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(54) Title: FLORIGEN PATHWAY TOOLKIT

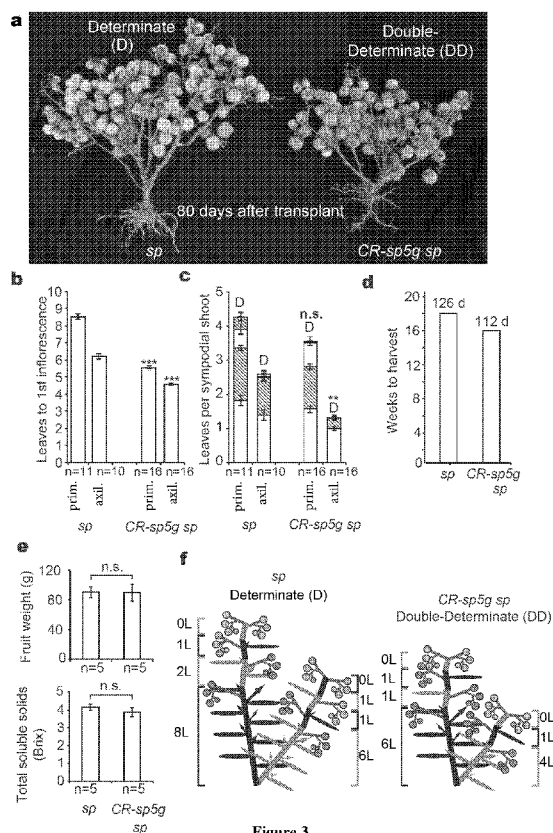


Figure 3

(57) Abstract: Described herein is a florigen pathway toolkit that comprises mutations in genes in the florigen pathway in Solanaceae, such as mutations in genes in the florigen pathway in tomatoes, that are useful to quantitatively modulate or adjust the balance of opposing flowering signals to customize and improve shoot architecture and flowering (flowering time) for tomato breeding, particularly for fresh market tomato breeding.



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FLORIGEN PATHWAY TOOLKIT

RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. 119(e) of U.S. provisional patent application serial number USSN 62/321,178, filed April 11, 2016, and entitled “FLORIGEN PATHWAY TOOLKIT.” The entire teachings of the referenced application are incorporated herein by reference.

FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

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BACKGROUND

One of the most important determinants of crop productivity is plant architecture. For many crops, artificial selection for modified shoot architectures provided critical first steps towards improving yield, followed by innovations in agronomic practices that enabled large- scale field production. However, for many other crops, architecture was not targeted during domestication. A prominent example is tomato, in which the major driver of yield was a drastic increase in fruit size. Shoot architecture, in contrast, remained largely unchanged until the discovery, 85 years ago, of the *self pruning* (*sp*) mutation, which transformed ‘indeterminate’ plants into a radically new ‘determinate’ form. The ability to manipulate tomato architecture beyond *sp* in a simple and predictable way remains limited, which means that the potential of new shoot architectures to further improve yield and reduce production costs remains untapped.

SUMMARY

Described herein is a florigen pathway toolkit that comprises mutations in genes in the florigen pathway in Solanaceae, such as mutations in genes in the florigen pathway in tomatoes, that are useful to quantitatively modulate or adjust the balance of opposing flowering signals to customize and improve shoot architecture and flowering (flowering time) for tomato breeding, particularly for fresh market tomato breeding. Members of the toolkit include suppressor of *sp* (*ssp*) gene mutants, which are useful to restore indeterminate

growth; Single Flower Truss (sft) gene mutants, which are useful to quantitatively manipulate determinacy to improve yield; sp gene mutants, the primary repressor of flowering and sympodial growth; and mutants of SP5G gene, an antagonist of florigen within the primary shoot. Members of the toolkit are chemically-induced alleles in genes that control the balance of opposing flowering signals; engineered alleles, such as CRISPR/Cas9 (CR-) engineered alleles, in such genes or a combination of these chemically-induced alleles and engineered alleles.

Described herein are heritable mutations in the SP5G gene in tomato plants, such as CRISPR/Cas9-induced heritable mutations in the SP5G gene, that alter flowering, shoot architecture and yield. Such heritable mutations have been shown to result in an “earliness” phenotype in tomato plants: tomato plants that are sp5g mutants, such as CR-sp5g mutants, flower earlier than corresponding WT tomato plants (corresponding tomato plants that do not comprise mutant sp5g/do not comprise mutant CR-sp5g). Flowering occurred earlier in sp5g mutants, such as in homozygous CR-sp5g mutants. However, the sympodial index (SI) or number of leaf nodes between successive inflorescences was not significantly different in sp5g mutant tomato plants and WT tomato plants. This earliness phenotype provided by sp5g mutations (e.g., by homozygous CR-sp5g mutants) can be valuable in breeding.

Also described here are early-yielding double-determinate sp5g sp double mutant plants (early-yielding double-determinate sp5g sp double mutant tomato plants) that result when sp5g mutations are combined with sp (when sp5g mutation is present in an sp mutant background). Sp5g mutations can be produced in any background (whether it is an sp mutant background or not); if it is not an sp background, CRISPR or other gene editing technology can be used to transform the determinate tomato plant into a double determinant by targeting both SP5G and SP and producing sp5g sp double mutants. Double determinate sp5g sp double mutant tomato plants are reduced in size, early-yielding and significantly more compact in size, compared, for example with corresponding sp (determinate) tomato plants. Double determinate tomato plants can be grown at higher density, for example, than is possible with other tomato plants, such as sp determinate plants. Further, at least in part because of the earliness effect, these tomato plants can be grown at higher latitudes, where the growing season is shorter; this is possible, in part, because of the earliness effect.

In one embodiment of an sp5g mutant, SP5G function has been eliminated; all or a portion of the SP5G gene has been deleted or altered and SP5G gene function (and that of the encoded protein) is eliminated. Mutations (deletions, alterations or both) can be made in any region or regions of the SP5G gene, such as in any portion of one or more of the exons

(e.g., exon 1) or in a sequence that encodes a conserved SP5G sequence or a sequence that encodes a specific domain, such as the sequence encoding a conserved PEBP domain comprising amino acids 23-164 of SP5G protein

(www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi?INPUT_TYPE=live&SEQUENCE=AAO31793.1 and www.ncbi.nlm.nih.gov/Structure/cdd/cddsrv.cgi?uid=176644) or the sequence encoding the 14 amino acid external loop domain, to produce a null/loss of function sp5g mutant. NCBI Gene ID 101254900 (updated 15 March 2016); ncbi.nlm.nih.gov/gene/101254900 and references cited. In an alternative embodiment, gene function and protein function are reduced. For example, the sp5g mutant results in production of functional protein at reduced levels (levels that result in the desired earliness phenotype).

SP5G gene function can be altered (eliminated or reduced) by any of a variety of types of mutations, as individual mutations or as a combination of mutations. Examples of different types of mutations include, but are not limited to, missense mutations, nonsense mutations, insertions, deletions, and duplications.

A missense mutation is a change in one DNA base pair of the SP5G gene; the codon that includes the change codes for a different (mutant) protein, and the substituted protein may be nonfunctional or may exhibit reduced activity (relative to a protein encoded by a non-mutated/wild-type SP5G gene). Thus, in some embodiments, the SP5G gene comprises a missense mutation that produces a gene encoding a non-functional protein. In some embodiments, the SP5G gene comprises a missense mutation that produces a gene encoding a functional protein that exhibits reduced activity relative to a protein encoded by the non-mutated/wild-type SP5G gene. In some embodiments, a missense mutation is in exon 1 of the SP5G gene.

A nonsense mutation or point mutation in the SP5G gene results in a premature stop codon or a nonsense codon in the transcribed mRNA; the encoded protein is truncated and typically is nonfunctional or exhibits reduced activity (relative to a protein encoded by a non-mutated/wild-type SP5G gene). In some embodiments, a mutant sp5g gene comprising a nonsense mutation encodes a truncated (shorter than full-length) nonfunctional protein. In some embodiments, a mutant sp5g gene comprising a nonsense mutation encodes a truncated (shorter than full-length) protein that exhibits reduced activity relative to a protein encoded by the non-mutated/wild-type SP5G gene. In some embodiments, a nonsense mutation or point mutation is in exon 1 of the SP5G gene.

An insertion changes the number of DNA bases in the SP5G gene by adding one or

more (e.g., 1, 2 or 3) nucleotides. As a result, the protein encoded by a mutant sp5g gene may be nonfunctional or exhibit reduced activity relative to a protein encoded by the non-mutated/wild-type SP5G gene. In some embodiments, an insertion is in exon 1 of the SP5G gene. By contrast, a deletion changes the number of DNA bases in the SP5G gene by deleting one or more (e.g., 1, 2 or 3) nucleotides. As a result, the protein encoded by the mutant sp5g gene may be nonfunctional or exhibit reduced activity relative to a protein encoded by the non-mutated/wild-type SP5G gene. In some embodiments, a deletion is in exon 1 of the SP5G gene.

Insertions and/or deletions, collectively referred to as “indels,” may result in a frameshift mutation, which changes the SP5G open reading frame. A frameshift mutation is caused, in some embodiments, by insertion or deletion of a number of nucleotides that is not divisible by three. In some embodiments, a frameshift mutation in the SP5G gene produces a gene encoding a nonfunctional protein. In some embodiments, a frameshift mutation in the SP5G gene produces a gene encoding a protein that exhibits reduced activity relative to a protein encoded by the non-mutated/wild-type SP5G gene.

In some embodiments, a mutation in a critical functional domain of the SP5G gene produces a gene that encodes a nonfunctional protein. For example, a mutation that alters a nonconserved amino acid in a critical functional domain of the SP5G gene may produce a gene encoding a nonfunctional protein.

In some embodiments, a mutation in a SP5G gene produces a gene that encodes a truncated protein. Preferably, truncation results in production of a nonfunctional protein. In some embodiments, truncation disrupts or alters a region of the SP5G protein critical to its function. Such a truncation can occur, for example, in the conserved domain comprising amino acids 23-164 of SP5G protein, the sequence encoding the 14 amino acid external loop domain or in both. Alternatively, truncation can occur at any location prior to amino acid 164 of SP5G protein. Truncation can result in production of a shortened version of SP5G protein, which has reduced function. Insertion results in addition of one or more base pairs to the SP5G gene and production of an altered protein. Deletion results in removal of one or more base pairs from the SP5G gene, such as a large deletion (a substantial portion or all of an exon, such as exon 1) that renders the gene nonfunctional. The resulting protein, as well, is out of frame and not produced or produced in an altered form, which is nonfunctional or has reduced function.

Particularly useful are null mutations. Such alterations in the SP5G gene mean that transcription into RNA does not occur, translation into a functional protein does not occur or

neither occurs. A preferred embodiment is a null mutation of an exon, such as exon 1 of SP5G. One approach to creating null mutations is to target CRISPR-Cas9 mutagenesis to exons that encode functional protein domains. See, Shi, J. et al. (2015) *Nature Biotechnology*, 33(6): 661-667 and Online Methods.

The effects/advantages of sp5g mutants in breeding tomatoes can be assessed individually or in combination with other members of the florigen toolkit described herein, such as in combination with suppressor of sp (ssp) mutants, which are useful to modify determinate and indeterminate growth, including individual ssp mutants, individual sft mutants and combinations of ssp mutants and sft mutants, which are useful to quantitatively manipulate determinacy to improve yield; and mutants of sp, the primary repressor of flowering and sympodial growth. Assessment of the effects of sp5g mutants can be carried out individually (assessment of the effects of only an sp5g mutant) by focusing on evaluating earliness for yield in the standard Roma/plum background (M82). In addition, the easily genotyped CR-sp5g deletion allele can be introgressed into large-fruited inbred tomatoes and into small-fruited inbred tomatoes. The sp5g mutation can be generated by CRISPR in any genetic background, whether the plant (tomato plant) carries sp or not. In one embodiment, mutation in sp and mutation in SP5G can be generated in any indeterminate background, using CRISPR to target both sp and SP5G. Any indeterminate plant can be transformed into a double determinate plant by targeting both genes (sp and SP5G), thus removing the need for a time-consuming backcrossing/introgression program. Assessment of alleles and allelic combinations from the florigen pathway toolkit (e.g., any combinations of two, three or four of sp5g mutants, sft mutants, sp mutants and ssp mutants) will provide those that are useful and, in some embodiments, best, for fresh market tomato processing and processing tomato performance. For example, triple mutants (double-determinate sp5g sp and an additional, different mutation), such as sp5g sp sft-1906 and sp5g sp ssp-2129, can be produced to provide early-yielding tomato plants with enhanced yields. Quadruple mutants (double-determinate sp5g sp and two additional, different mutations), such as sp5g sp sft-1906 ssp-2129, can be produced, to provide early-yielding tomato plants with enhanced yields. Alternatively, the double mutant can be combined with heterozygosity for a mutation that would result in early and increased yield, such as combining sp5g sp with heterozygosity for sft-1906 mutation to produce sp5g sp sft-1906/+ (double mutant plus heterozygosity for sft-1906 mutation). A further example of the double mutant combined with heterozygosity for a mutation from the florigen pathway toolkit is sp5g sp ssp2129/+.

As further shown, plants (tomato plants) comprising mutations in SP5G exhibit early

flowering, compared to the corresponding plant (tomato plant) lacking the mutation in SP5G and grown under the same conditions as the sp5g mutant plants. Tomato plants comprising mutations in the first exon of SP5G showed early flowering of the primary shoot. Mutations can be created by a variety of known methods. For example, mutations can be created by the CRISPR/Cas9 method, using one or two guide RNAs that target a portion of SP5G gene. In one embodiment, mutation in the first exon of the SP5G gene is created by targeting the first exon by CRISPR/Cas9 using two guide RNAs (see, for example, Figure 2 (a and b); gRNA, Target 1 and Target 2). CR-sp5g alleles a1 and a2 were identified from two independent first-generation (T0) transgenic plants. Representative plants comprising a mutant allele (e.g., CR-sp5g allele a1) show early flowering of the primary shoot. See Figure 2c. CR-sp5g-a2 plants also show early flowering of the primary shoot, compared to the WT plant. In Figure 2c, a red arrowhead marks an open primary inflorescence at anthesis in CR-sp5g-a1 plants after the sixth leaf (L6). WT plants have not yet flowered. (d, e) Primary shoot flowering time (Figure 2d) and sympodial shoot flowering time (Figure 1-SP5G-CRISPR e) from both primary and basal axillary shoots were compared in WT and CR-sp5g plants. As shown in Figure 2d, the number of leaves to first inflorescence (an indicator of time) was significantly reduced in both primary shoots and basal axillary shoots in plants comprising SP5G with mutant first exon, compared to WT plants. As shown in Figure 2e, the number of leaves per sympodial shoot in both primary and basal axillary shoots did not differ in sp5g mutants and WT plants. Mean values ($\pm$ s.e.m.) were compared to WT using two-tailed, two-sampled t tests (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). ID: indeterminate. These results indicate that SP5G functions as a repressor of flowering and an antagonist of the flowering hormone florigen (encoded by SFT), but only within the primary shoot. SP, on the other hand, represses flowering and antagonizes florigen (SFT) activity during sympodial shoot cycling. Results show that mutations in SP5G alter flowering time in tomato, but not the SI.

As further shown here, early-yielding double determinate sp5g sp double mutant plants result when CR-sp5g mutations are combined with sp (e.g., classic sp breeding allele). See Figure 3. Shown (a) are representative sp determinate (D) and CR-sp5g sp double determinate (DD) double mutant plants 80 days after they were transplanted to the field. Flowering time of primary (b) and sympodial shoots (c) in sp and CR-sp5g sp plants was compared and in both primary shoots and sympodial shoots, flowering time (as indicated by number of leaves to first inflorescence) was significantly earlier in CR-sp5g sp double determinate double mutant plants than in sp determinate plants. In addition, the CR-sp5g sp

plants reach final harvest stage (90% red fruit) at least two weeks sooner than the *sp* determinate plants. (d) Important fruit quality traits include fruit weight and Brix (total soluble solids). Assessment of fruit weight and Brix showed that early yielding double determinate *sp5g* double mutant plants did not differ significantly from *sp* determinate plants in either characteristic. As shown, the overall shoots, whether from main or side shoots, flower earlier in the *sp5g sp* double mutants, causing the double determinate habit, still with high yields. The more compact plant will allow growth at higher latitudes where the growing season is shorter; this is possible, in part, because of the earliness effect. In addition, because the unexpected synergistic effect of combining *sp5g* and *sp* mutations together results in more compact plants that are still high yielding, the plants can be grown at higher density. For example, four *sp5g sp* double mutant tomato plants grown in the same square footage area as two *sp* single mutant tomato plants will produce a higher yield than the two *sp* single mutant plants.

Work described here has important implications in at least the following ways. First, results have shown that CRISPR/Cas9 can be used to create heritable mutations in florigen pathway family members that result in phenotypic effects. In the case of CR-*sp5g* mutants, the phenotypic effect is early flowering. Second, the different roles shown for SP5G and SP in primary and sympodial shoot flowering, respectively, suggest that homologous florigen antagonists have been selected to function in different shoot systems. The finding that *sp5g* mutants affect only flowering time of the primary shoot is surprising, as is the double mutant effect of more compact growth habit. Finally, the absence of phenotypic changes in CR-*sp5g* plants beyond flowering indicates potential for application. This can be evaluated, using known methods, such as in elite germplasm. This can be done by integrating CR-*sp5g* mutations into an assessment of which alleles and allelic combinations from the florigen pathway toolkit are useful/best for fresh market tomato performance and processing tomato performance. This can be done, for example, by focusing on evaluating earliness in the standard Roma background (M82) and by introgression of the easily genotyped CR-*sp5g* deletion allele into large-fruited inbred tomatoes and small-fruited inbred tomatoes.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. CRISPR/Cas9 engineered mutations in SP5G. CRISPR/Cas9 targeting of SP5G, a CETS family homolog, showing sgRNAs for exon1, and generation of a desired large deletion in multiple T0 chimeric plants.

Figure 2. CRISPR/Cas9-engineered mutations in *SP5G* result in early flowering. (a)

The first exon of *SP5G* was targeted by CRISPR/Cas9 using two guide RNAs (gRNA, Target 1 and Target 2). (b) *CR-sp5g* alleles a1 and a2 identified from two independent first-generation (T0) transgenic plants. Note the insertion in a2, indicating a range of deletion and insertion alleles of various sizes can be generated (c) Representative plants of WT and *CR-sp5g-a1* showing early flowering of the primary shoot. *CR-sp5g-a2* plants are also early flowering. Red arrowhead marks an open primary inflorescence at anthesis in *CR-sp5g-a1* plants after the sixth leaf (L6). WT plants have not yet flowered. (d, e) Primary shoot flowering time (d) and sympodial shoot flowering time (e) from both primary and basal axillary shoots in WT and *CR-sp5g* plants. Mean values ($\pm$ s.e.m.) were compared to WT using two-tailed, two-sampled t tests (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). ID: indeterminate.

Figure 3. Combining *CR-sp5g* mutations with *sp* results in early yielding double determinate *sp5g sp* double mutant plants. (a) Representative *sp* determinate (D) and *CR-sp5g sp* double-determinate (DD) double mutant plants 80 days after transplanting to the field. (b, c) Flowering time of primary (b) and sympodial shoots (c) in *sp* and *CR-sp5g sp* plants. Notably, flowering time is significantly earlier in primary, axillary, and sympodial shoots compared to *sp* and *sp5g* single mutants alone, or what would be expected from combining the additive effects of each mutation. (d) Number of weeks to final harvest (90% red fruits) in the *sp* and *CR-sp5g sp* genotypes. The *CR-sp5g sp* plants reach final harvest stage 2 weeks faster than *sp* plants. (e) Statistical comparisons of mean values for fruit weight (top) and total soluble solids (Brix) content (bottom) from *sp* and *CR-sp5g sp* plants showing fruit weight and brix are unaffected in *CR-sp5g sp* plants. Ten fruits were measured per replicate. Mean values ($\pm$ s.e.m.) were compared to *sp* controls using two-tailed, two-sampled t tests (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). (f) Diagrams depicting shoot architectures of *sp* and *CR-sp5g sp* plants. Black bars and ovals represent shoot units and associated leaves. Alternating grey and black bars represent successive sympodial shoots. Arrows represent axillary shoots and green lines depict inflorescences. Colored circles represent maturing fruits. The number of leaves on primary, axillary, and sympodial shoots is indicated.

DETAILED DESCRIPTION

Described herein are induced mutations in the florigen pathway in plants, such as in Solanaceae, including tomato, that are useful to balance growth and termination to generate shoot architectures that result in increased yields of fruits (e.g., tomatoes) by modifying determinate growth. In specific embodiments, the mutations are in the *SP5G* gene and result in early flowering (e.g., Figure 2). Combining *sp5g* mutations with *sp* results in early

yielding double determinate *sp5g sp* double mutant plants (e.g., Figure 3). The *sp5g* effects shown are in an *sp* mutant background (e.g., classic *sp* mutant background; e.g. M82), whether or not other mutant alleles (e.g. *ssp*, *sft*) are present in homozygous or heterozygous condition.

Several types of mutant alleles and allelic combinations, which comprise what is referred to herein as the florigen pathway toolkit, modify the opposing activities of SFT and SP to achieve better (e.g., enhanced or optimized) plant architectures for fresh market and processing field performance, such as in large-fruited and small-fruited tomatoes. Mutations in positive regulators of the florigen pathway in large-fruited and small-fruited elite plants create homozygous and heterozygous (hybrid) mutant genotypes with architectural modifications and yield improvements. Mutations can be made in any tomato plant in which the *sp* gene is mutated (in any plant in which there is *sp* background). For example, any of the many available tomato varieties in which *sp* is mutated can be used. Alternatively, any tomato variety or type in which *sp* can be mutated (e.g., an indeterminate tomato plant) such as through the use of CRISPR to target the *sp* gene, can be used. CRISPR technology can be used to target and alter both *sp5g* and *sp* genes in an indeterminate plant and produce a double determinate plant. Newly discovered alleles of *sp*, the primary repressor of flowering and sympodial shoot growth, are used to quantitatively modify determinacy and yield fresh market inbreds and hybrids. Dosage sensitivity of the florigen pathway has been shown and this has been used to modulate yield and plant architecture of Solanaceae and to produce gradients of yield and structure, ranging from classic mutant *sp* background through increasing strength of alleles (weak to stronger alleles), alone (single heterozygote) or in combinations (e.g., double or triple heterozygotes). Flowering time showed a gradient of delays that increased from single to double heterozygotes and with increasing allelic strength, due to combined dosage effects. All determinate phenotypes were higher yielding than *sp* plants. The florigen pathway toolkit includes chemically induced alleles and engineered alleles, such as CRISPR/Cas9 engineered alleles, useful for further fine-tuning of flowering signals, shoot architecture and yield. These tools are useful to quantitatively modify determinacy and yield of plants, such as tomatoes.

Applicant's previous work has provided a general model for increasing tomato productivity in which enhanced yields can be achieved in the field by balancing flowering signals to shift the determinate growth of *sp*-classic toward indeterminate growth. Applicant has been successful in modifying antagonistic SFT/SP flowering signals to improve

architecture and yield in a Roma cultivar and describes here modified forms of determinate growth that improve yields in both large-fruited and small-fruited fresh market breeding lines. See, PCT Application Publication Number WO 2014/081730 A1; Mutations in Solanaceae Plants That Modulate Shoot Architecture and Enhance Yield-Related Phenotypes and US Application Publication Number US 2014/0143898 A1, Mutations in Solanaceae Plants That Modulate Shoot Architecture and Enhance Yield-Related Phenotypes. Each of these cited applications is incorporated by reference herein in its entirety.

Components of the florigen pathway toolkit can be used in various combinations to create a range of shoot architectures in Solanaceae, particularly in tomato, such as fresh market tomatoes. For example, heterozygosity for *ssp* mutations can be used to manipulate the balance of flower promoting and repressing signals (SFT/SP ratios) in new ways and achieve improved architectures with higher yields. For example, *ssp* mutations and *sft* mutations are used to create a range of shoot architectures. *sft* mutant heterozygosity improves inflorescence (flower) production and yield as a result of dose-dependent suppression of *sp*. Activities of protein complexes are sensitive to changes in the dosage of their component parts, which can be adjusted accordingly to have a desired effect on inflorescence production, yield or both inflorescence production and yield. Heterozygosity for *ssp* mutations can be selected to influence/determine the balance of flower-promoting signals and repressing signals (expressed, in one form, as the SFT/SP ratio) in order to produce plants, such as tomato plants (e.g., fresh market tomato plants), with improved architecture and higher yield. Alternatively, heterozygosity for *ssp* mutations can be selected to reduce flowering, yield or both flowering and yield. A long-desired architecture for greenhouse tomato production—indeterminate varieties with two leaves between inflorescences (SI = 2)—is produced using components of the florigen pathway toolkit. In one embodiment, *ssp* homozygotes in an *sp* background, such as *ssp*-2129 homozygotes in an *sp* background and *ssp*-610 homozygotes in an *sp* background, provide a genetic scheme to develop indeterminate varieties with two leaves between inflorescences (SI=2). Sympodial cycling in the *sp* background is sensitive to changes in florigen activity. Thus, the *sft* mutations and the *ssp* mutations can be used to modulate determinate growth quantitatively by means of allele-specific dosage effects. Further, because *ssp* and *sft* mutations affect different components of the same complex, individual dosage effects can be combined, making it possible to establish a set of genotypes that provide a gradient of *sp* suppression that results in highly desirable new architectural structures.

Exploiting dosage-sensitivity of the florigen pathway leads to a new optimum for processing tomato yield. When determinacy is gradually relieved, yields reach a new maximum before a further loss of flower-promoting signals results in a new balance that approaches wild type, resulting in a switch to indeterminate growth and progressively lower yields.

The florigen pathway toolkit is useful for improving architecture and yield in Solanaceae plants, such as tomato plants, including elite fresh market inbreds and hybrids. A variety of combinations of alleles, including homozygotes of each allele, single heterozygotes of each allele and double heterozygotes of two alleles, results in modified architecture, yield or both. Specific alleles that can be used include, but are not limited to, *sft-1906*, *ssp-2129*, *ssp-610* and *sft-tmf*; resulting combinations include single homozygotes of each of *sft-1906*, *ssp-2129*, *ssp-610* and *sft-tmf*; single heterozygotes of each of *sft-1906*, *ssp-2129*, *ssp-610* and *sft-tmf*; and double heterozygotes of two alleles, viz., *sft-1906/+ ssp-2129/+*; *sft-1906/+ ssp-610/+*; *sft-tmf/+ ssp-2129/+*; and *sft-tmf/+ ssp-610/+*. Further mutant alleles that can be used are *sft-4537* and *sft-7187*.

As described here, SP5G, which is a paralog of SFT in the Arabidopsis FT clade, is similar to SP in that its external loop domain is divergent from SFT/FT, which suggests it has repressive flowering activity. Thus, SP5G likely encodes an antagonist of florigen function in parallel with SP. SP5G has effects on flowering time and forces early inflorescence production of the primary shoot, without affecting sympodial index/with little effect on leaf architecture. In tomato plants, *sp5g* mutant results in production of fewer leaves on the primary shoot; additional leaves do not have time to form before the shoot ends in inflorescence. In the wild type plant, earlier flowering caused by the *sp5g* mutation occurs without an effect on shoot architecture of the entire plant. In contrast, a homozygous double *sp sp5g* mutant tomato plant does not grow as tall because the primary shoot stops growing earlier than in wild type plants. The availability of *sp5g* mutants in the context of the *sp* mutant background provides a new toolkit component that can be used to modulate/fine tune additional characteristics—flowering time and compactness. For example, combinations with other mutants, such as *sft* and *ssp1*, may be useful in those situations in which *sft* and *ssp1* mutations have an effect on flowering time, which can be compensated using *sp5g*.

Additional mutations, such as mutations in new regulators, including mutations outside the florigen pathway, are assessed to determine additive effects in combination with *sft/+* or *ssp/+* heterozygosity, providing even subtler fine-tuning of architecture and yield.

Use of the florigen pathway toolkit, alone or in combination with mutations outside the florigen pathway, results in a larger suite of novel germplasm for breeding higher yielding semi-determinate and indeterminate tomato varieties for both field and greenhouse production.

Described herein are induced mutations in the florigen pathway in Solanaceae, such as in tomato, that are useful to balance growth and termination to generate shoot architectures that result in increased yields of fruits (e.g., tomatoes) by modifying determinate growth. Several types of mutant alleles and allelic combinations from the florigen pathway toolkit described here can modify the opposing activities of SFT and SP to achieve better/optimized plant architectures for fresh market and field performance, such as in large- and small-fruited tomatoes. Mutations in positive regulators of the florigen pathway into large-fruited and small-fruited elite plants create homozygous and heterozygous (hybrid) mutant genotypes with architectural modifications and yield improvements. Newly discovered alleles of *sp*, the primary repressor of flowering and sympodial shoot growth, are used to quantitatively modify determinacy and yield in fresh market inbreds and hybrids. The toolkit includes chemically induced alleles and engineered alleles, such as CRISPR/Cas9 engineered alleles, which make possible further fine-tuning of flowering signals, shoot architecture, and yield.

Example CRISPR/Cas9-induced heritable mutations in SP5G

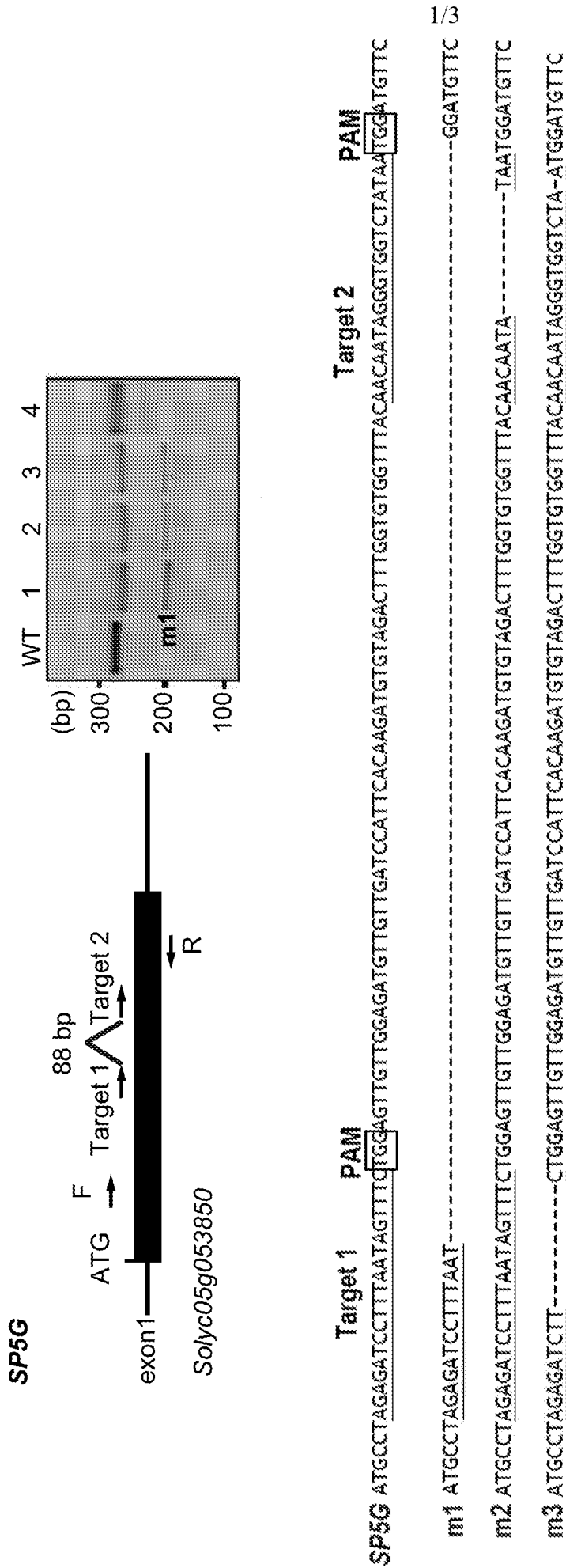
SP5G is the closest homolog of SFT (florigen), a tomato homologue of a key flower-promoting gene, FLOWERING LOCUS (FT). However, divergence between the functionally important external loop domain shared by SFT and SP5G suggests that SP5G may repress—not promote--flowering in tomatoes, in parallel with SELF PRUNING (SP) gene. Null alleles of SP5G were evaluated to assess their effects on flowering. In particular, CRISPR/Cas9-induced null alleles of SP5G were evaluated. For example, phenotypic changes caused by a large deletion detected by PCR in 3 out of 4 T0 CR-sp5g plants were assessed (Fig. 1). Initially, CR-spg5g T0 plants were outcrossed to wild type plants; heritability of the deletion allele was confirmed by PCR and sequencing. PCR was used to select plants lacking the Cas9 transgene, and as expected for a single hemizygous transgene insertion event in T0 plants, half of the F1 progeny were Cas9-free. Cas9-free F1 plants heterozygous for the large deletion were self-pollinated; and the phenotypes of resulting F2 progeny homozygous for the CR-sp5g deletion were evaluated (compared to wild type siblings). Homozygous CR-sp5g mutants flowered earlier than wild type siblings and quantification of this effect revealed that the primary inflorescence developed 2 leaves faster (sooner) in CR-sp5g mutants, resulting in an “earliness” phenotype. In contrast, the sympodial index of CR-sp5g plants was identical to that of wild-type plants. These results indicate that SP5G functions as a repressor of flowering and an antagonist of florigen (SFT), but only within the primary shoot. SP, on the other hand, is a repressor of flowering that acts only during sympodial shoot cycling.

CLAIMS

What is claimed is:

1. A tomato plant that is an sp5g sp double mutant and that flowers earlier than the corresponding sp [sibling] tomato plant, as measured with reference to the number of leaves produced prior to appearance of first inflorescence, wherein the sp5g sp double mutant comprises a mutation in exon 1 of SP5G.
2. The tomato plant of claim 1, wherein the mutation in exon 1 of SP5G comprises a missense mutation, nonsense mutation, insertion, deletion or duplication
3. The tomato plant of claim 2, wherein the insertion produces a gene comprising a frameshift or the deletion produces a gene comprising a frameshift.
4. The tomato plant of any one of claim 1 to 3, which is a homozygous CR-sp5g mutant.
5. The tomato plant of claim 4, wherein the CR-sp5g mutant is CR-sp5g allele a1 or CR-sp5g allele a2.
6. The tomato plant of any one of claims 1 to 5, in which the sympodial index is the same as the sympodial index of the corresponding sp [sibling] tomato plant.
7. The tomato plant of any one of claims 1 to 6, which is a Roma (M82) tomato plant, a large-fruited inbred tomato plant or a small-fruited inbred tomato plant.
8. A tomato plant that is homozygous for a mutated SP5G gene and flowers earlier than the corresponding [sibling] tomato plant, as measured with reference to the number of leaves produced prior to appearance of the first inflorescence.
9. The tomato plant of claim 8, wherein the mutated SP5G gene is a CRISPR/Cas9-induced heritable allele.
10. The tomato plant of claim 8 or 9, wherein the mutated SP5G is CR-sp5g.

11. The tomato plant of any one of claims 8 to 10, in which the sympodial index is the same as the sympodial index of the corresponding [sibling] tomato plant.
12. The tomato plant of any one of claims 8 to 11, which is a Roma (M82) tomato plant, a large-fruited inbred tomato plant, or a small-fruited inbred tomato plant.
13. A method of reducing flowering time (time to production of first inflorescence) in a tomato plant, as measured with reference to the number of leaves produced prior to appearance of the first inflorescence, comprising producing a tomato plant, tomato seed or tomato plant part that is homozygous for a mutated SP5G gene in an sp background and maintaining the tomato plant, tomato seed or tomato plant part under conditions under which the tomato plant, the tomato seed or the tomato plant part grows.
14. The method of claim 13, wherein the mutated SP5G gene is a CRISPR/Cas9-induced heritable allele.
15. The method of claim 13 or 14, wherein the mutated SP5G is CR-sp5g .
16. The method of any one of claims 13 to 15, in which the sympodial index of the tomato plant homozygous for a mutated SP5G gene is the same as the sympodial index of the corresponding [sibling] tomato plant.
17. The method of any one of claims 13 to 16, in which the tomato plant homozygous for a mutated SP5G gene is a Roma (M82) tomato plant, a large-fruited inbred tomato plant or a small-fruited inbred tomato plant.



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Figure 1

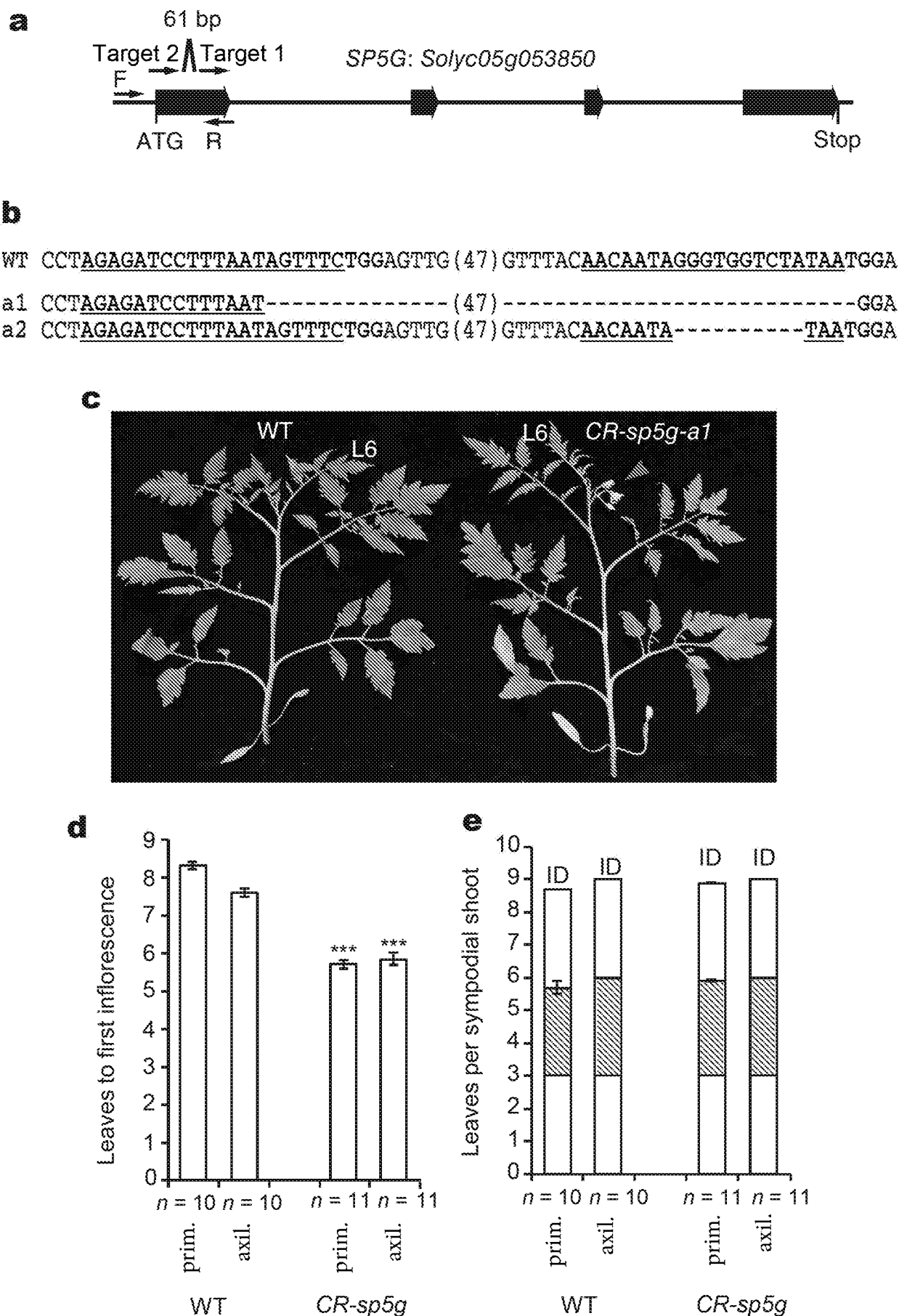


Figure 2

SUBSTITUTE SHEET (RULE 26)

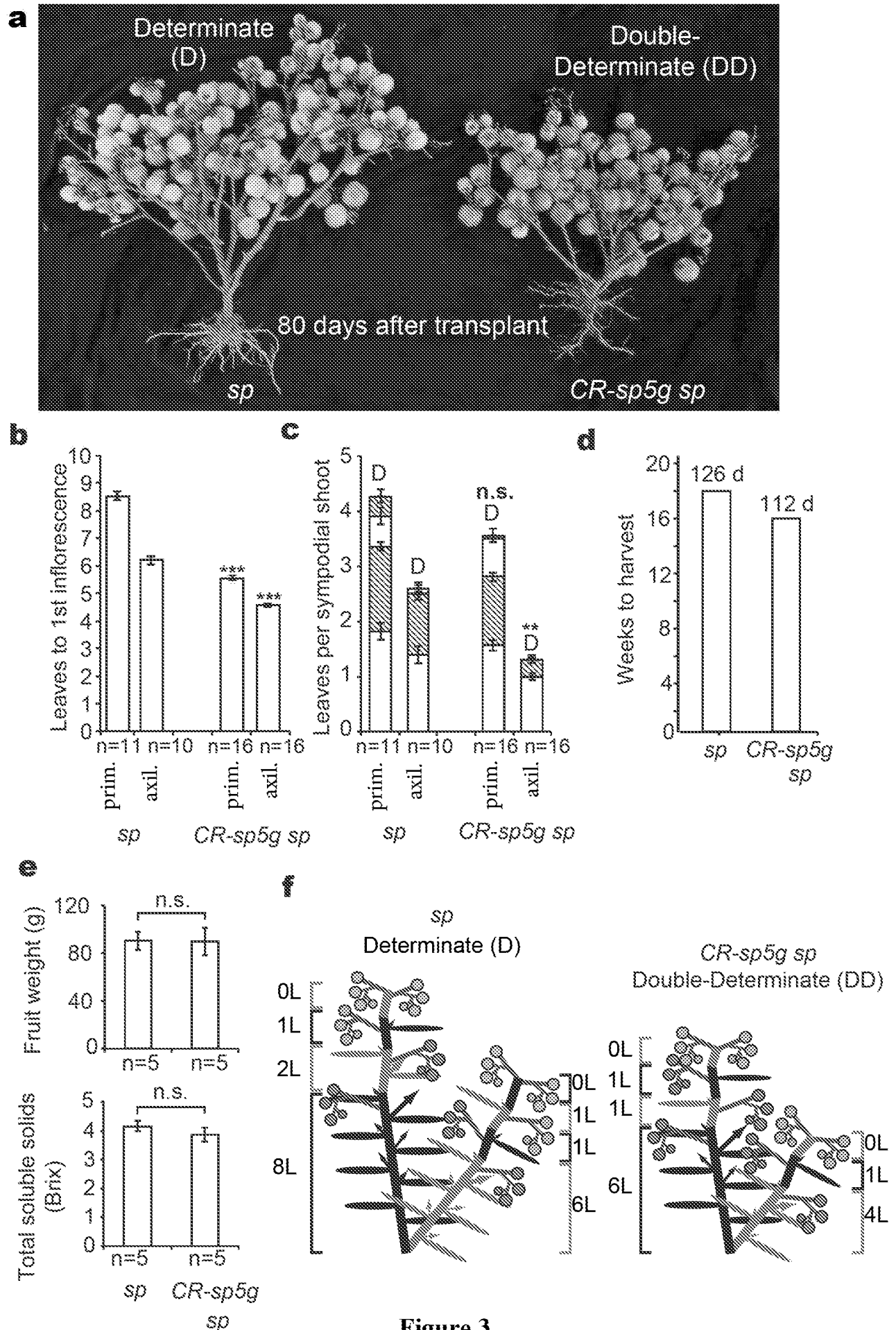


Figure 3

INTERNATIONAL SEARCH REPORTInternational application No.
PCT/US2017/026635**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 6-7, 11-12, 16-17
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

A. CLASSIFICATION OF SUBJECT MATTER**C12N 15/82(2006.01)i**

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N 15/82; A01N 37/46; C07K 14/415

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

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

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

eKOMPASS(KIPO internal) & Keywords: tomato, mutation, self-pruning, SP5G, early flowering

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2015-0284732 A1 (COLD SPRING HARBOR LABORATORY) 08 October 2015 See paragraph [0069]; and claims 83, 92.	1-5, 8-10, 13-15
A	PARK et al., 'Optimization of crop productivity in tomato using induced mutations in the florigen pathway', Nature Genetics, 02 November 2014(online), vol. 46, no. 12, pp. 1337-1342, Advanced online publication, internal pages 1-8 See abstract; and internal page 5.	1-5, 8-10, 13-15
A	JIANG et al., 'Tomato yield heterosis is triggered by a dosage sensitivity of the florigen pathway that fine-tunes shoot architecture', PLoS Genetics, 26 December 2013, vol. 9, issue 12, article no. e1004043, internal pages 1-13 See abstract.	1-5, 8-10, 13-15
A	TEO et al., 'New insights into the regulation of inflorescence architecture', Trends in Plant Science, March 2014, vol 19, no. 3, pp. 158-165 See abstract.	1-5, 8-10, 13-15



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

10 July 2017 (10.07.2017)

Date of mailing of the international search report

11 July 2017 (11.07.2017)

Name and mailing address of the ISA/KR

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/026635

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2015-0011393 A1 (NATIONAL UNIVERSITY CORPORATION NARA INSTITUTE OF SCIENCE AND TECHNOLOGY) 08 January 2015 See abstract; and paragraphs [0037], [0052].	1-5, 8-10, 13-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2017/026635

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2015-0284732 A1	08/10/2015	CA 2892012 A1 EP 2922960 A1 US 2014-0143898 A1 WO 2014-081730 A1	30/05/2014 30/09/2015 22/05/2014 30/05/2014
US 2015-0011393 A1	08/01/2015	EP 2813142 A1 EP 2813142 A4 JP 6074771 B2 WO 2013-118863 A1	17/12/2014 21/10/2015 08/02/2017 15/08/2013