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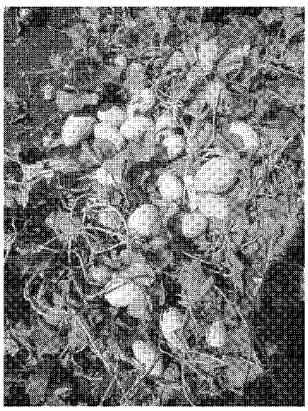
[Continued on next page]

(54) Title: MELON PLANTS WITH ENHANCED FRUIT YIELDS

FIG. 1



(57) Abstract: A *Cucumis melo* plant or a part thereof, the plant bearing more than 12 fruit, the fruit being seedless. Methods of generating same and breeding same are also disclosed.

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MELON PLANTS WITH ENHANCED FRUIT YIELDS

FIELD AND BACKGROUND OF THE INVENTION

The present invention, in some embodiments thereof, relates to melon plants, having small seedless fruit with enhanced fruit yields and methods of generating same.

Parthenocarpy, the production of seedless fruits, can be achieved by the addition of the plant growth regulators auxin, cytokinin or gibberellin in many crops. It has been shown that the exogenous application of auxin or gibberellin to unfertilized flowers in a number of plant species, induces fruit set in the absence of pollination, resulting in the production of parthenocarpic fruit. In previous efforts to produce seedless fruits, traditional plant breeding and exogenous application of hormones have been used with some success. However, the exogenous application of plant hormones is a labor-intensive process, and traditional plant breeding is a long term process. Moreover, at least some of the previous attempts to produce certain seedless fruits have resulted in low numbers of seedless fruits and/or in relatively small seedless fruits as compared with the normal, seeded fruits. In most cases, this led to a significant yield reduction in small fruit varieties.

Cucumis melo exhibits extreme diversity for fruit traits. Melon fruit vary in size, shape, external color, aroma and flesh characters such as sugar content, acidity and pigmentation. Still, there is an increasing demand for new fruit types by modern food markets. In melon, fruit set and number is a trait that is mostly governed at the hormonal level. Fruit set is affected by hormonal talk that results from the success or failure of the previous female flower on the branch to develop a fruit however the general number of fruit per plant is pretty constant. Typically, most melon varieties will produce 1-5 fruit per plant in the field.

There is a long felt need in the art for an effective and economical means and methods for the production of seedless fruit, particularly in good yield and quality as compared with prior art seedless fruits.

Background art includes U.S. Patent Application No. 20120324597 and WO 2011/018785.

SUMMARY OF THE INVENTION

According to an aspect of some embodiments of the present invention there is provided a *Cucumis melo* plant or a part thereof, the plant bearing more than 12 fruit, said fruit being seedless.

According to an aspect of some embodiments of the present invention there is provided a *Cucumis melo* plant having a *MELO3C009603/melo3c009603* genome such that upon self-pollination, 25 % of F1 bear more than 12 fruit, said fruit being seedless.

According to an aspect of some embodiments of the present invention there is provided a cutting of a *C. melo* plant of the plants described herein.

According to an aspect of some embodiments of the present invention there is provided a seed of a *Cucumis melo* plant having a *MELO3C009603/melo3c009603* genome such that upon self-pollination, 25 % of F1 bear more than 12 fruit, said fruit being seedless.

According to an aspect of some embodiments of the present invention there is provided a cell having the genome of the any of the plants described herein.

According to an aspect of some embodiments of the present invention there is provided a culture comprising a plurality of the cells described herein.

According to an aspect of some embodiments of the present invention there is provided a method of breeding a first *C. melo* comprising crossing the plants described herein with a second *C. melo* plant, thereby breeding the *C. melo*.

According to an aspect of some embodiments of the present invention there is provided a plurality of *C. melo* seeds which are heterozygotic for a MELO3C009603 mutation which upon planting brings about an enhanced fruit crop phenotype in 25 % of the plants derived therefrom.

According to an aspect of some embodiments of the present invention there is provided a hybrid seed produced by the methods described herein.

According to an aspect of some embodiments of the present invention there is provided a hybrid plant, or parts thereof, produced by growing the hybrid seed described herein.

According to an aspect of some embodiments of the present invention there is provided a method of growing any of the plants described herein comprising vegetatively propagating the plant, thereby growing the plant.

According to an aspect of some embodiments of the present invention there is provided an isolated polynucleotide comprising the sequence as set forth in SEQ ID NO: 9.

According to an aspect of some embodiments of the present invention there is provided an isolated polypeptide comprising a sequence as set forth in SEQ ID NO: 8.

According to an aspect of some embodiments of the present invention there is provided a method of marker assisted selection of a *C. melo* plant having improved fruit yield or having a progeny with improved yield, the method comprising analyzing for the presence of a loss of function mutation in at least one MELO3C009603 allele, wherein the presence of said mutation is indicative that the plant or progeny thereof will bear more than 5 seedless fruit.

According to an aspect of some embodiments of the present invention there is provided a food or processed product comprising the plants described herein or parts thereof.

According to some embodiments of the invention, the plant bears more than 15 fruit.

According to some embodiments of the invention, the plant bears more than 20 fruit.

According to some embodiments of the invention, the plant has a similar total soluble solid (TSS) content and β-carotene content as a wild-type *Cucumis melo* plant.

According to some embodiments of the invention, the weight of total fruit of the plant is greater than the weight of total fruit of a wild-type *Cucumis melo* plant.

According to some embodiments of the invention, the plant is of a variety *C. melo* Cantalupensis.

According to some embodiments of the invention, both alleles of MELO3C009603 of the genome of the plant have a loss of function mutation that results in a seedless trait.

According to some embodiments of the invention, both alleles of said MELO3C009603 have an F/I mutation at position 97 thereof.

According to some embodiments of the invention, the polynucleotide sequence of said MELO3C009603 is as set forth in SEQ ID NO: 7.

According to some embodiments of the invention, the polypeptide sequence of MELO3C009603 is as set forth in SEQ ID NO: 8.

According to some embodiments of the invention, the plant part is selected from the group consisting of roots, stems, leaves, cotyledons, flowers, fruit, embryos and pollen.

According to some embodiments of the invention, the crossing comprising pollinating.

According to some embodiments of the invention, the subspecies of said *melo* plant is selected from the group consisting of *melo* Cantalupensis, Noy Yizre'el, Ein Dor and Piel De Sapo.

According to some embodiments of the invention, the second *C. melo* plant is not any of the plants described herein (e.g. doesn't have a mutation in MELO3C009603).

According to some embodiments of the invention, 25 % of the plants bear more than 5 fruit, the fruit being seedless.

According to some embodiments of the invention, the marker assisted selection is conducted using an assay selected from the group consisting of single base extension (SBE), allele-specific primer extension sequencing (ASPE), DNA sequencing, RNA sequencing, microarray-based analyses, universal PCR, allele specific extension, hybridization, mass spectrometry, ligation, extension-ligation, and Flap Endonuclease-mediated assays.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING(S)

Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for

purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

In the drawings:

FIG. 1 is a photograph of a 'superfruiter' ('sf') plant bearing fruit in the field.

FIG. 2 is a photograph of the interior of various 'sf' fruit types.

FIG. 3 is a photograph of all the fruit of a representative single 'sf' plant.

FIGs. 4A-C are bar graphs illustrating the field performance of 'sf' and wild type F_2 segregants derived from four independent crosses. Horizontal continuous and broken lines represents mean values of all wild types and 'sf' respectively. A – average fruit number; B – average fruit weight; C- average fruit weight per plant (yield).

FIGs. 5A-B are bar graphs illustrating the quality of 'sf' and wild type F_2 segregants from CEZ x 'sf' cross. A- Average Brix (TSS) of 10 fruit of CEZ, 'sf' and wildtype segregants; B – Average β-carotene content of 10 fruit of CEZ, 'sf' and wildtype segregants.

FIG. 6 is a photograph of *Apo*I digestion products of a 213 base pairs (bp) PCR product amplified from DNA of (from left to right) 'sf', 'CEZ' and their F_1 plants. The size in base pairs (bp) of the DNA fragments appears on the left. Forward primer TAGACATGAGCCGCATCTGA (SEQ ID NO: 3) and reverse primer GAACGTGGCAACAACAACAA (SEQ ID NO: 4) were utilized for the PCR amplification.

FIG. 7 is an alignment of the 'Zing Finger' (ZF) motif of '*sf*' (MELO3C009603) showing the 'F^{97}' to 'I' amino acid change (red frame). The two Cysteine and Histidine amino acids of the C_2H_2 are bolded. CHYCCRNFPTSQALGGHQNAH (SEQ ID NO: 5); CHYCCRNIPTSQALGGHQNAH (SEQ ID NO: 6).

FIGs. 8A-B are graphs illustrating the expression of MELO3C009603 (*sf* gene). Figure 8A – RNA-Seq based digital expression of MELO3C009603 (*sf* gene) in 'sf' and wild type bulks; Figure 8B – qualitative-RTPCR analysis of relative expression (APR1 gene as a reference) of MELO3C009603 in the tissues that comprised each of the bulks.

FIG. 9 is a Venn diagram showing all differentially expressed genes (DEG) in the three segregating populations and the overlapping ones.

FIG. 10A is a graph illustrating RNA-Seq based digital expression of MELO3C021150 (seed nucellus gene) in isogenic 'sf' and wild type (CEZ) bulks;

FIG. 10B is a graph illustrating relative expression of MELO3C021150 in the tissues that comprised the isogenic bulks, analyzed by qRTPCR (APR1 gene as a reference).

FIGs. 11A-B provides the wild type complementary DNA sequence (FIG. 11A - SEQ ID NO: 7) and the mutated amino acid sequence (FIG. 11B - SEQ ID NO: 8) of MELO3C009603 in the mutated plants. Figure 11A. The first ATG is highlighted in yellow and the TAA stop codon is highlighted in red. 'TTC' that codes for 'F' in wild type is bolded and the 'T' that is mutated to an 'A' in 'sf' is colored red; Figure 11B - Protein sequence of 'sf' gene. The zinc finger domain colored blue, C_2 and H_2 green and the mutated amino acid 'I' in red. QAALGH motif within the ZF domain is underlined.

DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to melon plants, having enhanced fruit yields and methods of generating same.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways.

Among Cucurbitaceae, *C. melo* is one of the most important cultivated cucurbits. They are grown primarily for their fruit, which generally have a great diversity in size (500 g to 5 kg), flesh color (orange, green, and white), rind color (green, yellow, white, orange, and gray), shape (round, oval, and elongated), and dimension (5 to 25 cm wide;10 to 50 cm long).

Whilst attempting to create novel variations of melon plant, the present inventors treated CEZ (a 'charantais' type melon, developed by ARO) melon seeds with the chemical mutagen ethyl methanesulfonate (EMS) and selected 'superfruiter' melon plants (referred to herein as "sf" plants) which had enhanced fruit number and yield (Figures 1 and 3). Inspection of the fruit from these plants, revealed that their fruit was seedless or had tiny empty undeveloped seeds (Figure 2). Analysis of carotenoids in the mutated fruit by HPLC revealed similar beta carotene content as compared to the non-

mutated isogenic counterpart, CEZ. Furthermore, the total soluble solids (TSS) content revealed that the mutated fruit had a similar sugar content as compared to into non-mutated isogenic counterpart.

Wild type plants of 'CEZ' will develop an average of four fruit per plant and only one fruit will be developed on a branch while the 'sf' is capable of producing multiple fruit on each branch. In wild type plants the successful fertilization of a female flower and the initiation of fruit development will suppress the development of additional fruit from the next female flower on the same branch. This suppression mechanism is inactivated in 'sf'. Reciprocal crosses made with 'sf' indicated that its pollen is fully fertile, fruit will develop only upon fertilization however no seeds or small empty seeds will be contained in the small fruit of 'sf'. Thus 'sf' suffers from seed abortion that does not prevent development of fruit and does not inhibit the production of many additional fruit on the same branch.

This unique yield increase was shown to be governed by a single recessive gene (MELO3C009603, encoding a Cys_2His_2 (C_2H_2) type zinc finger protein) as demonstrated by segregation analysis of 'sf' in four independent F2 segregating populations.

Thus, according to one aspect of the present invention there is provided a *C. melo* plant or a part thereof, the plant bearing more than 12 fruit, said fruit being seedless.

The term '"plant" as used herein encompasses whole plants, ancestors and progeny of the plants and plant parts, including seeds, fruits, shoots, stems, roots (including tubers), and plant cells, tissues and organs. The plant may be in any form including callus tissue, suspension culture, embryos, meristematic regions, leaves, gametophytes, sporophytes, pollen, ovules and microspores.

The term “melon” as used herein refers to the species *Cucumis melo* L. including subspecies *agrestis* (vars. *conomon*, *makuwa, momordica* and *acidulous*) and *melo* (vars. *cantalupensis, reticulatus, adana, chandalok, ameri, inodorus, flexuosus, chate, tibish, dudaim* and *chito.*

The term “cultivar” is used herein to denote a plant having a biological status other than a “wild” status, which “wild” status indicates the original non-cultivated, or natural state of a plant or accession. The term “cultivar” (for cultivated variety) includes, but is not limited to, semi-natural, semi-wild, weedy, traditional cultivar, landrace, breeding material, research material, breeder's line, synthetic population, hybrid, founder

stock/base population, inbred line (parent of hybrid cultivar), segregating population, mutant/genetic stock, and advanced/improved cultivar. Examples of cultivars include such cultivated varieties that belong to the taxonomic groups *Cucumis melo* var. *cantalupensis* (the Charantais and Italian cantaloupe fruit types), *Cucumis melo* var. *reticulatis* (the Galia and Ananas fruit types), and *Cucumis melo* var. *inodorus* (including Piel de Sapo, Yellow Canary, Branco and Honeydew fruit types). Therefore, a plant of the present in invention is a plant of any *C. melo* var. The term "var." indicates a *varietas* (a taxonomic level below that of the species as detailed above).

According to one embodiment, the plant of this aspect of the present invention has an enhanced fruit yield compared to wild-type plants of the same genetic background grown under the same conditions (i.e. bear more than 10 fruit, 12 fruit, 15 fruit, 20 fruit, 25 fruit or even more than 30 fruit at one particular time). Typically, the average weight of each of the fruit is 350gm with a range of 100-600gm, depending on the genetic background (Figure 4B). The plant may comprise at least 2, at least 3, at least 5 fruit per branch at one particular time.

Most of the fruit on the plant are at the same stage of ripening depending on the degree of climacteric/non-climacteric mode of fruit ripening.

It will be appreciated that the weight of total fruit (i.e. crop) of said plant is greater than the weight of total fruit of a wild-type *Cucumis melo* plant.

As used herein the phrase "wild-type *Cucumis melo* plant" refers to the *Cucumis melo* plant having a non-mutated, naturally occurring genome.

The present inventors have shown that the sugar content and the beta carotene content of the melons of the plant of this aspect of the present invention are similar to their non-mutated (wild-type) counterpart. It will be appreciated that the wild-type counterparts do not have a mutation in other genes affecting beta carotene content such as CRTISO, as disclosed in U.S. Patent Application No. 20120324597. Thus, melons of the plant of this aspect of the present invention may be bred to be edible and of high quality in various genetic backgrounds. They are suitable as fresh produce, as fresh cut produce, or for processing such as, for example, canning.

As mentioned, the melons of the plant of this aspect of the present invention are seedless.

As used herein, the term "seedless melon" refers to a melon that does not contain fertilized mature seeds. While the melons of the present invention do not contain fertilized mature seeds, the melons may contain unfertilized ovaries, which are small and white in color. These unfertilized ovaries are not considered to be true seeds. The seed content in the fruit is reduced by at least 80 % as compared to that of a wild type melon of the same genetic background and growth conditions.

According to this aspect of the present invention the seedless trait is controlled by a genetic determinant and is independent of exogenous treatment with parthenocarpy-inducing plant hormones. Thus the seedless trait is obtained by stenospermocarpy and not by parthenocarpy.

According to one embodiment, at least 80 % of the fruits of a given plant have a seed content reduced by at least 80 %, at least 90% to about 99% or even 100 %.

According to another embodiment, at least 85 % of the fruits of a given plant have a seed content reduced by at least 80 %, at least 90% to about 99% or even 100 %.

According to another embodiment, at least 90 % of the fruits of a given plant have a seed content reduced by at least 80 %, at least 90% to about 99% or even 100 %.

According to another embodiment, at least 55 % of the fruits of a given plant have a seed content reduced by at least 80 %, at least 90% to about 99% or even 100 %.

According to another embodiment, at least 99 % of the fruits of a given plant have a seed content reduced by at least 80 %, at least 90% to about 99% or even 100 %.

Melon plants of this aspect of the present invention are characterized by having both alleles of the MELO3C009603 gene (wild type cDNA sequence - SEQ ID NO: 7) having a loss of function mutation that results in an enhanced fruit crop trait (and optionally a seedless trait). The MELO3C009603 may have a single mutation which brings about both the traits, or two mutations - one which brings about the enhanced fruit crop trait and the other that brings about the seedless trait. According to a particular embodiment the mutated MELO3C009603 amino acid sequence is set forth in SEQ ID NO: 8.

MELO3C009603 may be in a homozygous form or in a heterozygous form. According to this embodiment, homozygosity is a condition where both alleles at the MELO3C009603 locus are characterized by the same nucleotide sequence. Heterozygosity refers to different conditions of the gene at the MELO3C009603 locus.

The term "allele" as used herein, refers to any of one or more alternative forms of a gene locus, all of which alleles relate to a trait or characteristic. In a diploid cell or organism, the two alleles of a given gene occupy corresponding loci on a pair of homologous chromosomes.

The term "gene" as used herein refers to an inherited factor that determines a biological characteristic of an organism (i.e. a melon plant), an "allele" is an individual gene in the gene pair present in the (diploid) melon plant.

A plant is called "homozygous" for a gene when it contains the same alleles of said gene, and "heterozygous" for a gene when it contains two different alleles of said gene. The use of capital letters indicates a dominant (form of a) gene and the use of small letters denotes a recessive gene: "X,X" therefore denotes a homozygote dominant genotype for gene or property X; "X,x" and "x,X" denote heterozygote genotypes; and "x,x" denotes a homozygote recessive genotype. As commonly known, only the homozygote recessive genotype will generally provide the corresponding recessive phenotype (i.e. lead to a plant that shows the property or trait "x") whereas the heterozygotic and homozygote dominant genotypes will generally provide the corresponding dominant phenotype (i.e. lead to a plant that shows the property or trait "X"), unless other genes and/or factors such as multiple alleles, suppressors, codominance etc. (also) play a role in determining the phenotype.

A "loss-of-function mutation" is a mutation in the sequence of a gene, which causes the function of the gene product, usually a protein, to be either reduced or completely absent. A loss-of-function mutation can, for instance, be caused by the truncation of the gene product because of a frameshift or nonsense mutation or by an alteration of a single or more amino acids. A phenotype associated with an allele with a loss of function mutation is usually recessive but can also be dominant.

According to a particular embodiment, both alleles of MELO3C009603 carry an A to T mutation at position 3,450,971 on scaffold 11 of the melon genome, leading to a F/I amino acid change at position 97 of the predicted MELO3C009603 protein. An exemplary polynucleotide sequence of a mutated MELO3C009603 is set forth in SEQ ID NO: 9. An exemplary polypeptide sequence of a mutated MELO3C009603 is set forth in SEQ ID NO: 8.

It will be appreciated that the present invention also contemplates generating the *Cucumis melo* fruit by taking cuttings from 'sf' melon plants and performing vegetative propagation.

Vegetative propagation may be effected using methods well-known in the art, for example in-vitro plant tissue culture, rooting side shoot or protoplast fusion. In one embodiment, a method of vegetatively propagating a plant of the present invention comprises: a) collecting tissue of a plant of the present invention; b) cultivating said tissue to obtain proliferated shoots; c) rooting said proliferated shoots to obtain rooted plantlets; and d) growing plants from said rooted plantlets.

Cuttings according to this aspect of the present invention may include roots, stems, leaves, cotyledons, flowers, fruit, embryos and pollen. Preferably, the cuttings comprise stems and epical or side shoot meristem.

According to one embodiment, the plants of the present invention are of a hybrid variety – i.e. are generated following the crossing (i.e. mating) of two non-isogenic plants. The hybrid may be an F_1 Hybrid or an open-pollinated variety.

An F_1 Hybrid" as used herein, refers to first generation progeny of the cross of two non-isogenic plants.

The development of melon hybrids of the present invention requires the development of stable parental lines while at least one of them is heterozygous to *sf* gene. In breeding programs desirable traits from two or more germplasm sources or gene pools are combined to develop superior breeding varieties. Desirable inbred or parent lines are developed by continuous self-pollinations and selection of the best breeding lines, sometimes utilizing molecular markers to speed up the selection process.

Once the parental lines that give the best hybrid performance have been identified e.g., both carrying the mutation in the MELO3C009603, the hybrid seed can be produced indefinitely, as long as the homogeneity of the parents are maintained. According to one embodiment the melon plants of the present invention are stable parent plant lines (carrying a loss of function mutation in the MELO3C009603 gene in a heterozygous form).

As defined herein, the phrase "stable parental lines" refers to open pollinated, inbred lines, stable for the desired plants over cycles of self-pollination and planting.

Typically, 95 % of the genome is in a homozygous form in the parental lines of the present invention.

According to another aspect, the present invention provides a method for producing first generation (F_1) 'sf' hybrid melon plantlets.

According to one embodiment, the present invention provides a method for producing first generation 'sf' hybrid plantlets (and also seeds) comprising crossing (e.g. pollinating) a first stable parent melon plant which is seedless and has an enhanced fruit crop (e.g. either homozygote or heterozygote for the mutation MELO3C009603) with a second stable 'sf' heterozygous parent melon plant.

It will be appreciated that 25 or 50% of the F_1 hybrid melon seeds are homozygous for a MELO3C009603 mutation, depending if only one or both parental lines are heterozygous 'sf'.

According to another embodiment, the present invention also provides a DNA marker that enables selecting the F_1 'sf' plantlets.

Thus, according to another aspect of the present invention, there is provided a *Cucumis melo* plant having a *MELO3C009603/melo3c009603* genome such that upon selfing 25 % of F1 bear more than 5 fruit, said fruit being seedless.

The present invention also relates to seeds harvested from these F_1 hybrid melon plants and plants grown from these seeds.

A common practice in plant breeding is using the method of backcrossing to develop new varieties by single trait conversion.

The phrase "single trait conversion" as used herein refers to the incorporation of new single gene into a parent line wherein essentially all of the desired morphological and physiological characteristics of the parent lines are recovered in addition to the single gene transferred.

The term "backcrossing" as used herein refers to the repeated crossing of a hybrid progeny back to one of the parental melon plants. The parental melon plant which contributes the gene for the desired characteristic is termed the non-recurrent or donor parent. This terminology refers to the fact that the non-recurrent parent is used one time in the backcross protocol and therefore does not recur. The parental melon plant to which the gene from the non-recurrent parent are transferred is known as the recurrent parent as it is used for several rounds in the backcrossing protocol.

In a typical backcross protocol, a plant from the original varieties of interest (recurrent parent) is crossed to a plant selected from second varieties (non-recurrent parent) that carries the single gene of interest to be transferred. The resulting progeny from this cross are then crossed again to the recurrent parent and the process is repeated until a melon plant is obtained wherein essentially all of the desired morphological and physiological characteristics of the recurrent parent are recovered in the converted plant, in addition to the single transferred gene from the non-recurrent parent.

Thus, near-isogenic lines (NIL) may be created by many backcrosses to produce an array of individuals that are nearly identical in genetic composition except for the trait or genomic region under interrogation in this case 'sf' mutation in *MELO3C009603*.

Backcrossing methods can be used with the present invention to improve or introduce a characteristic into the parent lines.

Marker assisted selection of *C. melo* plantlets that will bear more than 5 seedless fruit (or parts thereof that are capable of producing a plant that bears more than 5 seedless fruit) may be performed. This is especially advantageous for selecting cuttings or during a backcrossing process. The method comprises analyzing for the presence of the A/T mutation that leads to F/I amino acid change at position 97 of MELO3C009603 predicted protein, wherein the presence of the mutation is indicative that the plant will bear more than 5 seedless fruit or the part thereof will produce a plant that bears more than 5 seedless fruit.

Many methods are known in the art for analyzing for mutations including for example single base extension (SBE), allele-specific primer extension sequencing (ASPE), DNA sequencing, RNA sequencing, microarray-based analyses, universal PCR, allele specific extension, hybridization, mass spectrometry, ligation, extension-ligation, Flap Endonuclease-mediated assays, restriction fragment length polymorphism (RFLP), electrophoresis, sequence alignment, allelic specific oligonucleotide hybridization (ASO) and random amplified polymorphic DNA (RAPD).

Thus, the present invention contemplates oligonucleotides (e.g. Primers) that can be used to distinguish between the mutated and non-mutated form of MELO3C009603. An exemplary set of primers is described in the Example section - SEQ ID NOs: 3 and 4.

The present inventors contemplate both chemical mutagenesis and recombinant techniques for the generation of the melon plants of the present invention.

Thus, the melon plants of the present invention may be generated by exposing the melon plant or part thereof to a chemical mutagen. Examples of chemical mutagens include, but are not limited to nitrous acid, alkylating agents such as ethyl methanesulfonate (EMS), methyl methane sulfonate (MMS), diethylsulfate (DES), and base analogs such as 5-bromo-deoxyuridine (5BU). An exemplary method for generating the melon plants of the present invention using chemical mutagenesis includes soaking melon seeds for 12 hours in water followed by additional 12 hours in EMS (e.g. 1 %). The treated seeds (M_1) are then planted and self pollinated to prepare M_2 families.

As mentioned, the melon plant of the present invention may also be generated using other techniques including but not limited to (a) deletion of the MELO3C009603 gene; (b) transcriptional inactivation of the MELO3C009603 gene (c) antisense RNA mediated inactivation of transcripts of the MELO3C009603 gene; (d) translational inactivation of transcripts of the MELO3C009603 gene; and (e) genome editing of MELO3C009603 gene.

Thus, for example, gene knock-in or gene knock-out constructs including sequences homologous with the MELO3C009603 gene can be generated and used to insert an ancillary sequence into the coding sequence of the enzyme encoding gene, to thereby inactivate this gene.

These construct preferably include positive and negative selection markers and may therefore be employed for selecting for homologous recombination events. One ordinarily skilled in the art can readily design a knock-in/knock-out construct including both positive and negative selection genes for efficiently selecting transformed plant cells that underwent a homologous recombination event with the construct. Such cells can then be grown into full plants. Standard methods known in the art can be used for implementing knock-in/knock out procedure. Such methods are set forth in, for example, U.S. Pat. Nos. 5,487,992, 5,464,764, 5,387,742, 5,360,735, 5,347,075, 5,298,422, 5,288,846, 5,221,778, 5,175,385, 5,175,384, 5,175,383, 4,736,866 as well as Burke and Olson, Methods in Enzymology, 194:251-270, 1991; Capecchi, Science 244:1288-1292, 1989; Davies et al., Nucleic Acids Research, 20 (11) 2693-2698, 1992; Dickinson et al.,

Human Molecular Genetics, 2(8):1299-1302, 1993; Duff and Lincoln, "Insertion of a pathogenic mutation into a yeast artificial chromosome containing the human APP gene and expression in ES cells", Research Advances in Alzheimer's Disease and Related Disorders, 1995; Huxley et al., Genomics, 9:742-750 1991; Jakobovits et al., Nature, 362:255-261 1993; Lamb et al., Nature Genetics, 5: 22-29, 1993; Pearson and Choi, Proc. Natl. Acad. Sci. USA, 1993, 90:10578-82; Rothstein, Methods in Enzymology, 194:281-301, 1991; Schedl et al., Nature, 362: 258-261, 1993; Strauss et al., Science, 259:1904-1907, 1993, WO 94/23049, WO93/14200, WO 94/06908 and WO 94/28123 also provide information.

Thus according to a particular embodiment of the present invention, the melon plant is generated by introduction thereto of a nucleic acid construct, the nucleic acid construct comprising a nucleic acid sequence encoding a polynucleotide agent which up-regulates an expression of MELO3C009603 having a mutation which brings about an enhanced crop yield trait (and optionally a seedless trait) and a cis-acting regulatory element capable of directing an expression of the polynucleotide agent in the plant.

Constructs useful in the methods according to the present invention may be constructed using recombinant DNA technology well known to persons skilled in the art. The gene constructs may be inserted into vectors, which may be commercially available, suitable for transforming into plants and suitable for expression of the gene of interest in the transformed cells. The genetic construct can be an expression vector wherein the nucleic acid sequence is operably linked to one or more regulatory sequences allowing expression in the plant cells.

The polynucleotide according to this aspect of the present invention may encode MELO3C009603 having for example an F/I mutation at position 97. The polypeptide sequence of an exemplary MELO3C009603 having an a F/I mutation at position 97 is typically at least 90 % homologous, at least 91 % homologous, at least 92 % homologous, at least 93 % homologous, at least 94 % homologous, at least 95 % homologous, at least 96 % homologous, at least 97 % homologous, at least 98 % homologous, at least 99 % homologous, or 100 % homologous to the sequence set forth in SEQ ID NO: 8. The nucleic acid sequence of an exemplary polynucleotide which encodes such a protein may be at least 90 % homologous, at least 91 % homologous, at least 92 % homologous, at least 93 % homologous, at least 94 % homologous, at least

95 % homologous, at least 96 % homologous, at least 97 % homologous, at least 98 % homologous, at least 99 % homologous, or 100 % homologous to the nucleic acid sequence set forth in SEQ ID NO: 9.

In a particular embodiment of the present invention the regulatory sequence is a plant-expressible promoter.

As used herein the phrase "plant-expressible" refers to a promoter sequence, including any additional regulatory elements added thereto or contained therein, is at least capable of inducing, conferring, activating or enhancing expression in a melon cell, tissue or organ.

The promoter may be a regulatable promoter, a constitutive promoter or a tissue-associated promoter.

As used herein, the term "regulatable promoter" refers to any promoter whose activity is affected by specific environmental or developmental conditions.

As used herein, the term "constitutive promoter" refers to any promoter that directs RNA production in many or all tissues of a plant transformant at most times.

As used herein, the term "tissue-associated promoter" refers to any promoter which directs RNA synthesis at higher levels in particular types of cells and tissues (e.g., a fruit-associated promoter).

Exemplary promoters that can be used to express an operably linked nucleic acid sequence (i.e. transgene) include the cauliflower mosaic virus promoter, CaMV and the tobacco mosaic virus, TMV, promoter.

Other promoters that can be used in the context of the present invention include those described in U.S. Patent No. 20060168699 and by Hector G. Numez-Palenius et al. [Critical Reviews in Biotechnology, Volume 28, Issue 1 March 2008, pages 13 – 55], both of which are incorporated herein by reference.

Plant cells may be transformed stably or transiently with the nucleic acid constructs of the present invention. In stable transformation, the nucleic acid molecule of the present invention is integrated into the plant genome and as such it represents a stable and inherited trait. In transient transformation, the nucleic acid molecule is expressed by the cell transformed but it is not integrated into the genome and as such it represents a transient trait.

There are various methods of introducing foreign genes into both monocotyledonous and dicotyledonous plants (Potrykus, I., Annu. Rev. Plant. Physiol., Plant. Mol. Biol. (1991) 42:205-225; Shimamoto et al., Nature (1989) 338:274-276).

The principle methods of causing stable integration of exogenous DNA into plant genomic DNA include two main approaches:

(i) Agrobacterium-mediated gene transfer: Klee et al. (1987) Annu. Rev. Plant Physiol. 38:467-486; Klee and Rogers in Cell Culture and Somatic Cell Genetics of Plants, Vol. 6, Molecular Biology of Plant Nuclear Genes, eds. Schell, J., and Vasil, L. K., Academic Publishers, San Diego, Calif. (1989) p. 2-25; Gatenby, in Plant Biotechnology, eds. Kung, S. and Arntzen, C. J., Butterworth Publishers, Boston, Mass. (1989) p. 93-112.

(ii) direct DNA uptake: Paszkowski et al., in Cell Culture and Somatic Cell Genetics of Plants, Vol. 6, Molecular Biology of Plant Nuclear Genes eds. Schell, J., and Vasil, L. K., Academic Publishers, San Diego, Calif. (1989) p. 52-68; including methods for direct uptake of DNA into protoplasts, Toriyama, K. et al. (1988) Bio/Technology 6:1072-1074. DNA uptake induced by brief electric shock of plant cells: Zhang et al. Plant Cell Rep. (1988) 7:379-384. Fromm et al. Nature (1986) 319:791-793. DNA injection into plant cells or tissues by particle bombardment, Klein et al. Bio/Technology (1988) 6:559-563; McCabe et al. Bio/Technology (1988) 6:923-926; Sanford, Physiol. Plant. (1990) 79:206-209; by the use of micropipette systems: Neuhaus et al., Theor. Appl. Genet. (1987) 75:30-36; Neuhaus and Spangenberg, Physiol. Plant. (1990) 79:213-217; glass fibers or silicon carbide whisker transformation of cell cultures, embryos or callus tissue, U.S. Pat. No. 5,464,765 or by the direct incubation of DNA with germinating pollen, DeWet et al. in Experimental Manipulation of Ovule Tissue, eds. Chapman, G. P. and Mantell, S. H. and Daniels, W. Longman, London, (1985) p. 197-209; and Ohta, Proc. Natl. Acad. Sci. USA (1986) 83:715-719.

The Agrobacterium system includes the use of plasmid vectors that contain defined DNA segments that integrate into the plant genomic DNA. Methods of inoculation of the plant tissue vary depending upon the plant species and the Agrobacterium delivery system. A widely used approach is the leaf disc procedure

which can be performed with any tissue explant that provides a good source for initiation of whole plant differentiation. Horsch et al. in Plant Molecular Biology Manual A5, Kluwer Academic Publishers, Dordrecht (1988) p. 1-9. A supplementary approach employs the Agrobacterium delivery system in combination with vacuum infiltration. The Agrobacterium system is especially viable in the creation of transgenic dicotyledenous plants.

There are various methods of direct DNA transfer into plant cells. In electroporation, the protoplasts are briefly exposed to a strong electric field. In microinjection, the DNA is mechanically injected directly into the cells using very small micropipettes. In microparticle bombardment, the DNA is adsorbed on microprojectiles such as magnesium sulfate crystals or tungsten particles, and the microprojectiles are physically accelerated into cells or plant tissues.

Following stable transformation plant propagation is exercised. The most common method of plant propagation is by seed. Regeneration by seed propagation, however, has the deficiency that due to heterozygosity there is a lack of uniformity in the crop, since seeds are produced by plants according to the genetic variances governed by Mendelian rules. Basically, each seed is genetically different and each will grow with its own specific traits. Therefore, it is preferred that the transformed plant be produced such that the regenerated plant has the identical traits and characteristics of the parent transgenic plant. Therefore, it is preferred that the transformed plant be regenerated by micropropagation which provides a rapid, consistent reproduction of the transformed plants.

Micropropagation is a process of growing new generation plants from a single piece of tissue that has been excised from a selected parent plant or cultivar. This process permits the mass reproduction of plants having the preferred tissue expressing the fusion protein. The new generation plants which are produced are genetically identical to, and have all of the characteristics of, the original plant. Micropropagation allows mass production of quality plant material in a short period of time and offers a rapid multiplication of selected cultivars in the preservation of the characteristics of the original transgenic or transformed plant. The advantages of cloning plants are the speed of plant multiplication and the quality and uniformity of plants produced.

Micropropagation is a multi-stage procedure that requires alteration of culture medium or growth conditions between stages. Thus, the micropropagation process involves four basic stages: Stage one, initial tissue culturing; stage two, tissue culture multiplication; stage three, differentiation and plant formation; and stage four, greenhouse culturing and hardening. During stage one, initial tissue culturing, the tissue culture is established and certified contaminant-free. During stage two, the initial tissue culture is multiplied until a sufficient number of tissue samples are produced to meet production goals. During stage three, the tissue samples grown in stage two are divided and grown into individual plantlets. At stage four, the transformed plantlets are transferred to a greenhouse for hardening where the plants' tolerance to light is gradually increased so that it can be grown in the natural environment.

Although stable transformation is presently preferred, transient transformation of leaf cells, meristematic cells or the whole plant is also envisaged by the present invention.

Transient transformation can be effected by any of the direct DNA transfer methods described above or by viral infection using modified plant viruses.

Viruses that have been shown to be useful for the transformation of plant hosts include CaMV, TMV and BV. Transformation of plants using plant viruses is described in U.S. Pat. No. 4,855,237 (BGV), EP-A 67,553 (TMV), Japanese Published Application No. 63-14693 (TMV), EPA 194,809 (BV), EPA 278,667 (BV); and Gluzman, Y. et al., Communications in Molecular Biology: Viral Vectors, Cold Spring Harbor Laboratory, New York, pp. 172-189 (1988). Pseudovirus particles for use in expressing foreign DNA in many hosts, including plants, is described in WO 87/06261.

As used herein the term “about” refers to ± 10 %.

The terms "comprises", "comprising", "includes", "including", “having” and their conjugates mean "including but not limited to".

The term “consisting of” means “including and limited to”.

The term "consisting essentially of" means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.

As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions illustrate some embodiments of the invention in a non-limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific

American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Culture of Animal Cells - A Manual of Basic Technique" by Freshney, Wiley-Liss, N. Y. (1994), Third Edition; "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

MATERIALS AND METHODS

Plant material

Seeds of 'CEZ', a 'Charentais' type melon (*Cucumis melo* subsp. *melo* Cantalupensis Group), were subjected to EMS mutagenesis, M_1 plants were self-pollinated, M_2 families were visually phenotyped and mutant lines were selected as described previously (Tadmor et al., 2007). Plants were grown under conventional conditions in the field and in the greenhouse.

Fruit carotenoid analysis

Five mature fruit were harvested, peeled, sliced and a central slice was cut into small cubes and immediately frozen in liquid nitrogen. Frozen fruit samples were ground to a fine powder by A11 analytical grinding mill (Ika) in the presence of liquid nitrogen. Carotenoids were extracted from 0.5g ground tissue in hexane:acetone:ethanol (50:25:25, v/v/v) mixture as described in Tadmor et al., (2005) and were analyzed, identified, and quantified with a Waters (Milford, MA) 2695 HPLC apparatus equipped with a Waters 996 PDA detector and Millennium software (Waters), as described previously (Tadmor et al., 2000).

RNA extraction

RNA extraction for RNA-seq analysis was performed according to Portnoy et al 2011, as described below. Additional RNA extraction for RT-PCR used the same protocol, downscaled by 1/20, starting with about 100mg frozen tissue in 1.5ml tubes.

Frozen fruit rind tissues (about 5g of 1.5mm wide fruit rind) were pulverized with a mortar and pestle in liquid nitrogen. Pulverized tissue was mixed well by vortexing in a 50mL tube with 10 mL of extraction buffer containing 0.2M Tris-HCl (pH 9.0), 0.2M ethylenediaminetetraacetic acid (EDTA), 0.4M NaCl, and 2% (w/v) SDS, and incubated at 65°C for 5 min. Then 30% (w/v) sodium lauroylsarcosine was added to a final concentration of 2% (v/v), and the mixture was vortexed and incubated at 65°C for 2 to 3 min. An equal volume of phenol was added to the solution, vortexed, and centrifuged at 5000g for 5 min. The aqueous phase was transferred to a new 50mL tube on ice, following three rounds of chloroform-isoamyl alcohol (24:1, v/v) extractions. Nucleic acids were precipitated with 1/10 volume of 3M sodium acetate (NaAc) (pH 5.3) and 2 volumes of 95% (v/v) EtOH. The resulting nucleic acid pellet was dissolved in 10 mL 2M LiCl at 4°C overnight. Total RNA was precipitated by centrifugation at 15,000 g for 10 min at 4°C and dissolved in 0.5 mL diethylpyrocarbonate (DEPC) water. After reprecipitation with 1/10 volume of 3M NaAc (pH 5.3) and 2 volumes of 95% EtOH, the pellet was dissolved in 50 to 100 μL DEPC water. RNA was further treated with DNase I (Thermo scientific) according to the manufacturer's protocol. After DNase reaction, samples were cleaned by chloroform-isoamyl alcohol extraction, precipitated with 1/10 volume of 3M NaAc and 2 volumes of 95% EtOH, cleaned with 70% EtOH, air dried for 5 min and diluted in

water. The quality of the RNA was analyzed by ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE), electrophoresis in SB 1% Agarose gel, and PCR of intron flanking primers to check if presence of DNA contamination is observed on Agarose gel.

Leaf RNA was extracted with triReagent (Sigma), according to manufacturer's instructions. DNaseI (Thermo scientific) was applied according to manufacturer's protocol, and cleaned by the addition of chloroform-isoamyl alcohol, precipitated with Isopropanol, washed with 70% EtOH, left for 5 minutes to air dry and dissolved in ddH2O. Concentration of RNA was determined by NanoDrop.

RNA-Seq

After DNase treatment RNA samples were checked for integrity on a 1% Agarose gel, checked for purity in NanoDrop (260/280 ratio of around 2, 260/230 of around 2.4), and absence of DNA contamination was determined by PCR analysis with EF1α-intron primers.

F–AGGCTGATTGTGCTGTCCTT - SEQ ID NO: 1;

R-GATGGGAACGAAGGGAATTT - SEQ ID NO: 2.

Samples containing DNA contamination should yield amplicons of 391bp, in contrast to 303bp cDNA, when fractionated on an Agarose gel. Samples containing about 30μg RNA were precipitated with two volumes of EtOH and 1/10 volume of 3M NaAc and stored at (-20°C). Construction of strand-specific libraries was performed with TruSec RNA Samp Prep Kit FC-121-1031 (Illumina Inc) according to the manufacturer's instructions. Twelve libraries were shipped on dry ice for sequencing with the Illumina HiSeq2000. Each library was individually barcoded and all libraries were sequenced in one Illumina lane yielding and average of $17x10^6$ reads of 50bp per library. The Illumina reads were sorted to their libraries and barcodes were removed. Raw reads were trimmed for low quality bases at the end of the RNA-seq and low-quality reads were removed using the FASTX-toolkit. The resulting high quality reads were then mapped to the melon genome using TopHat version v2.0.10 (Kim et al., 2011) and were counted using HTseq v0.5.3p3. Bioconductor DESeq package (Anders, 2010) in the R environment was used to identify differentially expressed genes between 'sf' and 'wildtype' samples. Genes showing FDR < 0.05 were considered as differential expressed. SNP analysis was carried using the variant calling routines GATK Unified

Genotyper program (version 2.5-2) (DePristo et al., 2011) and filtered to achieve a high-confidence SNP set.

RT-PCR

1µg of RNA was used for cDNA synthesis using 'Verso system' (Thermo Scientific) according to the manufacturer's instructions. Reaction was performed in an Eco RT-PCR system (Illumina). Each sample contained: 1µl cDNA, 0.2µl of each primer (10mM), 5µl of FastSYBR green master mix reaction mix (Applied Biosystems) and 3.6µl ddH2O. The machine was programmed as specified by the enzyme manufacturer. Each analysis was conducted in relation to the housekeeping gene ARP1, and analyzed in Eco version 4 software.

DNA extraction

Young plant meristems (about 1gr) were ground in liquid nitrogen with mortar and pestle. DNA extraction solution was prepared by mixing extraction buffer (0.35 M Sorbitol, 0.1 M Tris, 5 mM EDTA, pH7, add 0.02 M NaBisulfite before use) : nucleic lysis buffer (0.35 M Sorbitol, 0.1 M Tris, 5 mM EDTA, pH7, add 2 % CTAB before use) : 5 % sarkosyl, in ratio of 1:1:0.4. All chemicals were supplied by Sigma. DNA extraction solution was incubated in 65 °C. 600 µl DNA extraction solution were added to 100 µg tissue weighted into 1.5 ml tube, mixed and incubated in 65 ^{0}C for 10 minutes. 600 µl chloroform:isoamylalcohol (ratio 24:1) were added, mixed for 5 minutes in 200RPM, centrifuged at 15,000g for 10 minutes and supernatant was removed into a new tube. Cold isopropanol (2/3 of supernatant volume) was added, mixed, incubated for 30 minutes to over-night in (-20 °C), centrifuged at 20,000g for 10 minutes, liquid phase removed, pellet washed with 70 % EtOH, precipitated again and EtOH was removed, pellet air dried for 5 minutes, dissolved with 50-200 µl water, 2 µl RNase were added, samples were incubated at 37 ^{0}C for 30 min, centrifuged at 15,000g for 3 min and supernatant was removed to a new tube. Concentration and purity of DNA were determined in NanoDrop, integrity of DNA was checked on a 0.8% Agarose gel.

RESULTS

Identification of Superfruiter

Visual phenotyping of 2,000 M_2 families derived from mutagenized seeds of the breeding line 'CEZ' was performed. Each M_2 family was represented in the field by 12

plants. One family segregated for a unique phenotype; 3 out of the 12 plants grown in the field carried more than 15 fruit per plant (Figures 1 and 2) as compared to the wild type plants which carried on average 3 fruit. Each of the mutants' fruit weighed around 300gr while the wild type fruit weighed about 900gr. Interestingly, the mutant's fruit had no seeds or very tiny empty seeds. This mutation is referred to herein as 'superfruiter' (Figure 3). When the fruit of a 'sf' plant was diluted, the fruit remained small.

Inheritance of super fruiter

Cuttings of superfruiter ('sf') plants were transferred to the greenhouse once they developed sufficient roots. The 'sf' plants developed from the cuttings could not be self-pollinated. However their pollen was used for successful pollination of 'CEZ', 'Noy Yizre'el', 'Ein Dor' and 'Piel De Sapo' lines and viable F_1 seeds were obtained. These seeds were planted and F_1 plants were successfully self-pollinated to yield F_2 seeds. 150-200 plants of each of the F_2 populations were planted in the field, allowed to grow under commercial production conditions and were open pollinated by bees. Once fruit was generated the present inventors could visually distinguish between 'sf' and wild type phenotypes. In all four F_2 populations 'sf' segregated as a single recessive gene (Table 1).

Table 1

F_2 population	n	phenotype	Observed	Expected	Chi square	P value
ED x sf	188	sf	43	47	0.454	0.5005
		WT	145	141		
NY x sf	166	sf	39	41.5	0.201	0.6541
		WT	127	124.5		
CEZ x sf	191	sf	48	47.75	0.002	0.9667
		WT	143	143.25		
PDS x sf	194	sf	38	48.5	3.031	0.0817
		WT	156	145.5		

Chi square value of the deviation of segregants from the 1:3 expected Mendelian ratio. 'n' – population size; P value that is larger than 0.05 indicates that there is a high probability that the observed deviations could be due to random chance alone.

To estimate the yield of 'sf' the present inventors measured the fruit number and weight of all 'sf' plants, divided the sum of fruit by plant number to obtain a mean fruit number per plant, divided the sum of fruit weight by plant number to obtain a mean yield per plant and divided the sum of fruit weight by fruit number to obtain a mean fruit weight. Similar measurements and calculations were conducted for wild type plants. In all tested genetic backgrounds 'sf' carried significantly more fruit (Figure 4A), significantly smaller fruit (Figure 4B) and significantly higher yield per plant (Figure 4C).

To determine the effect of 'sf' on fruit quality the present inventors randomly picked 10 mature fruit of CEZ and of CEZx'sf' wildtype and 'sf' phenotype segregants. Fruit were tasted, analyzed for total soluble solids (TSS) as an index for sugar content, HPLC analyzed for β-carotene content. No effect of 'sf' on melon fruit flavor that is mainly determined by sugar content was detected. No quality difference between 'sf', wild type segregants or 'CEZ' was detected including TSS or β-carotene content (Figures 5A-B).

To identify the gene that determines 'sf' phenotype, two replications of 10 plants showing either 'sf' or wild type phenotype in the 'sf' X NY and in the 'sf' X ED segregating F_2 populations, 'sf' phenotype from the 'sf' X CEZ segregating F_2 population and the 'sf' originator line, CEZ were selected. From each of these plants shoot meristems, stems, female flowers and young fruit at the age of 2-4 days after pollination were sampled. RNA was extracted from bulks of each tissue x phenotype combination. Equal amounts of RNA from all tissues were combined to develop two replications of 'sf' and wild type phenotypes of 'sf' X NY and of 'sf' X ED segregating F_2 populations (eight pools), 'sf' phenotype from the 'sf' X CEZ segregating F_2 population and the 'sf' originator line, CEZ (four pools). Twelve libraries were RNA-Seq analyzed with Illumina HiSeq 2000 yielding an average of $17X10^6$ reads for each library. Single Nucleotide Polymorphism (SNP) identified by comparing RNA-Seq data of phenotypic pools, derived from 'sf' X NY and of 'sf' X ED segregating F_2 populations, were scattered along the melon genome however most of them were located on scaffold 11. The present inventors then looked for SNP that were homozygous in 'CEZ', carried the alternative allele in all 'sf' phenotypes ' and carried mostly 'CEZ' allele in all wild type phenotypes. A single SNP in MELO3C009603 that is located on scaffold 11 of

chromosome 4 was fixed in all 'sf' material ('A') compared to 'CEZ' ('T') and was the minor allele in all 'wild type' phenotypes.

Primers were designed that PCR amplify a 213bp fragment that has an *Apo*I restriction site in the wild type allele that is mutated in sf.

F TAGACATGAGCCGCATCTGA - SEQ ID NO: 3

R GAACGTGGCAACAACAACAA - SEQ ID NO: 4

Conducting an *Apo*I digestion on the PCR amplified fragment yields a 140 bp and a 73bp fragments in wild type, a 213bp fragment when a homozygote 'sf' mutant is digested and all three bands in the heterozygote (Figure 6B). This marker showed complete co-segregation with 'sf' phenotype in four independent F_2 populations consisting of at least 300 plants each.

MELO3C009603 codes for a Cys_2 His_2 Zinc Finger (ZF) protein. The 'T' to 'A' transversion changed TTC, which codes for the highly conserved amino acid phenylalanine at position 97 (F^{97}), to ATC that codes for isoleucine (I) in the ZF motif (Figure 7).

RNA-Seq data indicated that the digital expression of MELO3C009603 is low, only 40-60 reads in each bulk, and similar in both bulks. These bulks included a mix of several tissues. The relative expression of MELO3C009603 in each of these tissues using quantitative RTPCR was analyzed and it was found that it has similar low expression in leaves and similar very low expression in all other analyzed tissues (Figures 8A-B).

Analysis of RNA-Seq data for differentially expressed genes (DEG) identified 103 genes that showed more than two fold change between 'CEZ' and 'sf', the isogenic comparison. Only 55 genes, out of these 103, showed significant difference while using the adjusted P value out of these 55 only 14 genes showed significant differential expression between 'sf' and 'wild type' bulks in all three analyzed segregating populations (Figure 9 and Table 2, herein below). Of these 14 genes only MELO3C021150 that codes for a seed nucellus-specific protein homolog was down regulated in 'sf' of all three analyzed population and MELO3C003230, which codes for a putative anthocyanin 5-aromatic acyltransferase homolog, was up regulated in 'sf' of the analyzed populations. The other twelve genes show similar direction of change in 'CEZ x sf' and in 'ED x sf' but opposite direction in 'NY x sf' F_2 population (Table 2,

herein below). Quantitative RT-PCR analysis of MELO3C021150 in different organs indicated that the digital expression found between the bulks is contributed by the young fruit tissue and that MELO3C021150 is not transcribed in all other tissues (Figure 10).

Table 2

C	Sub-C	ID	CEZsf	CEZWT	WT/sf	P	AdjP	EDsf	EDWT	WT/sf	P	AdjP	NYsf	NYWT	WT/sf	P	AdjP	ACC	DESC
1	AAA	MELO3C021150	2.5	139.5	56.5	0.00000	0.00000	1.9	20.4	10.6	0.00001	0.00130	1.1	36.8	32.2	0.00000	0.00000	AAB82329	seed nucellus-specific protein [Citrullus lanatus]
1	AAA	MELO3C003230	953.6	331.1	0.3	0.00000	0.00009	953.6	253.4	0.4	0.00000	0.00003	1,277.1	346.0	0.3	0.00000	0.00002	XP_002531355	Anthocyanin 5-aromatic acyltransferase, putative [Ricinus communis]
2	AA	MELO3C022716	3.9	247.2	63.4	0.00000	0.00000	1.4	7.8	5.6	0.00486	1.00000	5.2	46.9	9.0	0.00000	0.00003	AAB82329	seed nucellus-specific protein [Citrullus lanatus]
2	AA	MELO3C023027	2,064.6	401.0	0.2	0.00000	0.00000	2,064.6	668.2	0.5	0.00001	0.00239	821.0	437.8	0.5	0.00152	0.40811	AAM74923	17 kDa phloem lectin Lec17-1 [Cucumis melo]
2	AA	MELO3C015490	170.1	35.7	0.2	0.00000	0.00000	170.1	59.1	0.6	0.00486	1.00000	256.8	80.2	0.3	0.00001	0.00059	XP_002514129	calmodulin binding protein, putative [Ricinus communis]
2	AA	MELO3C027040	1,176.0	352.7	0.3	0.00000	0.00001	1,176.0	363.7	0.3	0.00000	0.00000	559.6	333.6	0.6	0.00500	0.83448	AAM74923	17 kDa phloem lectin Lec17-1 [Cucumis melo]
2	AA	MELO3C024762	365.0	111.0	0.3	0.00000	0.00004	365.0	70.4	0.3	0.00000	0.00010	176.8	80.6	0.5	0.00064	0.23000	AAW51125	putative alcohol acyl-transferases [Cucumis melo]
2	AA	MELO3C007337	54.3	10.5	0.2	0.00000	0.00017	54.3	25.7	0.5	0.00187	0.71667	132.5	45.1	0.3	0.00006	0.03744	ADL36665	COL domain class transcription factor [Malus x domestica]

C	Sub-C	ID	CEZsf	CEZWT	WT/sf	P	Adj P	EDsf	EDWT	WT/sf	P	Adj P	NYsf	NYWT	WT/sf	P	Adj P	ACC	DESC
2	AA	MELO3C006043	468.3	167.9	0.4	0.00000	0.00024	468.3	235.6	0.6	0.00062	0.37711	695.8	250.4	0.4	0.00002	0.01400	ADN34176	UDP-glucose:glucosyltransferase [Cucumis melo subsp. melo]
2	AA	MELO3C026488	468.8	198.2	0.4	0.00004	0.00201	468.8	113.2	0.4	0.00002	0.00299	401.8	191.0	0.5	0.00068	0.24044	O49858	RecName: Full=Cytochrome P450 82A3; AltName: Full=Cytochrome P450 CP6
2	A	MELO3C002560	452.8	66.6	0.1	0.00000	0.00000	452.8	59.1	0.6	0.00372	1.00000	151.3	92.8	0.6	0.00966	1.00000	AAY85179	fiber expressed protein [Gossypium hirsutum]
2	A	MELO3C007961	17.9	127.3	7.1	0.00000	0.00000	17.9	36.7	3.3	0.00010	0.10777	9.9	16.1	1.6	0.35211	1.00000	BAB40817	endochitinase MCHT-2 [Cucumis melo]
2	A	MELO3C008466	167.9	32.1	0.2	0.00000	0.00000	167.9	9.9	0.4	0.00304	0.93211	42.3	18.7	0.4	0.00363	0.69880	XP_002520298	calcium binding protein/cast, putative [Ricinus communis]
2	A	MELO3C013917	206.2	53.5	0.3	0.00000	0.00001	206.2	56.2	0.6	0.00296	0.91955	248.0	102.3	0.4	0.00021	0.00950	ADE41101	AP2 domain class transcription factor [Malus x domestica]
2	A	MELO3C023255	66.5	13.8	0.2	0.00000	0.00008	66.5	43.3	0.6	0.00665	1.00000	66.7	32.9	0.5	0.00358	0.69226	XP_002328473	predicted protein [Populus trichocarpa]
2	A	MELO3C005711	353.7	125.2	0.4	0.00000	0.00031	353.7	181.9	0.5	0.00017	0.15555	606.4	293.8	0.5	0.00075	0.25776	XP_002509857	metal ion binding protein, putative [Ricinus communis]
2	A	MELO3C011268	8.5	38.2	4.5	0.00003	0.01176	8.5	43.2	1.9	0.03800	1.00000	5.1	19.5	3.8	0.01173	0.44776	XP_002515504	ATP binding protein, putative [Ricinus communis]
2	A	MELO3C021821	198.7	77.7	0.4	0.00004	0.00233	198.7	187.6	1.7	0.00236	0.81441	257.9	127.4	0.5	0.00127	0.36333	XP_002328950	glycosyltransferase, CAZy family GT2 [Populus trichocarpa]
2	A	MELO3C026807	102.0	37.3	0.4	0.00007	0.00331	102.0	30.1	0.5	0.00412	1.00000	2.2	1.3	0.6	0.81200	1.00000		

C	Sub-C	ID	CEZsf	CEZWT	WT/sf	P	AdjP	EDsf	EDWT	WT/sf	P	AdjP	NYsf	NYWT	WT/sf	P	AdjP	ACC	DESC
1	BBB	MELO3C003917	924.3	42.2	0.0	0.00000	0.00000	924.3	14.1	0.0	0.00000	0.00000	60.4	3,450.8	57.1	0.0000	0.0000	ACC93947	heat-shock protein 70 [Hevea brasiliensis]
1	BBB	MELO3C027124	853.0	41.8	0.0	0.00000	0.00000	853.0	9.5	0.1	0.00000	0.00000	40.4	3,290.9	81.4	0.0000	0.0000	ADM47405	small molecular heat shock protein [Nicotiana tabacum]
1	BBB	MELO3C025139	1,852.7	189.9	0.1	0.00000	0.00000	1,852.7	157.4	0.4	0.00000	0.00013	223.6	5,792.0	25.9	0.0000	0.0000	ADM47405	small molecular heat shock protein [Nicotiana tabacum]
1	BBB	MELO3C021172	4,697.3	673.7	0.1	0.00000	0.00000	4,697.3	1,160.9	0.5	0.00002	0.00315	1,599.9	3,878.2	2.4	0.0007	0.0043 0	XP_002880227	BCL-2-associated athanogene 6 [Arabidopsis lyrata subsp. lyrata]
1	BBB	MELO3C002020	5,756.5	1,131.6	0.2	0.00000	0.00000	5,756.5	206.6	0.4	0.00000	0.00032	337.1	1,608.5	4.8	0.0000	0.0000	AAX08108	heat shock protein 101 [Vitis vinifera]
1	BBB	MELO3C026374	857.8	157.0	0.2	0.00000	0.00000	857.8	48.3	0.4	0.00003	0.00391	73.0	585.7	8.0	0.0000	0.0000	ADM47405	small molecular heat shock protein [Nicotiana tabacum]
1	BBB	MELO3C006933	252.0	37.4	0.1	0.00000	0.00000	252.0	1.7	0.1	0.00001	0.01192	11.6	88.8	7.7	0.0000	0.0000	XP_002318188	predicted protein [Populus trichocarpa]
1	BBB	MELO3C003195	107.3	16.5	0.2	0.00000	0.00000	107.3	6.1	0.0	0.00000	0.00000	13.4	57.2	4.3	0.0002	0.0017 9	XP_002534180	heat-shock protein, putative [Ricinus communis]
1	BBB	MELO3C006935	9,853.8	2,844.1	0.3	0.00000	0.00000	9,853.8	186.1	0.4	0.00001	0.00180	325.9	3,182.2	9.8	0.0000	0.0000	XP_002513649	heat shock protein, putative [Ricinus communis]
C	Sub-C	ID	CEZsf	CEZWT	WT/sf	P	AdjP	EDsf	EDWT	WT/sf	P	AdjP	NYsf	NYWT	WT/sf	P	AdjP	ACC	DESC
1	BBB	MELO3C022499	307.3	79.1	0.3	0.00000	0.00000	307.3	4.5	0.2	0.00003	0.00391	16.3	136.2	8.4	0.0000	0.0000	XP_002320721	predicted protein [Populus trichocarpa]
1	BBB	MELO3C010773	65.0	17.9	0.3	0.00002	0.00116	65.0	40.3	0.4	0.00001	0.00217	9.7	90.7	9.4	0.0000	0.0000	ABH02912	MYB transcription factor MYB71 [Glycine max]

C	Sub-C	ID	CEZsf	CEZWT	WT/sf	P	AdjP	EDsf	EDWT	WT/sf	P	AdjP	NYsf	NYWT	WT/sf	P	AdjP	ACC	DESC
1	BBB	MELO3C002508	866.4	366.8	0.4	0.0003	0.0166	866.4	295.6	0.5	0.0001	0.0164	302.3	988.6	3.3	0.0000	0.0011	AAD33596	thioredoxin h [Hevea brasiliensis]
2	BB	MELO3C022116	1,000.9	32.6	0.0	0.0000	0.0000	21.3	4.3	0.2	0.0020	0.17445	6.7	1,514.5	225.7	0.0000	0.0000	XP_002519929	heat-shock protein, putative [Ricinus communis]
2	BB	MELO3C018485	681.3	37.2	0.1	0.0000	0.0000	681.3	13.7	0.5	0.00565	1.00000	11.9	437.1	36.8	0.0000	0.0000	XP_002521274	heat-shock protein, putative [Ricinus communis]
2	BB	MELO3C025135	1,245.0	103.4	0.1	0.0000	0.0000	1,245.0	7.6	0.5	0.16667	1.00000	11.3	467.9	41.4	0.0000	0.0000	AAD49336	low molecular weight heat-shock protein [Nicotiana tabacum]
2	BB	MELO3C018023	1,232.5	184.1	0.1	0.0000	0.0000	1,232.5	59.2	0.5	0.00411	0.28115	108.7	295.7	2.7	0.0005	0.0291	ADN33815	sterol regulatory element-binding protein site 2 protease [Cucumis melo]
2	BB	MELO3C004433	1,794.3	279.2	0.2	0.0000	0.0000	1,794.3	352.3	0.6	0.00024	0.20144	532.9	1,263.8	2.4	0.0012	0.0608	XP_002531446	calcium ion binding protein, putative [Ricinus communis]
C	Sub-C	ID	CEZsf	CEZWT	WT/sf	P	AdjP	EDsf	EDWT	WT/sf	P	AdjP	NYsf	NYWT	WT/sf	P	AdjP	ACC	DESC
2	BB	MELO3C024086	1,469.6	255.5	0.2	0.0000	0.0000	1,469.6	73.1	0.6	0.00418	1.00000	122.4	446.5	3.6	0.0000	0.0006	XP_002526446	heat shock protein, putative [Ricinus communis]
2	BB	MELO3C006536	415.6	62.4	0.2	0.0000	0.0000	415.6	24.7	0.4	0.00037	0.26635	30.0	185.6	6.2	0.0000	0.0000	NP_001148098	heat shock 70 kDa protein 1 [Zea mays]
2	BB	MELO3C015515	60.2	393.2	6.5	0.0000	0.0000	60.2	1,846.2	1.7	0.00066	0.39588	456.6	1,031.6	2.3	0.0016	0.0774	Q9ZRA4	RecName: Full=Auxin-binding protein ABP19a; Flags: Precursor
2	BB	MELO3C003177	271.8	40.9	0.2	0.0000	0.0000	271.8	43.0	0.4	0.00026	0.21088	40.0	73.1	1.8	0.0759	1.0000	XP_002534074	Peptide transporter, putative [Ricinus communis]

C	Sub-C	ID	CEZsf	CEZWT	WT/sf	P	AdjP	EDsf	EDWT	WT/sf	P	AdjP	NYsf	NYWT	WT/sf	P	AdjP	ACC	DESC
2	BB	MELO3C016897	5,196.4	1,190.0	0.2	0.00000	0.00000	5,196.4	70.3	0.6	0.00156	0.65227	111.7	6,477.3	58.0	0.00000	0.00000	ACV93250	CII small heat shock protein 1 [Prunus salicina]
2	BB	MELO3C015804	1,495.2	372.0	0.2	0.00000	0.00000	1,495.2	19.5	0.6	0.00751	1.00000	29.8	500.7	16.8	0.00000	0.00000	XP_002517070	Heat shock factor protein HSF30, putative [Ricinus communis]
2	BB	MELO3C003331	283.8	62.0	0.2	0.00000	0.00000	283.8	31.0	0.6	0.1165	1.00000	24.3	29.0	1.2	0.65888	1.00000	XP_002873020	hypothetical protein ARALYDRAFT_486956 [Arabidopsis lyrata subsp. lyrata]
C	Sub-C	ID	CEZsf	CEZWT	WT/sf	P	AdjP	EDsf	EDWT	WT/sf	P	AdjP	NYsf	NYWT	WT/sf	P	AdjP	ACC	DESC
2	BB	MELO3C003331	283.8	62.0	0.2	0.00000	0.00000	283.8	31.0	0.6	0.1165	1.00000	24.3	29.0	1.2	0.65888	1.00000	XP_002873020	hypothetical protein ARALYDRAFT_486956 [Arabidopsis lyrata subsp. lyrata]
2	BB	MELO3C004434	685.6	185.5	0.3	0.00000	0.00000	685.6	227.2	0.4	0.00001	0.01555	308.5	697.6	2.3	0.00244	0.10176	ADN34124	serine-rich protein [Cucumis melo subsp. melo]
2	BB	MELO3C019254	90.8	284.0	3.1	0.00000	0.00009	90.8	48.8	1.6	0.1036	1.00000	82.6	49.9	0.6	0.1211	1.00000	BAC79616	putative Septum-promoting GTP-binding protein 1 (GTPase spg1) [Oryza sativa]
2	BB	MELO3C023497	25.5	2.0	0.1	0.00000	0.00012	25.5	11.1	0.6	0.2883	1.00000	13.0	22.9	1.8	0.2333	1.00000	XP_002531706	Nonspecific lipid-transfer protein precursor, putative [Ricinus communis]
2	BB	MELO3C005923	303.6	106.8	0.4	0.00000	0.00027	303.6	61.0	0.3	0.00000	0.00001	96.1	166.3	1.7	0.06433	0.93955	XP_002867485	chloroplast small heat shock protein [Arabidopsis lyrata subsp. lyrata]

C	Sub-C	ID	CEZsf	CEZWT	WT/sf	P	AdjP	EDsf	EDWT	WT/sf	P	AdjP	NYsf	NYWT	WT/sf	P	AdjP	ACC	DESC
2	BB	MELO3C025085	528.1	198.5	0.4	0.0001	0.0039	528.1	26.7	0.5	0.0131	0.58772	39.0	263.1	6.7	0.0000	0.0000	BAG09378	peroxisomal small heat shock protein [Glycine max]
2	BB	MELO3C005629	175.4	58.9	0.3	0.0001	0.0060	175.4	149.4	2.2	0.0010	0.10665	55.5	158.3	2.9	0.0004	0.02711	ABH08746	CBF/DREB-like transcription factor 1 [Citrus trifoliata]
2	BB	MELO3C002513	519.6	206.1	0.4	0.0001	0.0093	519.6	50.8	0.5	0.0077	0.04330	82.5	288.2	3.5	0.0000	0.0016	NP_567623	Aldolase-type TIM barrel family protein [Arabidopsis thaliana]
2	BB	MELO3C024263	182.0	67.9	0.4	0.0003	0.0167	182.0	54.9	0.2	0.0000	0.00000	79.7	154.0	1.9	0.0250	0.56632	XP_002515999	tonoplast intrinsic protein, putative [Ricinus communis]
2	B	MELO3C020963	338.8	87.0	0.3	0.0000	0.0000	338.8	155.9	1.7	0.0150	0.63559	510.2	106.9	0.2	0.0000	0.0000	XP_002533354	WRKY transcription factor, putative [Ricinus communis]
2	B	MELO3C021168	108.2	246.0	2.3	0.0011	0.0495	108.2	114.0	2.3	0.0009	0.09744	125.8	75.2	0.6	0.0921	1.00000	XP_002511954	Esterase precursor, putative [Ricinus communis]
2	B	MELO3C011948	68.4	20.8	0.3	0.0004	0.0201	68.4	21.4	1.7	0.22733	1.00000	33.6	13.0	0.4	0.0258	0.57667	XP_002530026	leucine-rich repeat-containing protein, putative [Ricinus communis]
2	B	MELO3C003911	261.9	112.4	0.4	0.0009	0.0401	261.9	294.5	1.8	0.0042	0.28511	463.2	268.1	0.6	0.0441	0.77227	XP_002512304	copine, putative [Ricinus communis]

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.

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WHAT IS CLAIMED IS:

1. A *Cucumis melo* plant or a part thereof, the plant bearing more than 12 fruit, said fruit being seedless.

2. A *Cucumis melo* plant having a *MELO3C009603/melo3c009603* genome such that upon self-pollination, 25 % of F1 bear more than 12 fruit, said fruit being seedless.

3. The plant of claim 1, bearing more than 15 fruit.

4. The plant of claim 1, bearing more than 20 fruit.

5. The plant of claim 1, having a similar total soluble solid (TSS) content and β-carotene content as a wild-type *Cucumis melo* plant.

6. The plant of claim 1, wherein the weight of total fruit of said plant is greater than the weight of total fruit of a wild-type *Cucumis melo* plant.

7. The plant of claim 1, being of a variety *C. melo* Cantalupensis.

8. The plant of claim 1, wherein both alleles of MELO3C009603 of the genome of the plant have a loss of function mutation that results in a seedless trait.

9. The plant of claim 8, wherein both alleles of said MELO3C009603 have an F/I mutation at position 97 thereof.

10. The plant of claim 8, wherein the polynucleotide sequence of said MELO3C009603 is as set forth in SEQ ID NO: 7.

11. The plant of claim 1, wherein the polypeptide sequence of MELO3C009603 is as set forth in SEQ ID NO: 8.

12. A cutting of a *C. melo* plant of the plant of any one of claims 1-11.

13. The plant part of any one of claims 1-11, being selected from the group consisting of roots, stems, leaves, cotyledons, flowers, fruit, embryos and pollen.

14. A seed of the plant of claim 2.

15. A cell having the genome of the plant of any one of claims 1-11.

16. A culture comprising a plurality of the cells of claim 15.

17. The plant part of any of claims 1-11, selected from the group consisting of roots, stems, leaves, cotyledons, flowers, fruit, embryos and pollen.

18. A method of breeding a first *C. melo* comprising crossing the plant of claims 1-11 with a second *C. melo* plant, thereby breeding the *C. melo*.

19. The method of claim 18, wherein said crossing comprising pollinating.

20. The method of claim 18, wherein the subspecies of said *melo* plant is selected from the group consisting of *melo* Cantalupensis, Noy Yizre'el, Ein Dor and Piel De Sapo.

21. The method of claim 18, wherein the second *C. melo* plant is not the plant of any one of claims 1-11.

22. A plurality of *C. melo* seeds which are heterozygotic for a MELO3C009603 mutation which upon planting brings about an enhanced fruit crop phenotype in 25 % of the plants derived therefrom.

23. The plurality of seeds of claim 22, wherein said 25 % of the plants bear more than 5 fruit, said fruit being seedless.

24. A hybrid seed produced by the method of claims 18 or 20.

25. A hybrid plant, or parts thereof, produced by growing the hybrid seed of claim 22 or 24.

26. A method of growing the plant of any one of claims 1 or 3-11 comprising vegetatively propagating the plant, thereby growing the plant.

27. An isolated polynucleotide comprising the sequence as set forth in SEQ ID NO: 9.

28. An isolated polypeptide comprising a sequence as set forth in SEQ ID NO: 8.

29. A method of marker assisted selection of a *C. melo* plant having improved fruit yield or having a progeny with improved yield, the method comprising analyzing for the presence of a loss of function mutation in at least one MELO3C009603 allele, wherein the presence of said mutation is indicative that the plant or progeny thereof will bear more than 5 seedless fruit.

30. The method of claim 29, wherein said marker assisted selection is conducted using an assay selected from the group consisting of single base extension (SBE), allele-specific primer extension sequencing (ASPE), DNA sequencing, RNA sequencing, microarray-based analyses, universal PCR, allele specific extension, hybridization, mass spectrometry, ligation, extension-ligation, and Flap Endonuclease-mediated assays.

31. A food or processed product comprising the plant of any one of claims 1-11 or parts thereof.

FIG. 1

FIG. 2

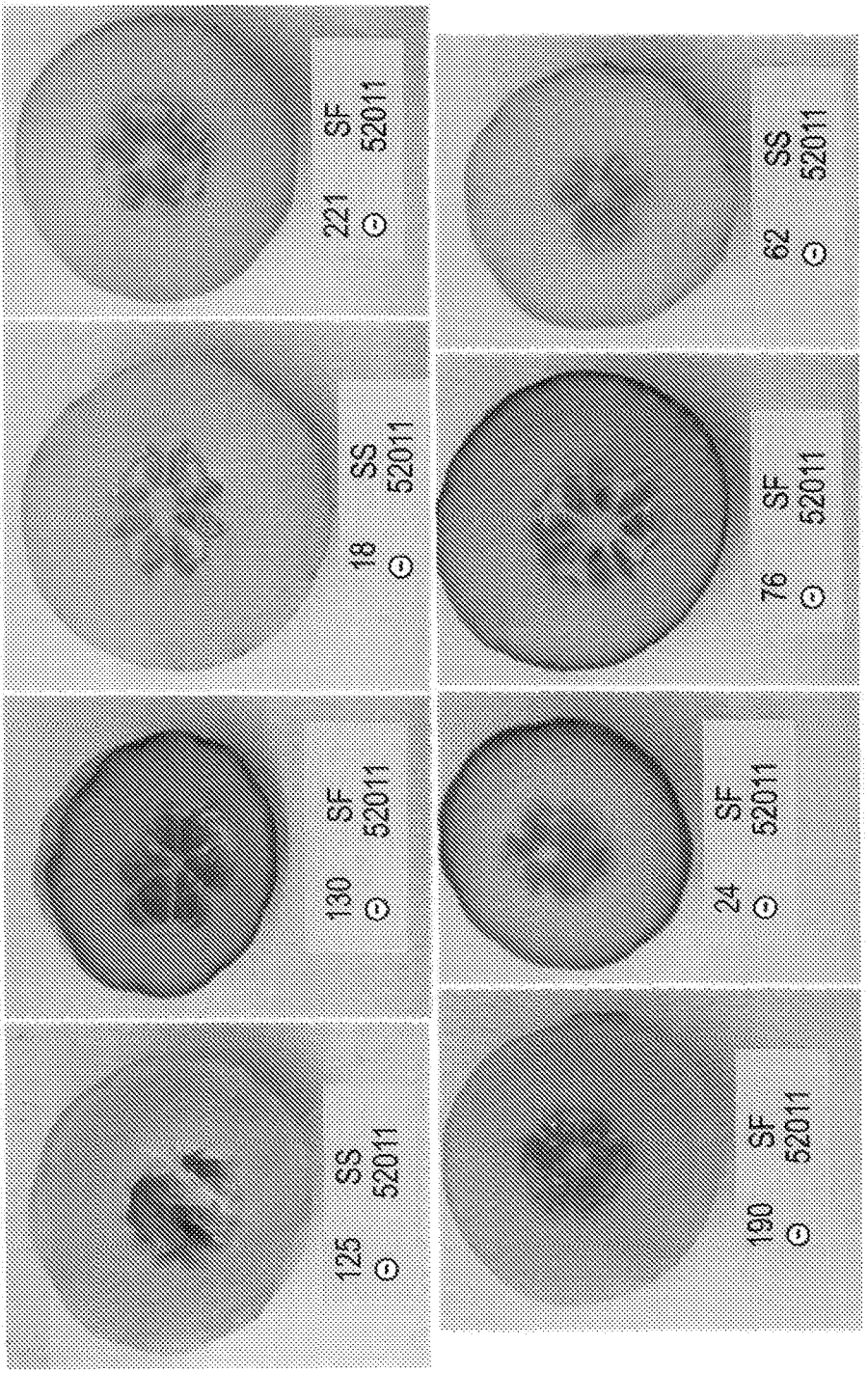

SF
52011
221
SS
52011
62
SS
52011
18
SF
52011
76
SF
52011
130
SF
52011
24
SS
52011
125
SF
52011
190

FIG. 3

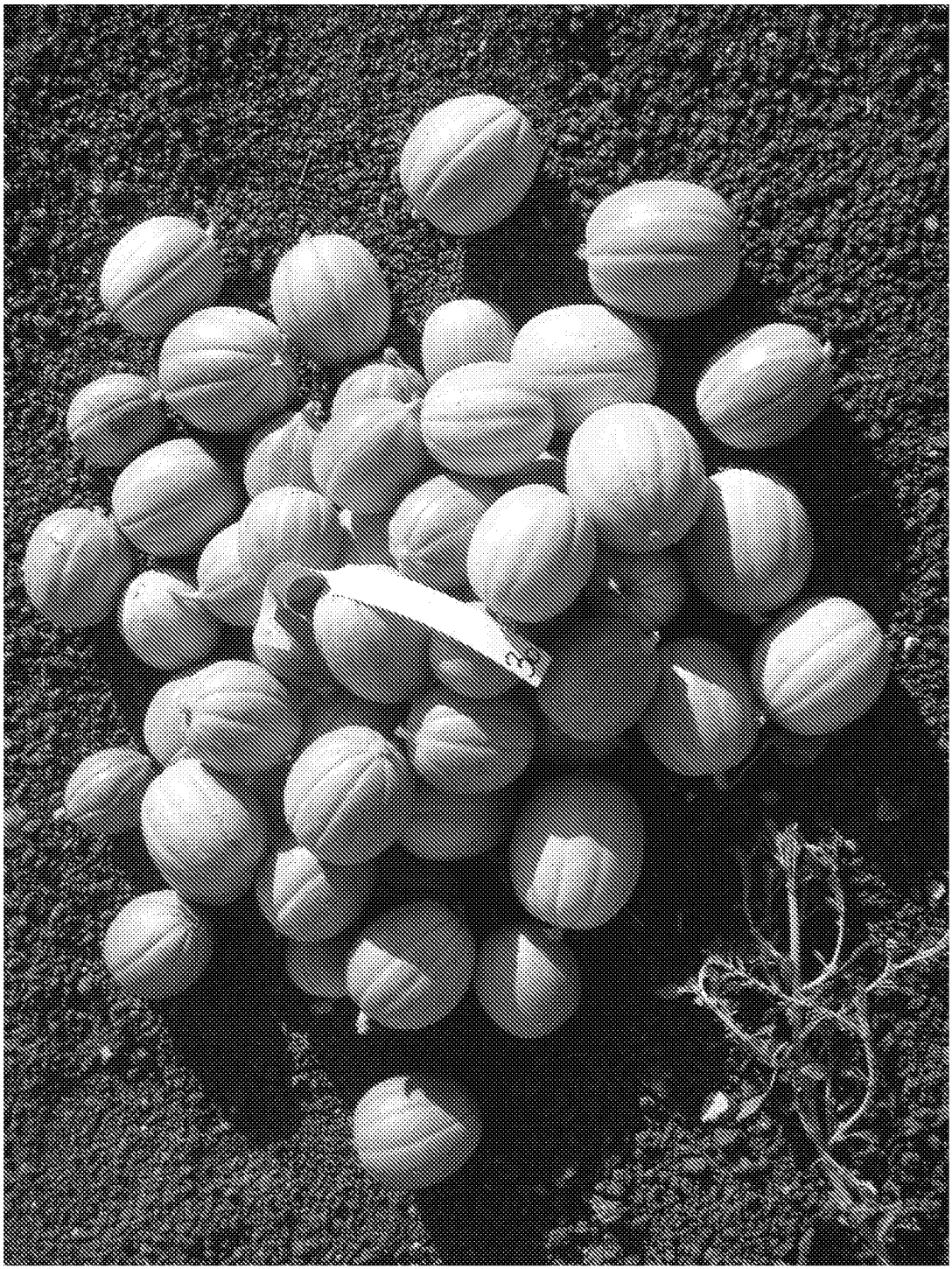


FIG. 4A

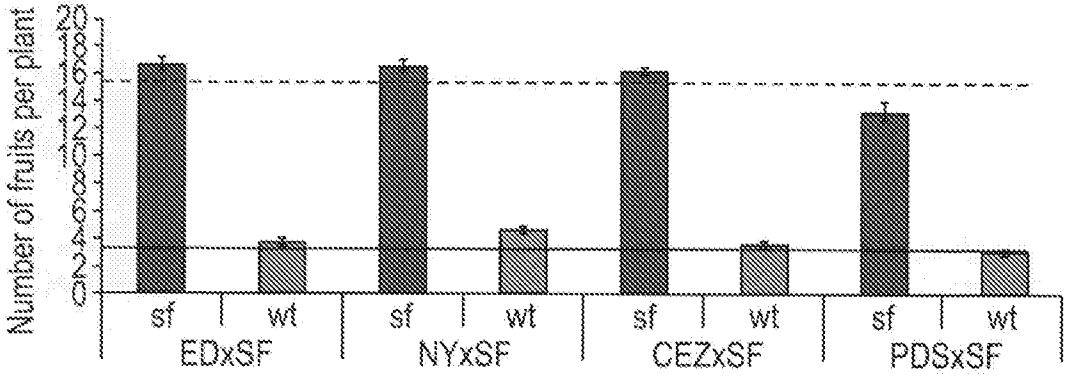


FIG. 4B

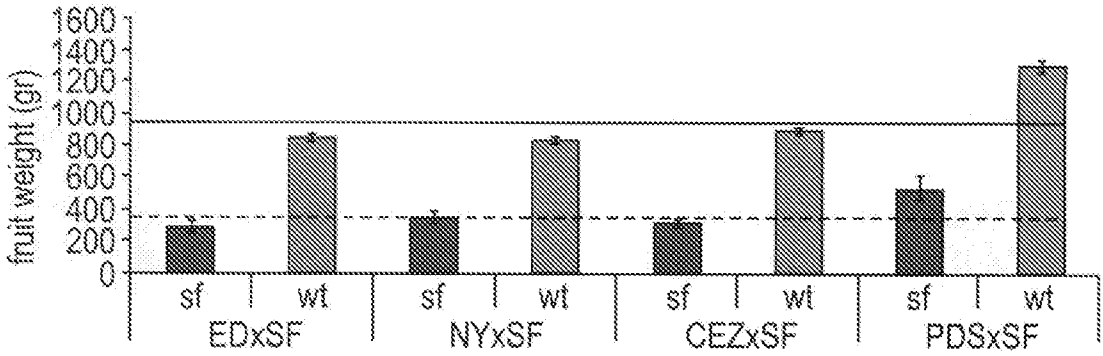
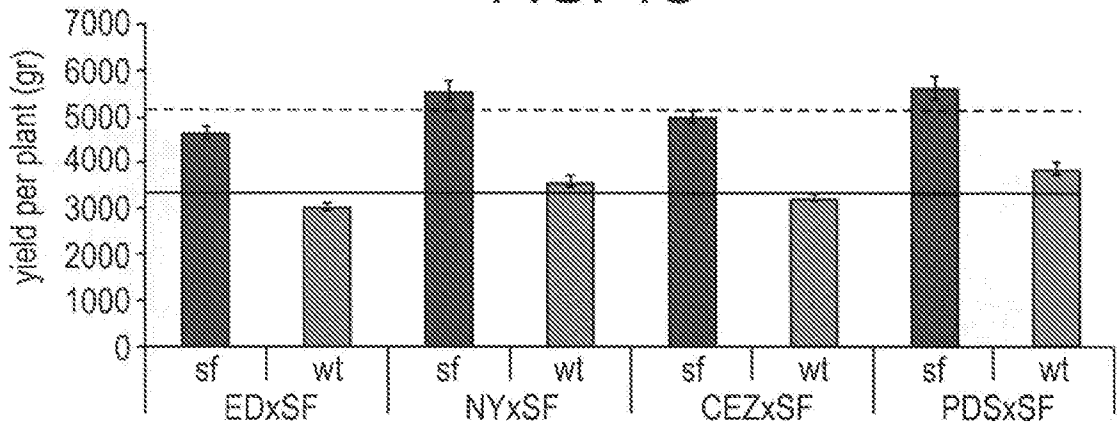


FIG. 4C



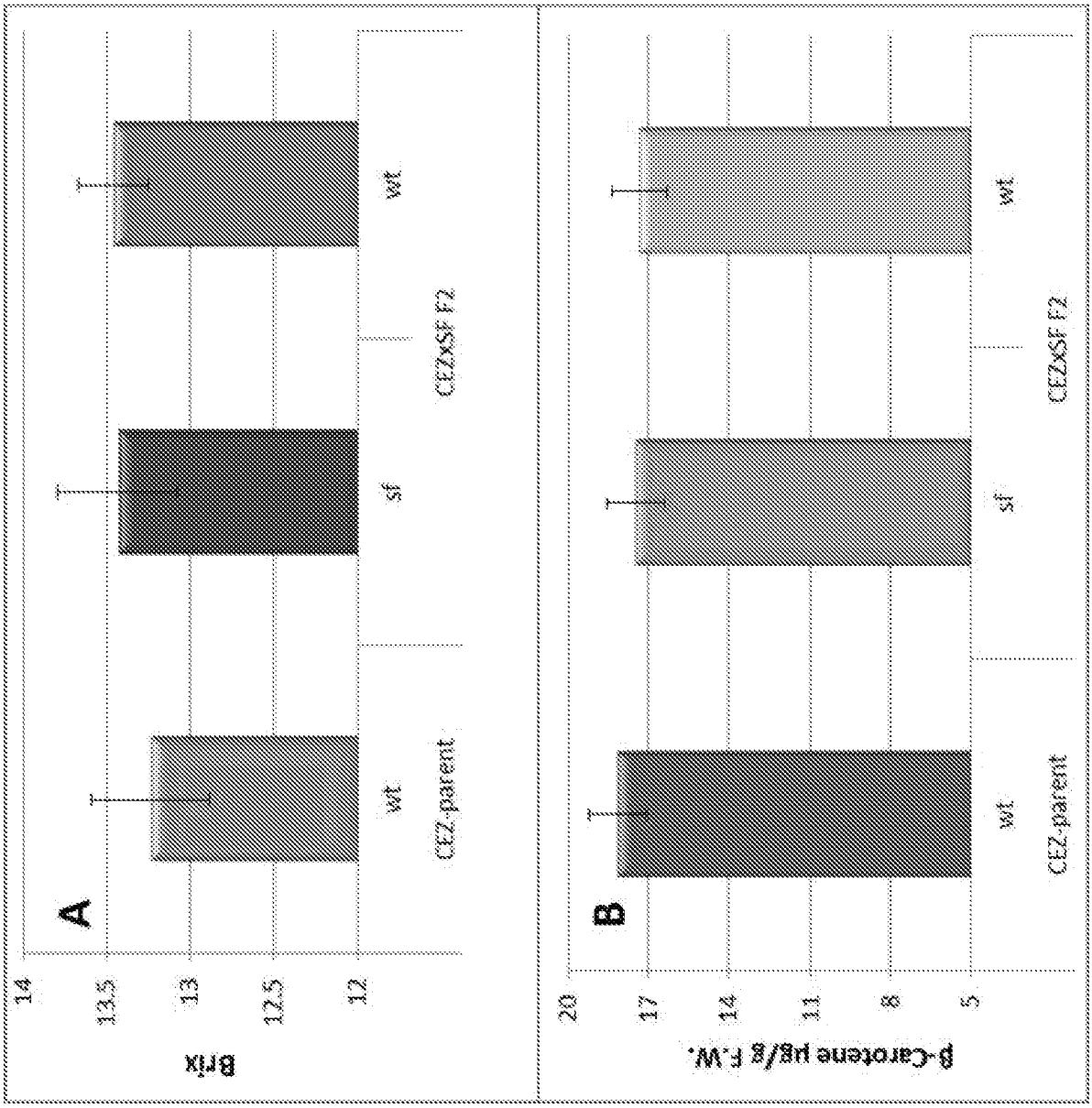

A
Brix
14
13.5
13
12.5
12
wt
sf
wt
CEZ-parent
CEZxSF F2
B
β-Carotene µg/g F.W.
20
17
14
11
8
5
wt
sf
wt
CEZ-parent
CEZxSF F2

FIG. 5A

FIG. 5B

FIG. 6

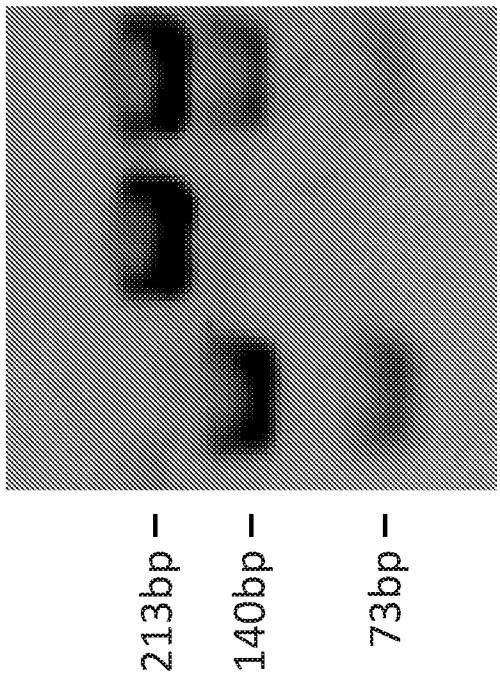


FIG. 7

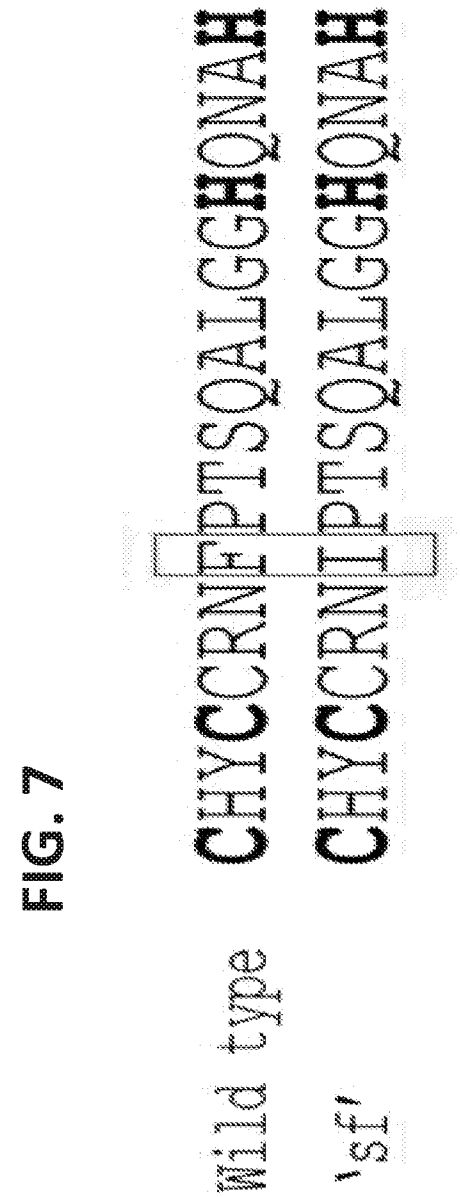

Wild type
CHYCCRNEPTSQALGGHQNAH
'sf'
CHYCCRNIPTSQALGGHQNAH

FIG. 8A

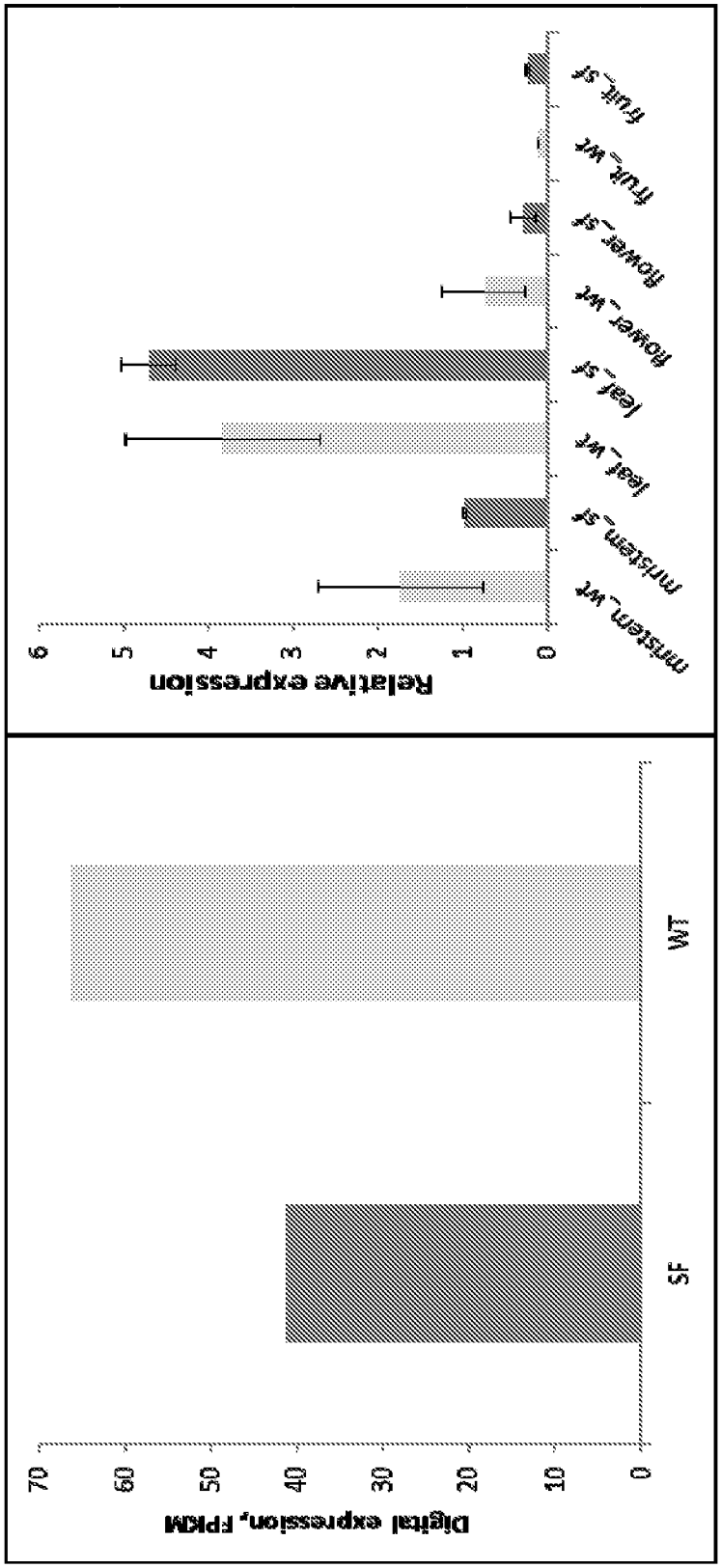

Digital expression, FPKM
0
10
20
30
40
50
60
70
WT
SF

FIG. 8B

Relative expression
0
1
2
3
4
5
6
mristem_wt
mristem_sf
leaf_wt
leaf_sf
flower_wt
flower_sf
fruit_wt
fruit_sf

FIG. 9

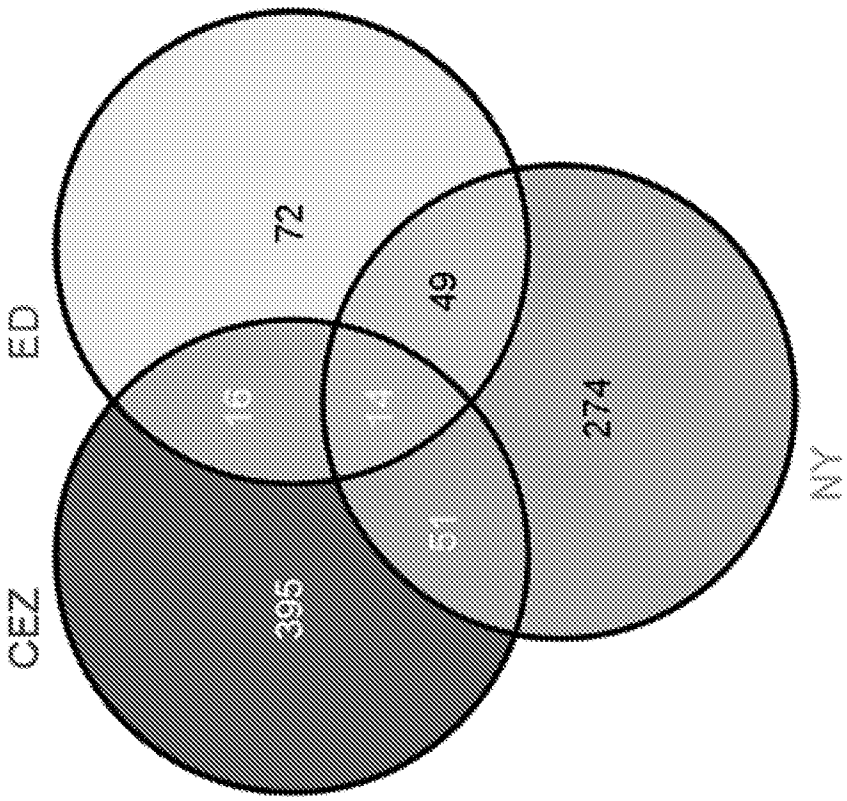


FIG. 10A

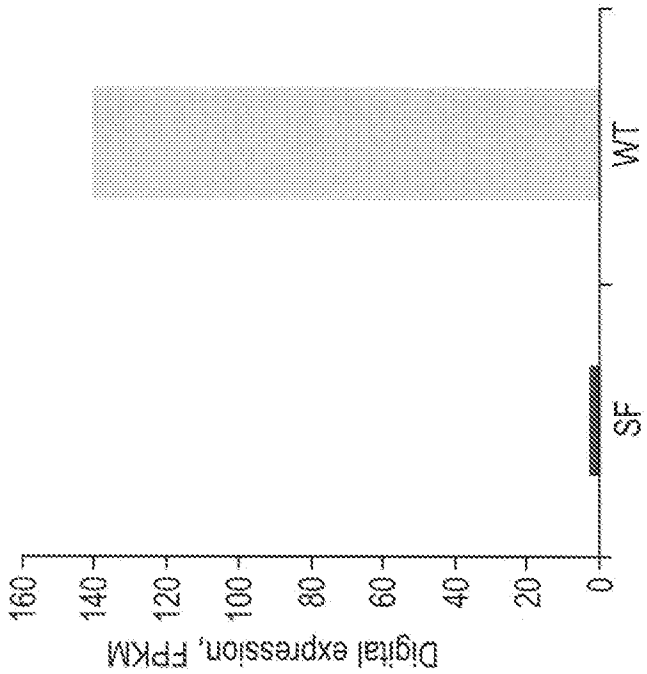
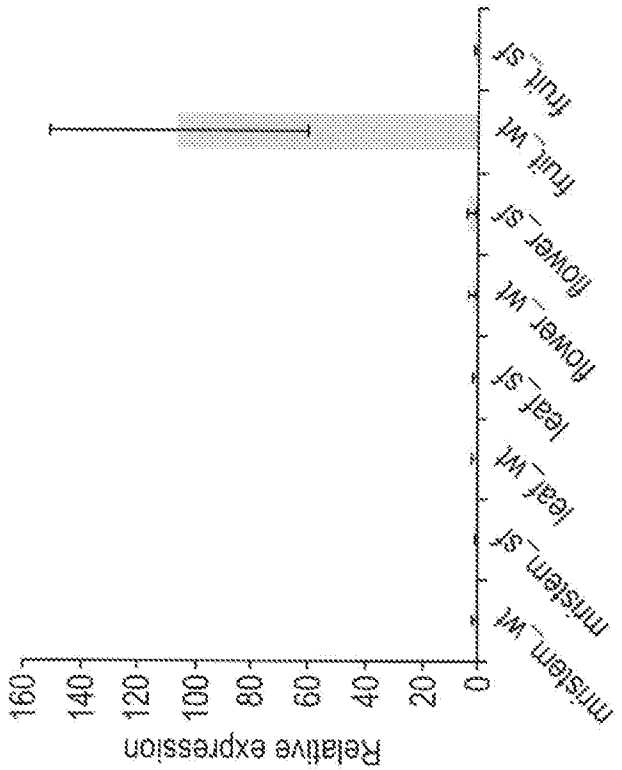


FIG. 10B



>MELO3C009603

TCTTCCTTTTCTTTCTTCTTCTTCTTCTTCTTCTGCCGTAACCGATTGATATTAATTCTCTCCTCGGTGG
CGGCATAGGTTTTTCAAAACCCCACGGCCCCAACACCACCATATACACTTTCATGGACAAGAGTACCAGT
GAACGAGAGACTCATGATTTCATGAACGTCGAGTCTTTCTCTCAACTTCCCTTCATCCGTCCTGCACCAA
AAGAAAAGGGCATTAGGCTTTTCGGGATAGAATTTGGAAGTCGAAACGCTGCTACCGTCTCCGCTTCTTC
CATTGAGGAATCAGAGTCGGGTGAAACTGTTATTGCATGCGAAGATGCGAAAGAAAACAACAACGATGGC
AACAACAACAACGGCGGAGAAAGTAGCCGGAGATTTGAATGTCATTACTGTTGTAGAAATTTCCCTACTT
CTCAAGCCTTAGGAGGACACCAAAATGCTCACAAAAGAGAGCGCCAACATGCAAAAAGGGCTCACCTTCA
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AACTACCAACGCCTAGGCGCTACTCACCTTAATAATTACCCTTCTTGGAATAGAAATTCGCCAGCAGCAA
CGGCTGCTGCTGCTGCTGCTACAAGATTTTACGGTAGCTCTGGTGGGCAGTATTCGTCGGCAGCAACCGC
GACGCCTATAAACGGGAGCCCGTTGGCGATGTGGAGAATCTCGGCCGTTCAAAATAGTAATGTACCGTCA
TCGTTTGGCGGTCGGGAGCGGTCGTCTCTACACCCGTTGCCGTTGTTTTCCGGAGATGAGATGATGAAAG
GCGCTGGTGGTGGTGGTGGCACCGCCGTTAGTGCCGGTGGGTCCGGCGGGTCTCATCAGACTGGCCGGTT
TGTTTACGAGGCAAAAACAGCGGACCAAGTGAGTTTGGATCTTCATCTGTAATAATTTAATTAATTGACA
AATTTCTAAATTGACATGAATTTTCCTATAGAGATGATGGAAATTTTTTCATCTTCTTTTATATGCATTT
GCAAAAGAGAGAAAAAAAAAAGAAAGAGACAAAATTCCGGCCGCTGGAGACGGATTTAATAAAACACTAA
TCGATCAAATATTTGTTTCTTC

FIG. 11A

MDKSTSERETHDFMNVESFSQLPFIRPAPKEKGIRLFGIEFGSRNAATVSASSIEESESGETVIACEDAK
ENNNDGNNNNGGESSRRFECRYCCRNIFTSQALGGHQNAHKRERQHAKRAHLQSNAAAMVHGIGPFSDAA
HVYGLMNYQRLGATHLNNYPSWNRNSPAATAAAAAATRFYGSSGGQYSSAATATPINGSPLAMWRISAVQ
NSNVPSSFGGRERSSLHPLPLFSGDEMMKGAGGGGGTAVSAGGSGGSHQTGRFVYEAKTADQVSLDLHL

FIG. 11B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL2015/050252

A. CLASSIFICATION OF SUBJECT MATTER

IPC (2015.01) A01H 3/00, A01H 5/10, C07K 14/415, C12N 15/29

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)

IPC (2015.01) A01H 3/00, A01H 5/10, C07K 14/415, C12N 15/29

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

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

Databases consulted: NCBI, Google Patents, CAPLUS, BIOSIS, EMBASE, REGISTRY, USGENE, DGENE, Google Scholar, PatBase
Search terms used: (Cucumis melo or muskmelon or cantaloupe) and (seedless or parthenocarpy) and (fruit set or fruit number or fruit load) and increase and (zinc finger or C2H2) and (NAA or BA or IAA or CPPU or GA or 4-CPA); Seq ID NO:8

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Valantin, M., Gary, C., Vaissiere, B. E., & Frossard, J. S. (1999). Effect of fruit load on partitioning of dry matter and energy in cantaloupe (Cucumis melo L.) .Annals of Botany, Vol. 84, no. 2, pages 173-181. 31 Dec 1999 (1999/12/31) pages 173-175	1-26,29-31
A	Grumet, R., Katzir, N. L., Little, H. A., Portnoy, V., & Burger, Y. (2007). New insights into reproductive development in melon (Cucumis melo L.). Int J Plant Dev Biol, Vol. 1, pages 253-264. 31 Dec 2007 (2007/12/31) pages 256-257	1-31
A	Hayata, Y., Niimi, Y., Inoue, K., & Kondo, S. (2000). CPPU and BA, with and without pollination, affect set, growth, and quality of muskmelon fruit. HortScience, Vol. 35, no. 5, pages 868-870. 31 Aug 2000 (2000/08/31) whole document	1-26,29-31

[X] 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	Date of mailing of the international search report
08 Jun 2015	08 Jun 2015

Name and mailing address of the ISA:	Authorized officer
Israel Patent Office Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel Facsimile No. 972-2-5651616	MAZEL Alexander Telephone No. 972-2-5651716

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL2015/050252

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	Database NCBI [online] 25 June 2014 (2014/06/2) "PREDICTED: zinc finger protein 8-like [Cucumis melo]" Database accession number: XP_008443018. 25 Jun 2014 (2014/06/25) whole document	27,28
A	Levi, A., Davis, A., Hernandez, A., Wechter, P., Thimmapuram, J., Trebitsh, T., & King, S. (2006). Genes expressed during the development and ripening of watermelon fruit. Plant cell reports, Vol. 25, no. 11, pages 1233-1245. 27 Jun 2006 (2006/06/27) abstract, table 1 (page 1241), page 1244 (left column)	2-31

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL2015/050252

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

a. [X] forming part of the international application as filed:

[X] in the form of an Annex C/ST.25 text file.

[ ] on paper or in the form of an image file.

b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

[ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

[ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments: