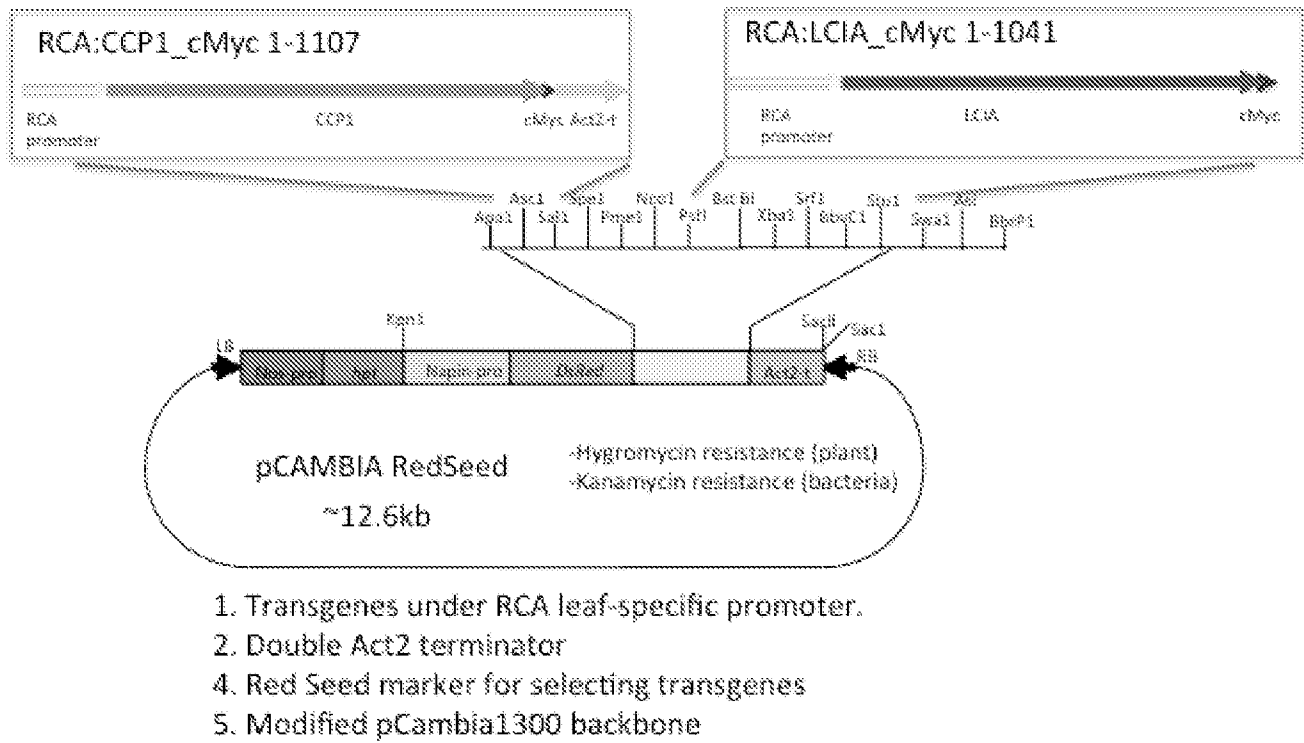


FIG. 2

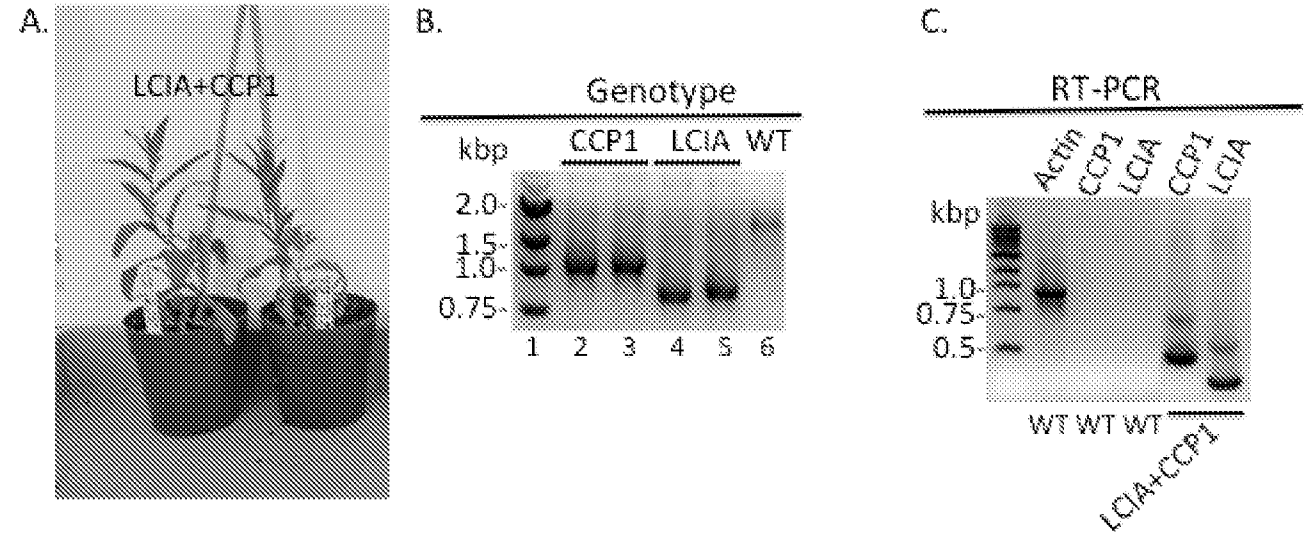


Fig. 3A

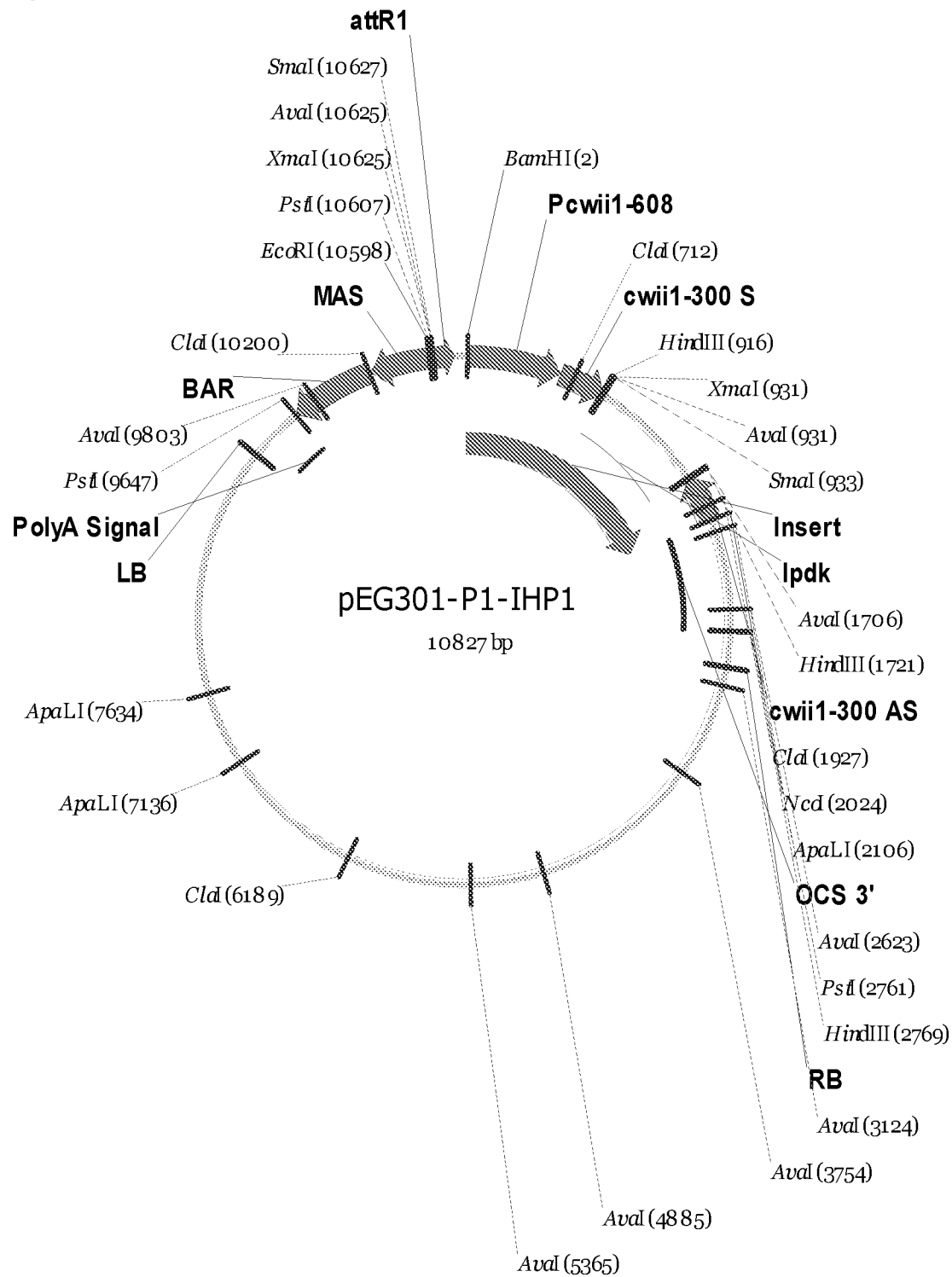


Fig. 3B

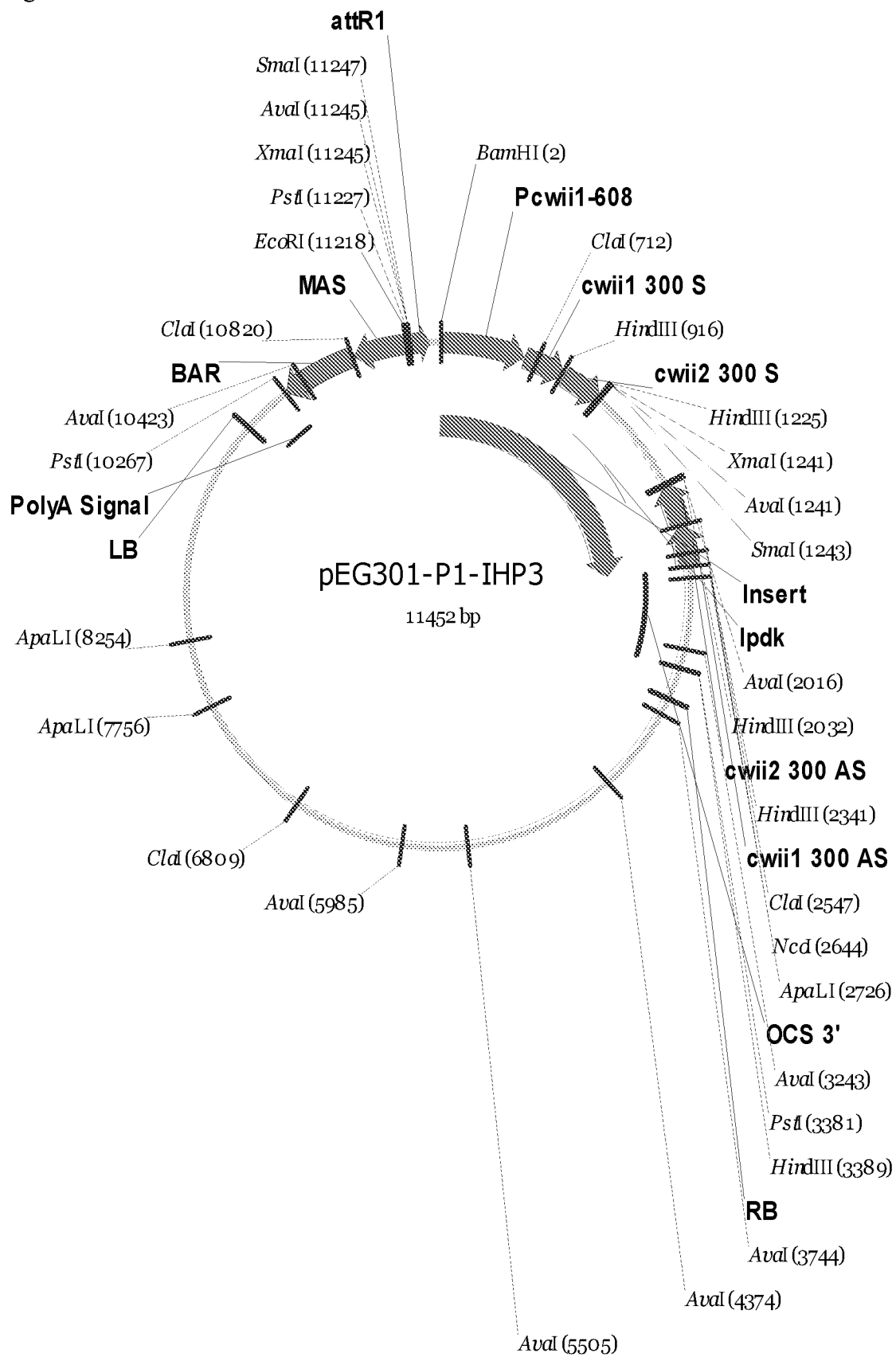


FIG. 3C

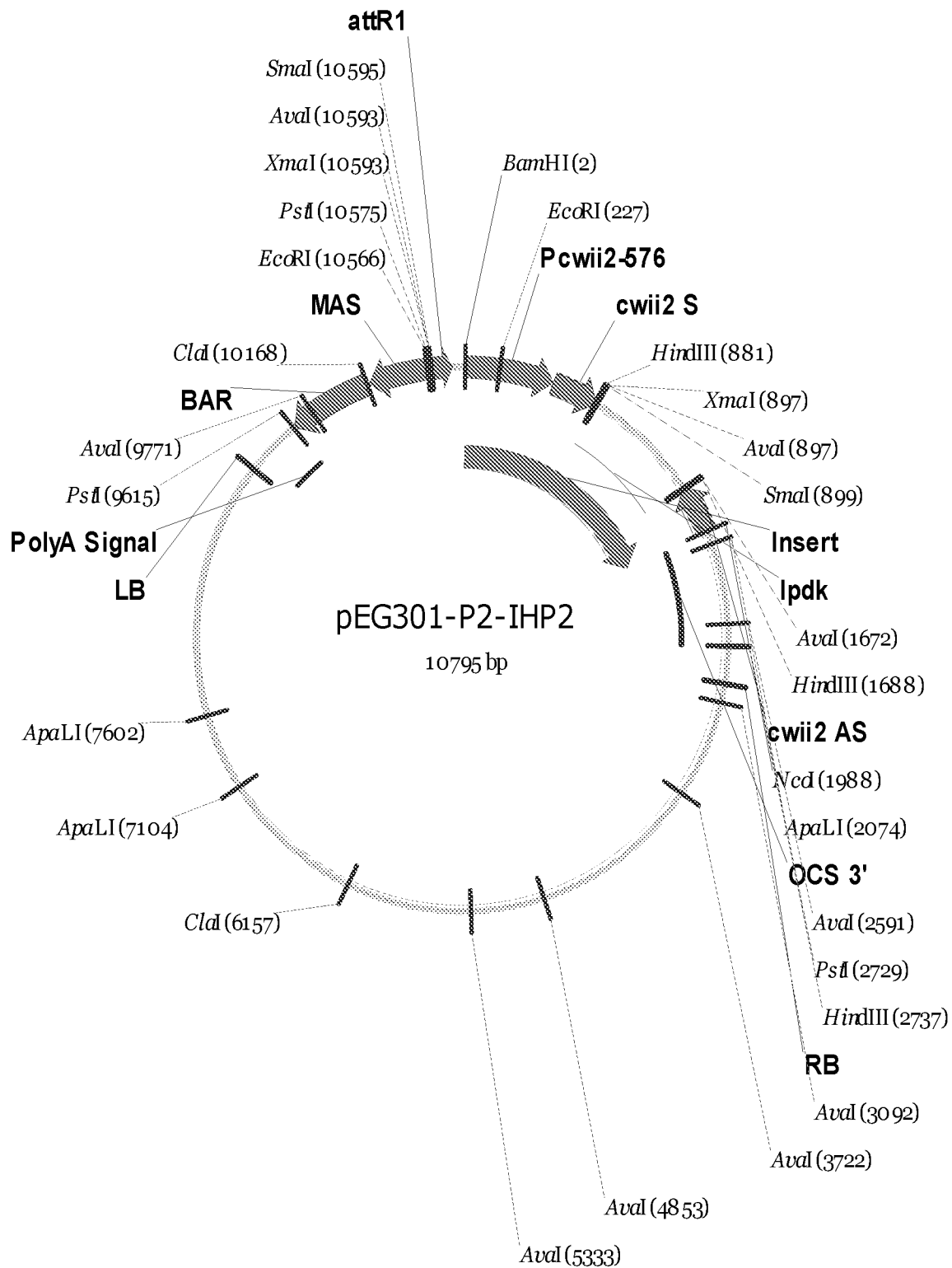


FIG. 3D

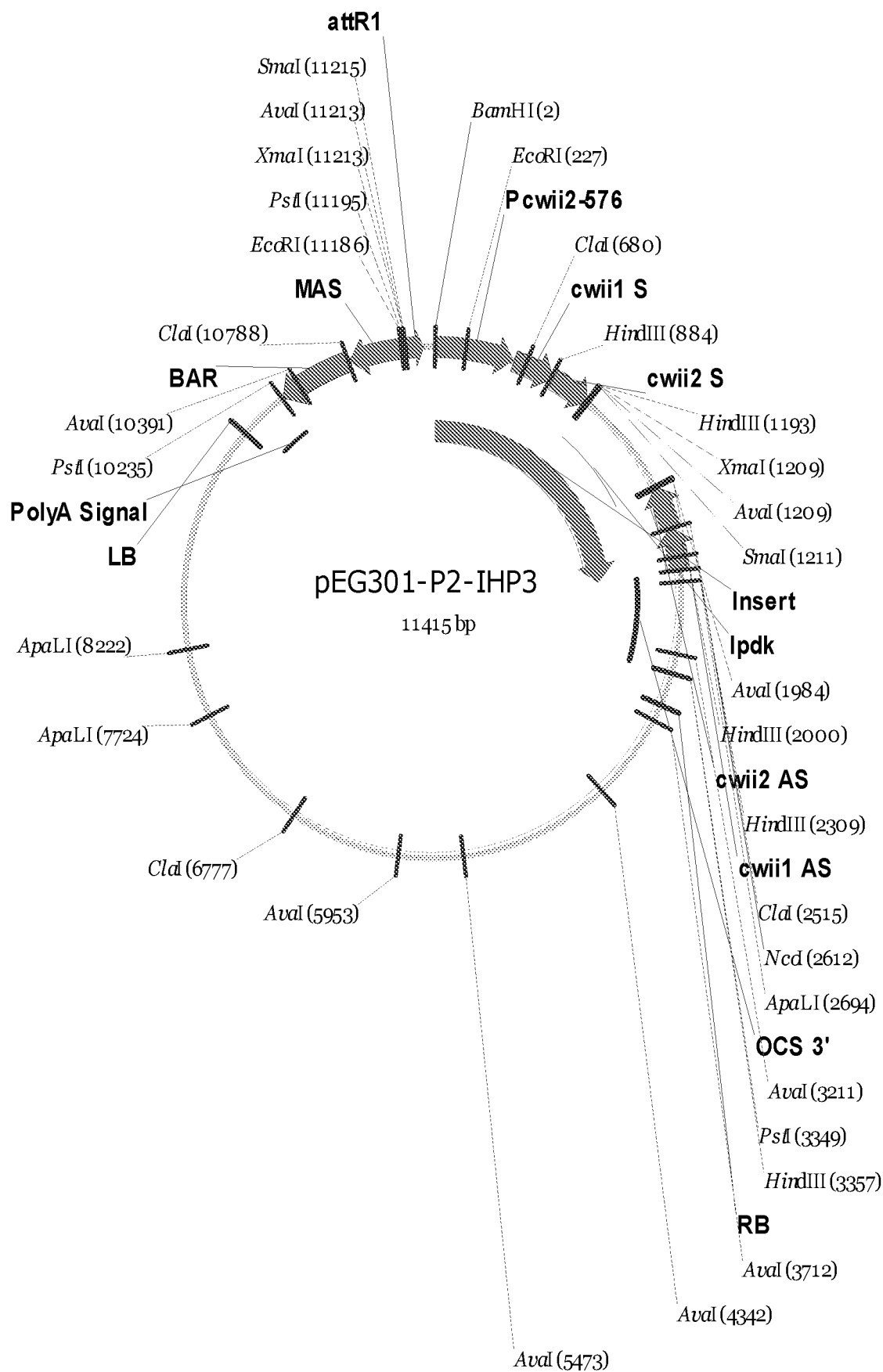


FIG. 4

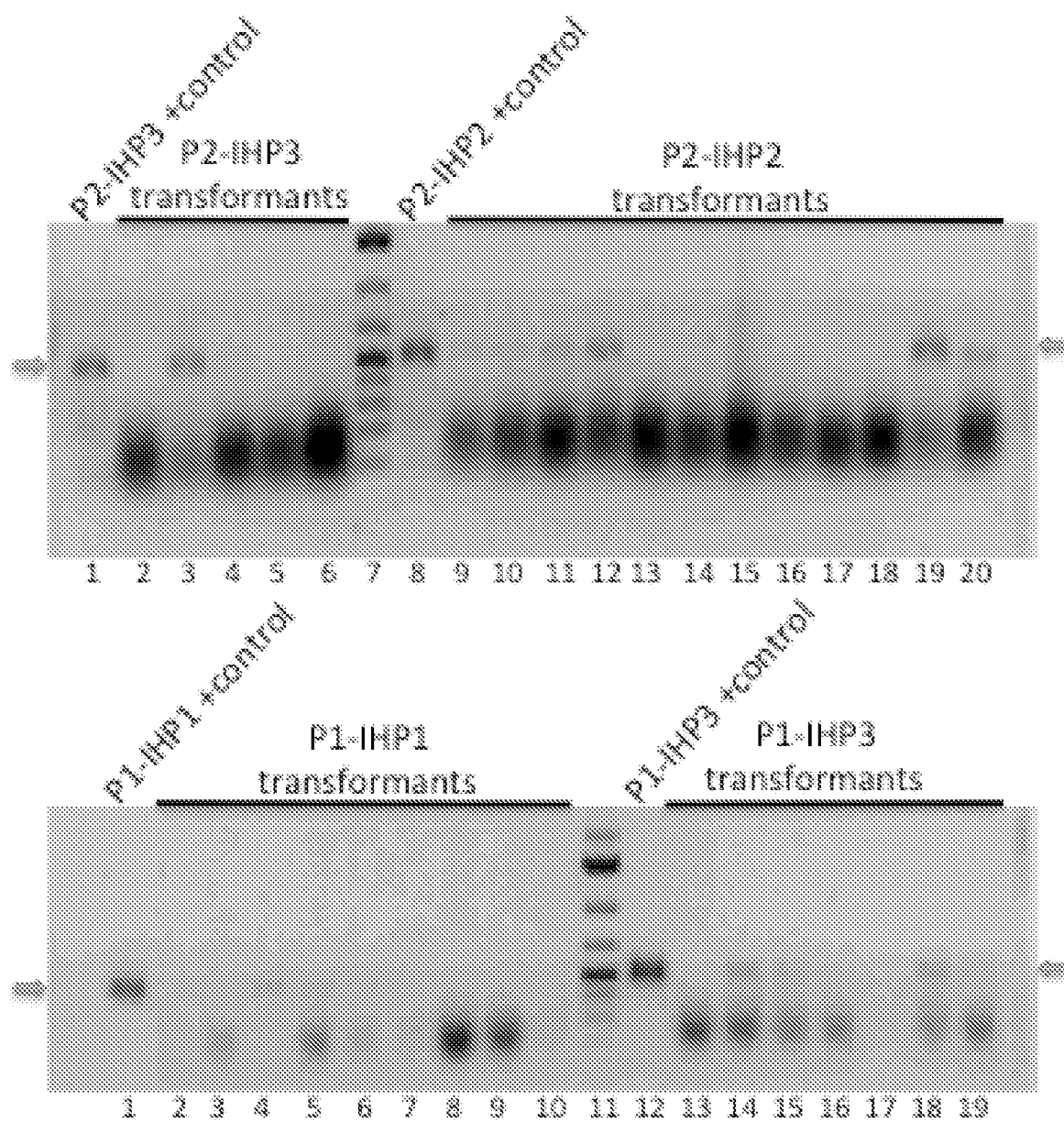


FIG. 5

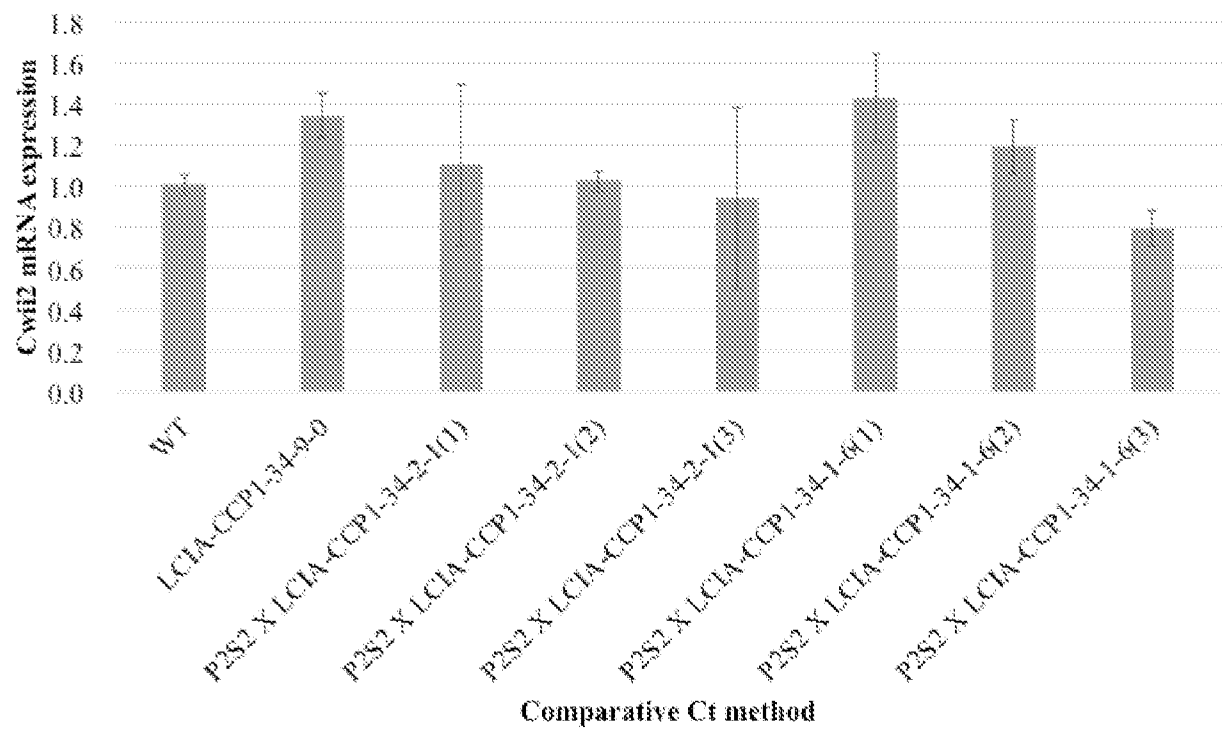
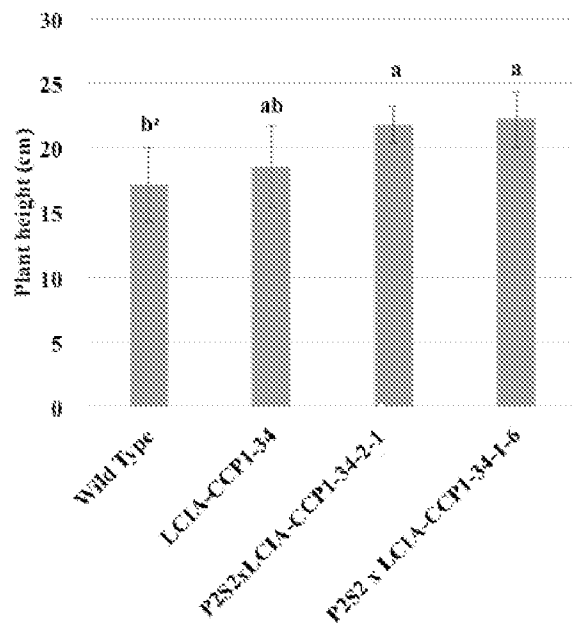


FIG 6A



Plant height at 27 days after sowing (cm)
 z: Tukey-Kramer test (n = 5, P < 0.05, bar: S.D.)

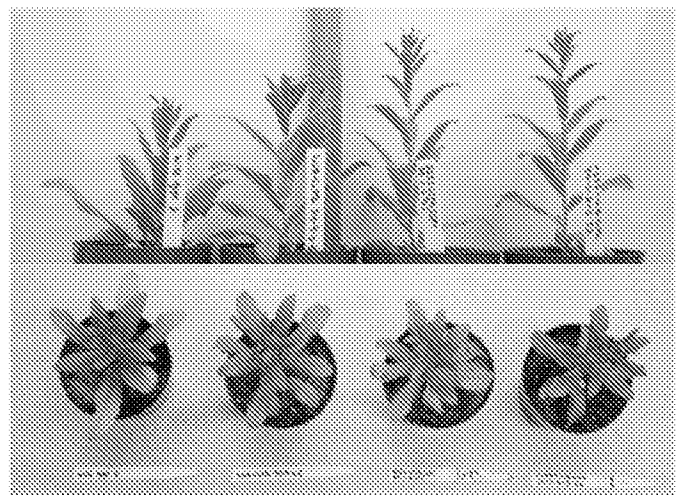


FIG. 6B

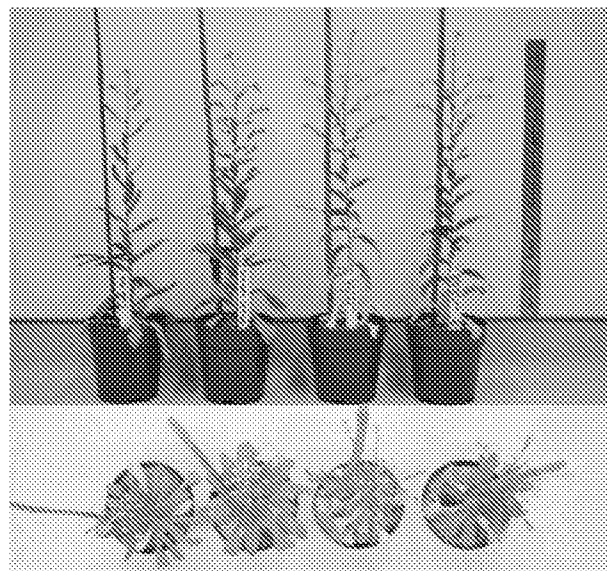
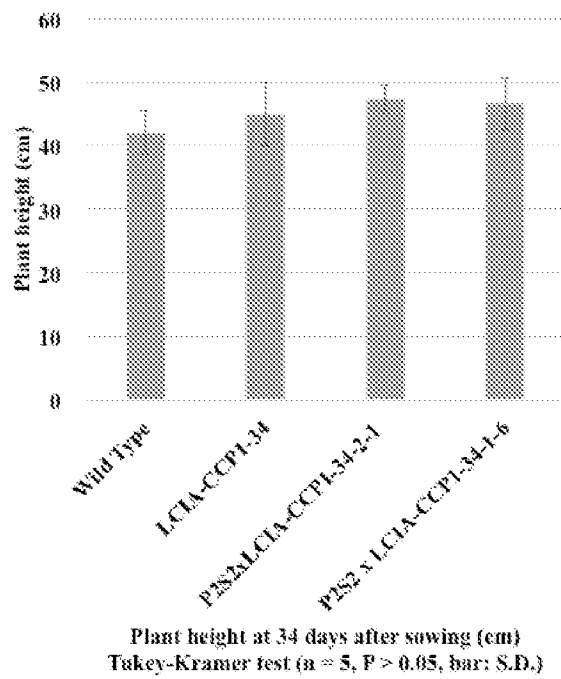


FIG. 6C

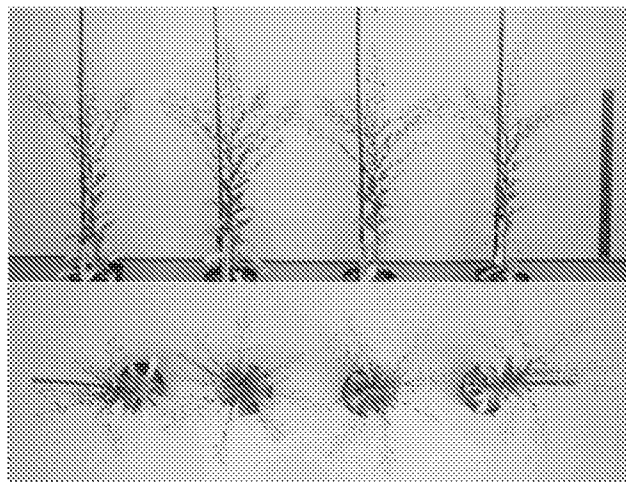
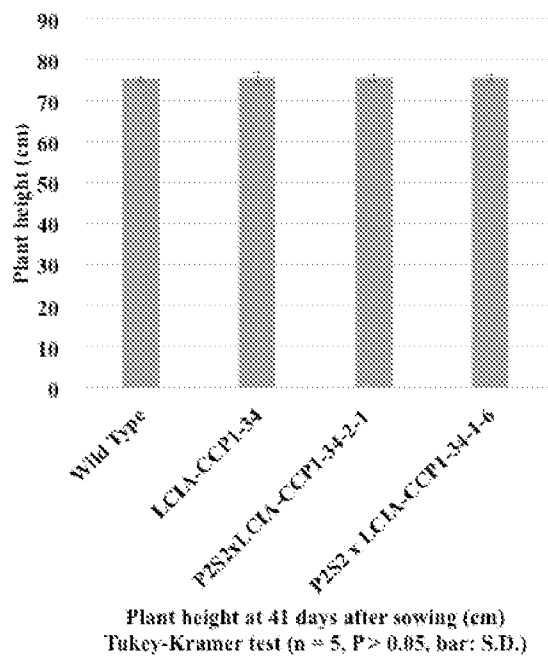


FIG. 7

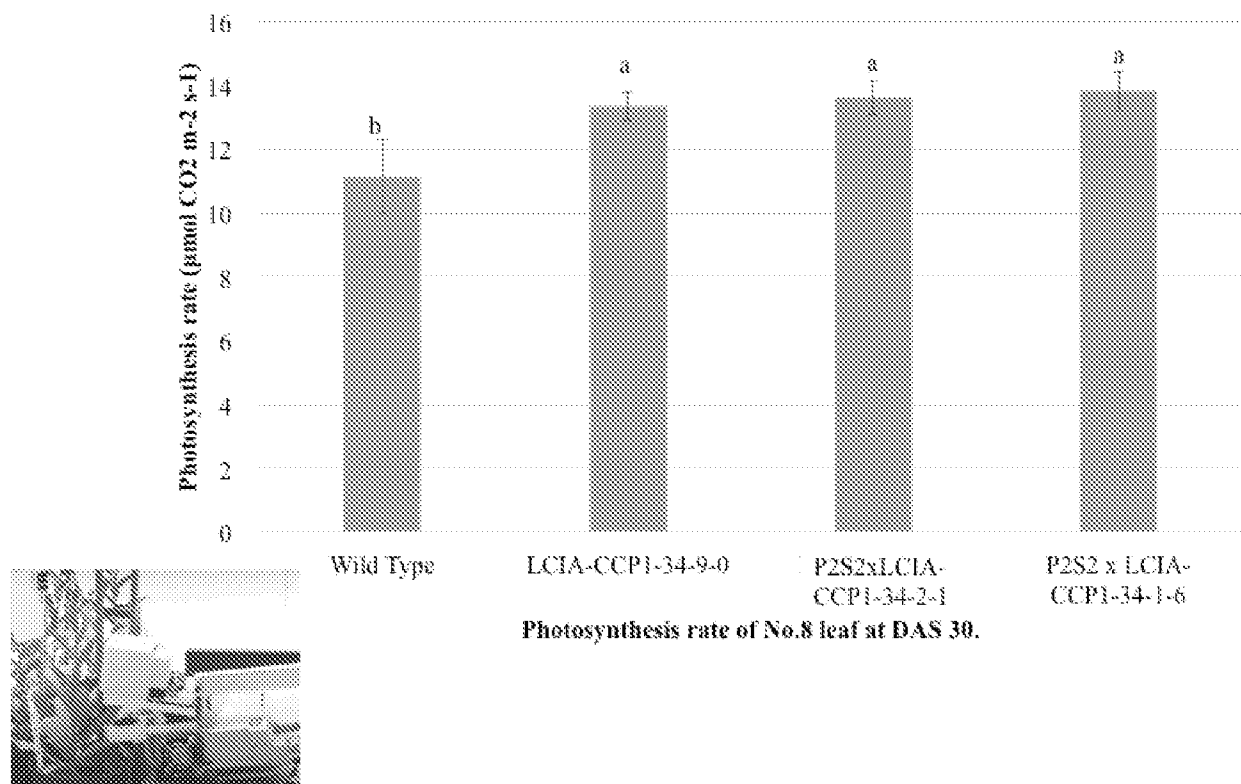


FIG. 8

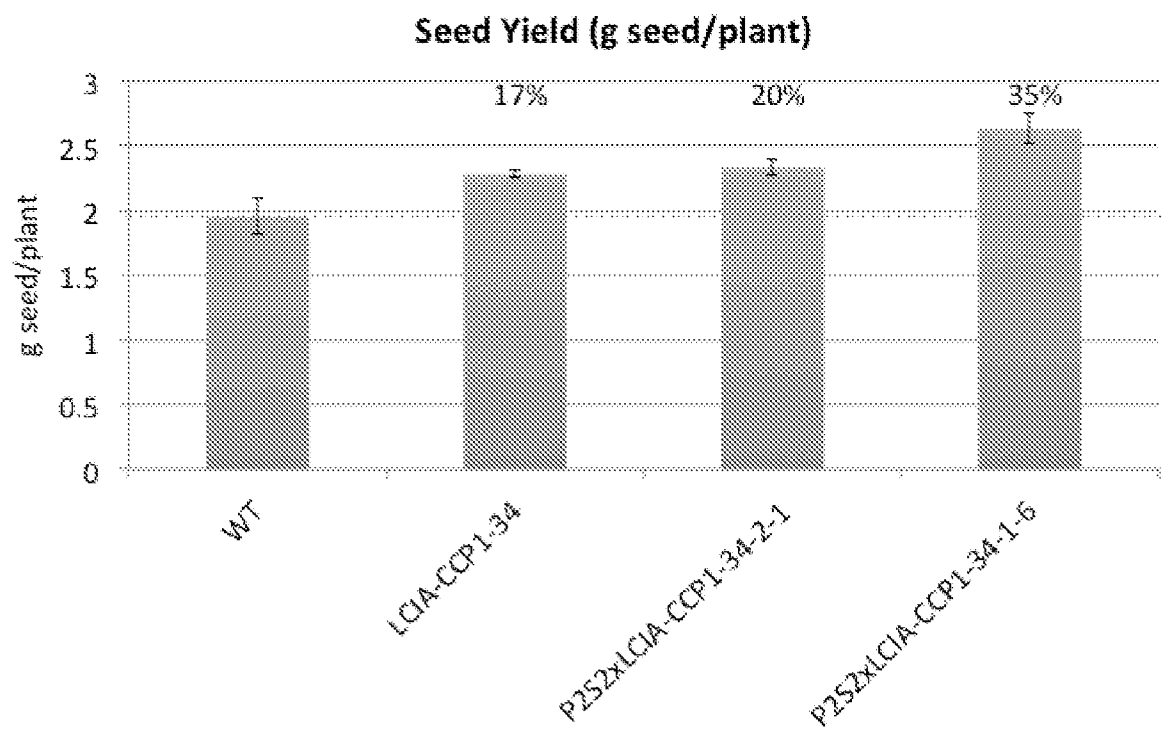


FIG. 9

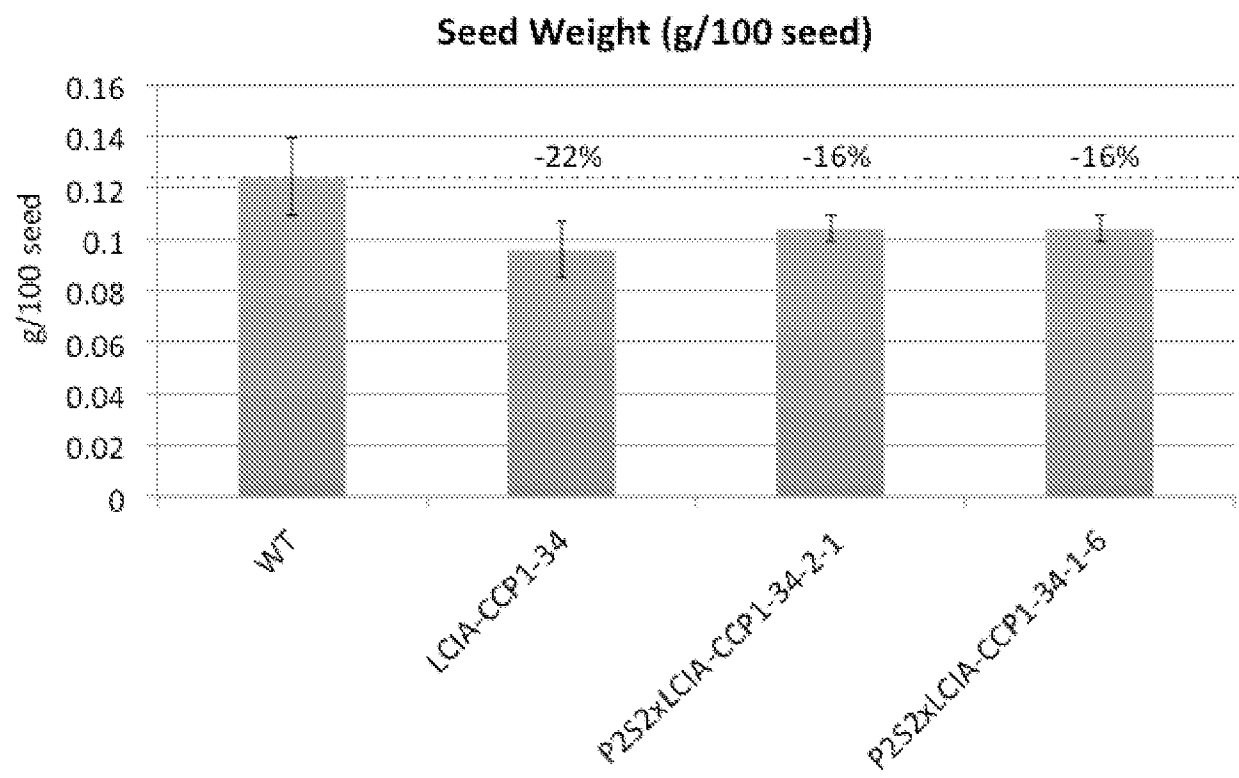


FIG. 10

