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(54) **RESTORER FACTOR FOR THE BACCATUM CYTOPLASMIC MALE STERILITY SYSTEM IN PEPPER**

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(57) **ABSTRACT**

The present disclosure provides *Capsicum annuum* BCMS plants comprising a male fertility restoration locus. Such plants comprise novel introgressed genomic regions associated with male fertility from *Capsicum annuum* on chromosome 6. In certain aspects, compositions and methods for producing, breeding, identifying, and selecting plants or germplasm with a male fertility phenotype are provided.

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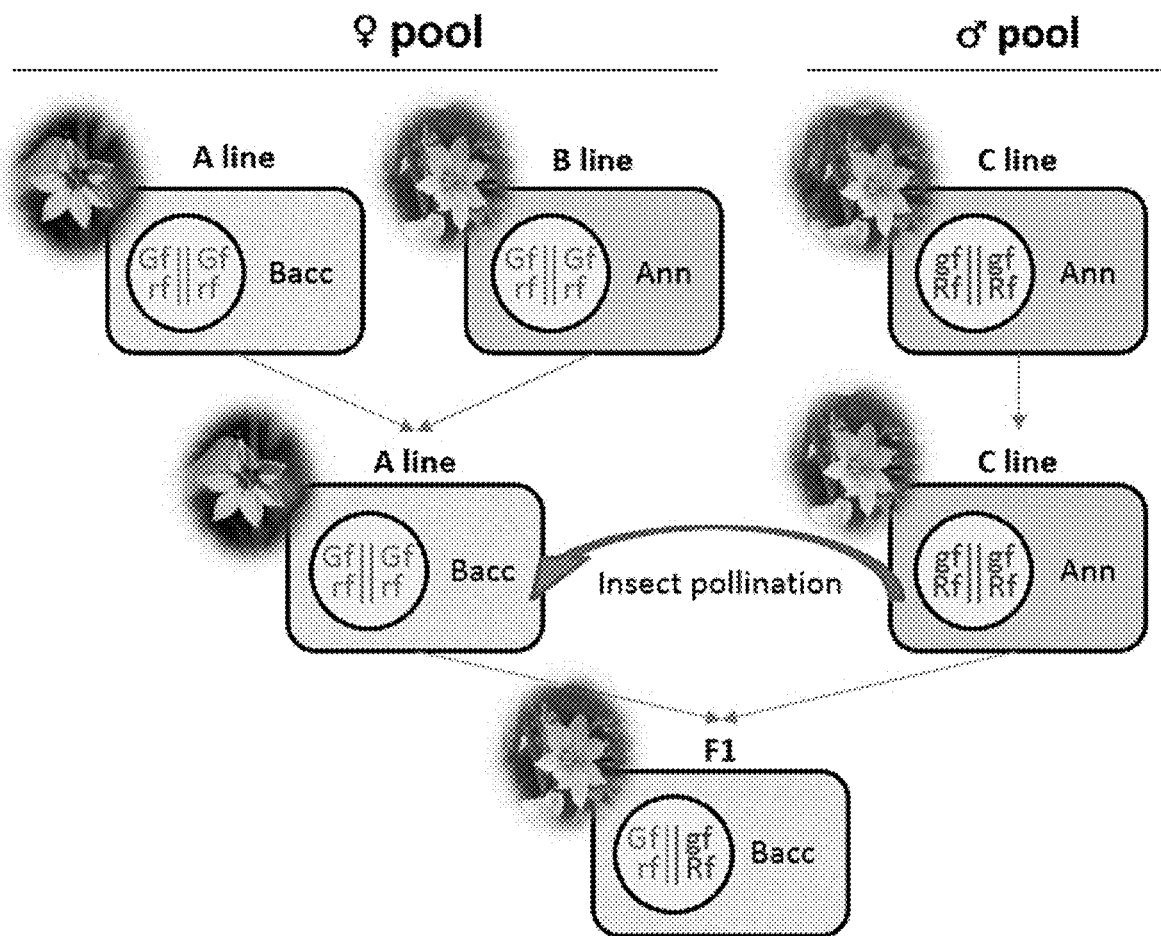
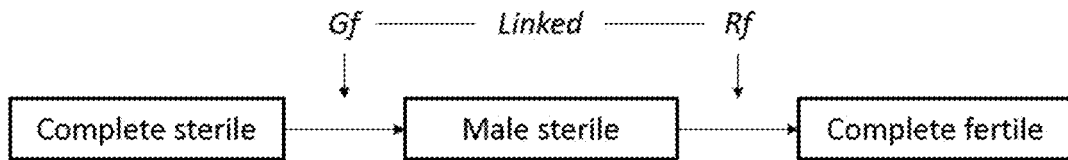


FIG. 1



gfrf = annuum restoration haplotype *gfrf* = annuum non-restoration haplotype
Gfrf = baccatum haplotype

Possible genotypes	Female fertility	Male fertility	Flower class
<i>Gfrf/Gfrf</i>	Fertile	Sterile	Male sterile
<i>Gfrf/gfrf</i>	Fertile	Sterile	Male sterile
<i>Gfrf/gfRf</i>	Fertile	Fertile	Complete fertile
<i>gfrf/gfrf</i>	Sterile	Sterile	Complete sterile
<i>gfrf/gfRf</i>	Sterile	Sterile	Complete sterile
<i>gfRf/gfRf</i>	Sterile	Sterile	Complete sterile

FIG. 2

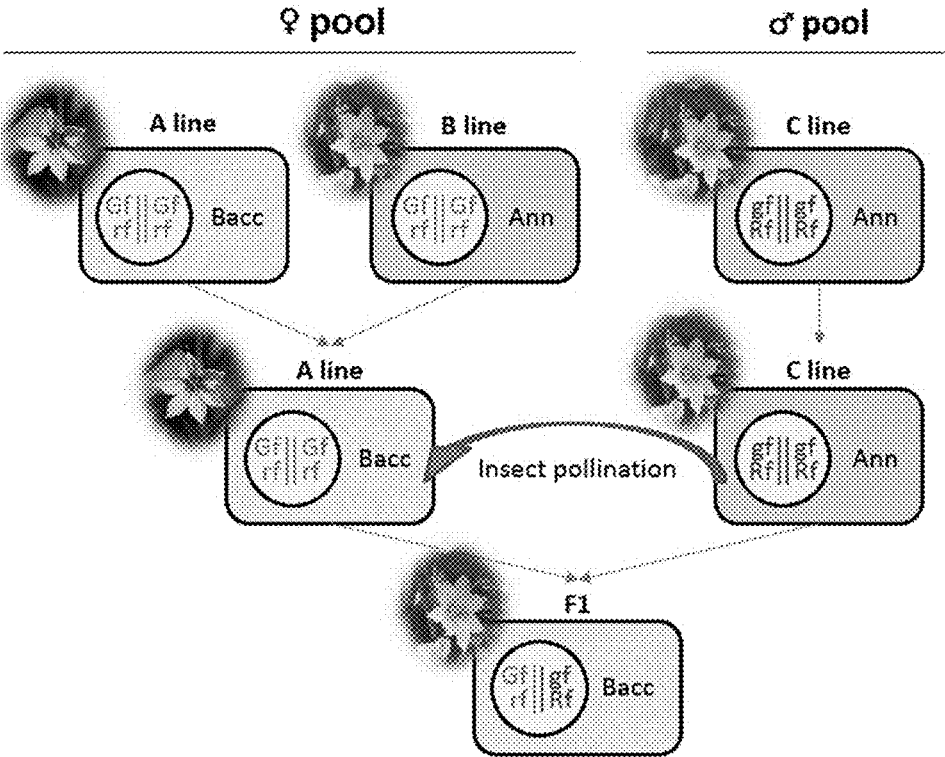


FIG. 3

Parental cross: BCMS Donor Line 1: (S)*Gfrf/gfrf* x 'Flame Fountain' (N)*gfRf/gfRf*

→ F₁: ½(S)*Gfrf/gfRf* (completely fertile) + ½(S)*gfrf/gfRf* (completely sterile)

→ F₂: ½(S)*Gfrf/gfRf* (completely fertile) + ¼(S)*Gfrf/Gfrf* (male sterile)

→ ...

→ F_n: ½(S)*Gfrf/gfRf* (completely fertile) + ½(S)*Gfrf/Gfrf* (male sterile)

* note that (S)*Gfrf/Gfrf* plants cannot be selfed and do not contribute to next selfing generation.

RESTORER FACTOR FOR THE BACCATUM CYTOPLASMIC MALE STERILITY SYSTEM IN PEPPER

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of U.S. Provisional Appl. Ser. No. 62/690,728, filed Jun. 27, 2018, the disclosure of which is hereby incorporated by reference in its entirety.

INCORPORATION OF SEQUENCE LISTING

[0002] The sequence listing that is contained in the file named "SEMB035US_ST25.txt", which is 50.8 kilobytes as measured in Microsoft Windows operating system and was created on Jun. 25, 2019, is filed electronically herewith and incorporated herein by reference.

FIELD OF THE INVENTION

[0003] The present invention relates to the field of agriculture and more specifically to methods and compositions for producing pepper plants exhibiting restored male fertility.

BACKGROUND OF THE INVENTION

[0004] The goal of vegetable breeding is to combine various desirable traits in a single variety/hybrid. Production of hybrid peppers may be carried out by hand-emasculation or by using male sterility. A number of male sterility systems have been identified for use in pepper production, however each system has limitations. Efforts to overcome these limitations are hindered by a lack of specific markers linked to the alleles associated with male sterility phenotypes. The use of marker-assisted selection (MAS) in plant breeding methods has made it possible to select plants based on genetic markers linked to traits of interest. However, accurate markers for identifying or tracking desirable traits in plants are frequently unavailable even if a gene associated with the trait has been characterized. These difficulties are further complicated by factors such as polygenic or quantitative inheritance, epistasis and an often incomplete understanding of the genetic background underlying expression of a desired phenotype.

SUMMARY OF THE INVENTION

[0005] In one aspect, the present invention provides a *Capsicum annuum* plant comprising a *Capsicum baccatum* cytoplasm and comprising a chromosomal segment on chromosome 6 that confers male fertility in a male sterile *Capsicum annuum* plant comprising a *Capsicum baccatum* cytoplasm relative to a plant lacking said chromosomal segment. In some embodiments, the chromosomal segment is flanked by Marker M29 (SEQ ID NO: 29) and a marker selected from the group consisting of Marker M14 (SEQ ID NO: 14), Marker M15 (SEQ ID NO: 15), Marker M16 (SEQ ID NO: 16), and Marker M17 (SEQ ID NO: 17) in said plant. In further embodiments, the chromosomal segment comprises a marker locus selected from the group consisting of Marker M18 (SEQ ID NO: 18), Marker M19 (SEQ ID NO: 19), Marker M20 (SEQ ID NO: 20), Marker M21 (SEQ ID NO: 21), Marker M22 (SEQ ID NO: 22), Marker M23 (SEQ ID NO: 23), Marker M24 (SEQ ID NO: 24), Marker M25

(SEQ ID NO: 25), Marker M26 (SEQ ID NO: 26), Marker M27 (SEQ ID NO: 27), and Marker M28 (SEQ ID NO: 28) on chromosome 6. In some embodiments, the chromosomal segment is located between 26,405 bp and 213,924,156 bp on chromosome 6 of public pepper genome sequence Pepper CM334 v.1.55. In some embodiments, the chromosomal segment comprises the haplotype of variety Ganti, wherein a representative sample of seed of said variety has been deposited under NCIMB accession number 43055. In other embodiments, the chromosomal segment comprises the haplotype of variety Flame Fountain, wherein a representative sample of seed of said variety has been deposited under NCIMB accession number 43054.

[0006] The present invention also provides a seed that produces a *Capsicum annuum* plant comprising a *Capsicum baccatum* cytoplasm and comprising a chromosomal segment on chromosome 6 that confers male fertility in a male sterile *Capsicum annuum* plant comprising a *Capsicum baccatum* cytoplasm relative to a plant lacking said chromosomal segment.

[0007] Additionally, the present invention provides a plant part of a *Capsicum annuum* plant comprising a *Capsicum baccatum* cytoplasm and comprising a chromosomal segment on chromosome 6 that confers male fertility in a male sterile *Capsicum annuum* plant comprising a *Capsicum baccatum* cytoplasm relative to a plant lacking said chromosomal segment. In certain embodiments, the plant part is a cell, a seed, a root, a stem, a leaf, a flower, a fruit, or pollen.

[0008] The present invention also provides a *Capsicum annuum* plant comprising a *Capsicum baccatum* cytoplasm and comprising a chromosomal segment on chromosome 6 that confers male fertility in a male sterile *Capsicum annuum* plant comprising a *Capsicum baccatum* cytoplasm relative to a plant lacking said chromosomal segment, wherein the plant is a sweet pepper variety.

[0009] Additionally, the present invention provides a *Capsicum annuum* plant comprising a *Capsicum baccatum* cytoplasm and comprising a chromosomal segment on chromosome 6 that confers male fertility in a male sterile *Capsicum annuum* plant comprising a *Capsicum baccatum* cytoplasm relative to a plant lacking said chromosomal segment, wherein the plant has a blocky type fruit shape.

[0010] The present invention also provides a *Capsicum annuum* plant comprising a *Capsicum baccatum* cytoplasm and comprising a chromosomal segment on chromosome 6 that confers male fertility in a male sterile *Capsicum annuum* plant comprising a *Capsicum baccatum* cytoplasm relative to a plant lacking said chromosomal segment, wherein said plant further comprises a chromosomal segment from *Capsicum baccatum* on chromosome 6 that confers uniform female fertility in a male sterile *Capsicum annuum* plant comprising a *Capsicum baccatum* cytoplasm relative to a plant lacking said chromosomal segment, wherein said chromosomal segment from *Capsicum baccatum* is flanked by Marker A12 (SEQ ID NO: 35) and Marker A35 (SEQ ID NO: 36) in said plant.

[0011] In another aspect, the present invention provides a method for producing a *Capsicum annuum* plant that confers male fertility in a male sterile *Capsicum annuum* plant comprising a *Capsicum baccatum* cytoplasm, comprising introgressing into said plant a chromosomal segment on chromosome 6 that confers male fertility in a male sterile *Capsicum annuum* plant comprising a *Capsicum baccatum* cytoplasm relative to a plant lacking said chromosomal

segment. In some embodiments, the introgressing comprises crossing a plant comprising said chromosomal segment with itself or with a second *Capsicum annuum* plant of a different genotype to produce one or more progeny plants, and selecting a progeny plant comprising said chromosomal segment. In further embodiments, the selecting a progeny plant comprises detecting at least one allele flanked by Marker M29 (SEQ ID NO: 29) and a marker selected from the group consisting of Marker M14 (SEQ ID NO: 14), Marker M15 (SEQ ID NO: 15), Marker M16 (SEQ ID NO: 16), and Marker M17 (SEQ ID NO: 17) on chromosome 6. In other embodiments, the progeny plant is an F₂-F₆ progeny plant. In particular embodiments, the crossing comprises backcrossing, which in certain embodiments comprises from 2-7 generations of backcrosses.

[0012] The present invention also provides a *Capsicum annuum* plant produced by a method comprising introgressing into said plant a chromosomal segment on chromosome 6 that confers male fertility in a male sterile *Capsicum annuum* plant comprising a *Capsicum baccatum* cytoplasm relative to a plant lacking said chromosomal segment. Thus, the present invention also provides a method of producing food or feed comprising obtaining a *Capsicum annuum* plant comprising a *Capsicum baccatum* cytoplasm and comprising a chromosomal segment on chromosome 6 that confers male fertility in a male sterile *Capsicum annuum* plant comprising a *Capsicum baccatum* cytoplasm relative to a plant lacking said chromosomal segment, or a part thereof, and producing said food or feed from said plant or part thereof.

[0013] In another aspect, the present invention provides a *Capsicum annuum* plant obtainable by a method comprising the step of introgressing into a plant a male fertility restoration locus allele for *Baccatum* cytoplasmic male sterility, wherein said male fertility restoration locus allele is defined as located in a chromosomal segment flanked by Marker M29 (SEQ ID NO: 29) and a marker selected from the group consisting of Marker M14 (SEQ ID NO: 14), Marker M15 (SEQ ID NO: 15), Marker M16 (SEQ ID NO: 16), and Marker M17 (SEQ ID NO: 17) on chromosome 6. In certain embodiments, the introgressing comprises backcrossing. In other embodiments, the introgressing comprises marker-assisted selection.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] FIG. 1: Shows an overview of the genetic model for the functioning of the *Baccatum* cytoplasmic male sterility (CMS) system. The phenotypic predictions are for plants with *Capsicum baccatum* cytoplasm.

[0015] FIG. 2: Shows a schematic of the hybrid production concept with CMS, based on *Capsicum baccatum* cytoplasm. The female pool comprises two lines that lack the male fertility restorer locus: the A-line, which is a male sterile line that serves as the female parent in the hybrid cross; and the B-line, which is a male fertile line that serves as the maintainer and enables crosses within the female breeding pool. The C-line serves as the male parent of the hybrid cross and generally is a normal *Capsicum annuum* line that lacks the Gf locus, but is fixed for the male fertility restorer locus allele Rf. "Bacc" indicates plants with a *Capsicum baccatum* cytoplasm, while "Ann" indicates plants with a *Capsicum annuum* cytoplasm.

[0016] FIG. 3: Shows a schematic of the genetic model for Gf and Rf transmission in pepper plants having a *Capsicum*

baccatum or a *Capsicum annuum* cytoplasm. The "(S)" denotes a *Capsicum baccatum* cytoplasm and the "(N)" denotes a *Capsicum annuum* cytoplasm. F_n progeny derived from restored F₁ plants (e.g. BCMS Donor Line 1×'Flame Fountain') segregate for 50% completely fertile and 50% male sterile plants.

DETAILED DESCRIPTION

[0017] Male sterility is used by breeders for two basic product concepts in a variety of crops. The first product concept is seedless fruit. Plants comprising the male sterility trait are crossed with plants comprising parthenocarp genes to produce hybrid seed. This hybrid seed produces plants bearing seedless fruit. Under normal circumstances, male sterile plants cannot set fruit in the absence of pollination. However, if the plant also contains parthenocarp genes, then fruit set occurs in the absence of pollination. In this product concept, it is possible to use different forms of male sterility without a restorer locus. However, only cytoplasmic male sterility will allow for production of seed from which 100% of the plants grown from this seed are sterile and bear seedless fruit. Using genic male sterility for this product concept requires an intermediate seedling selection step after showing the hybrid seed, followed by transplanting or grafting of the selected sterile plants. The second product concept is one where male sterility is used to easily develop hybrid seed. In the development of hybrid seed it is important to ensure genetic purity of a seed batch. This entails minimizing the number of seed that are the result of self-fertilization. Self-fertilization is prevented during seed production through physical removal of male sex organs in the flower before the flower opens, a process referred to as emasculation. This is a labor-intensive procedure that is not only costly, but also is not 100% effective. Genetic emasculation of the female line overcomes these limitations and ensures the genetic purity of the hybrid seed. However, successful hybrid production requires that the male sterility system used can be restored in the hybrid. Thus, the male parent of the hybrid will typically contain a dominant male fertility restorer locus. When a male parent comprising the restorer locus is crossed with the male sterile female parent, fully fertile hybrid plants will be produced. Given that resultant hybrid plants are heterozygous, it is essential that the restorer locus be dominant.

[0018] Genetic (or genic) male sterility (GMS) systems utilize male sterility loci that are often inherited in a recessive manner and encoded within the nuclear genome. An exception to this is, for example, a GMS system in rapeseed where both the sterility and sterility suppressor genes are dominant. A primary disadvantage with the genetic male sterility system is that only half of the progeny plants will be male sterile. A breeder would therefore have to select which plants are suitable for hybrid/seedless fruit production post-germination, resulting in at least half of the seedlings being discarded. A system that does not have this problem is the cytoplasmic male sterility (CMS) system. In this system, the male sterility loci are coded in the mitochondrial DNA and, in the absence of a nuclear male fertility restoration gene, 100% of the progeny plants are male sterile. In pepper, the Peterson's CMS system is widely used because a dominant male fertility restoration locus is available, making this system suitable for both hybrid production and the seedless fruit concept. The restorer locus for Peterson's CMS is often referred to as the CMS restorer locus or Rf. The CMS

restorer allele (Rf) was identified in pungent *Capsicum annuum* germplasm and has been mapped to a locus on the short arm of chromosome 6. Transferring a functioning restorer locus to sweet pepper types has proven difficult and remains the subject of much study and breeding efforts because the Peterson's CMS system is known to be unstable with respect to environmental conditions and genetic background.

[0019] An environmentally-stable alternative to the Peterson's CMS system is the *Baccatum* cytoplasmic male sterility (BCMS) system. This system was created by crossing a female *Capsicum baccatum* plant with a male *Capsicum annuum* plant. The resulting hybrid, which was obtained through a step of embryo rescue, contained a *Capsicum baccatum* cytoplasm and was male sterile. Through extensive backcrossing to the *Capsicum annuum* parent, the *Capsicum baccatum* genome was replaced with *Capsicum annuum* DNA. However, the BCMS system has some limitations. First, female sterility segregates within the population of BCMS lines and efforts to eliminate this negative trait through crossing and selection has not been successful. Second, there does not appear to be a dominant restorer locus for the BCMS, in the absence of a nuclear male fertility restoration gene.

[0020] The present inventors have found that the limitation of use of the BCMS system due to the lack of a dominant restorer locus can be overcome by use of a chromosomal segment from certain *Capsicum annuum* lines on the short arm of chromosome 6 that confers male fertility in BCMS pepper lines. This locus was identified in the varieties 'Ganti' (NCIMB 43055) and 'Flame Fountain' (NCIMB 43054) in a study of BCMS male fertility restoration using *Capsicum annuum* accessions. The variety 'Ganti' is a landrace from Hungary that is best described as a sweet Hungarian-white or Hungarian-wax type pepper. The variety 'Flame Fountain' is a hot Indian-type pepper that is characterized by a long and thin fruit that colors green to red. Surprisingly, although the novel male fertility restoration locus of the invention is located on the same chromosome as the CMS restorer allele used in the Peterson's CMS system, it was found that they are distinct, as crosses between BCMS plants and plants with the Peterson's CMS fertility restorer locus did not result in male fertility restoration. The invention therefore provides methods and compositions for restoring male fertility in BCMS pepper plants, as well as markers for tracking and identifying the novel chromosomal segment in plants during breeding. A non-limiting summary of useful markers is provided in Table 1. The chromosomal segment is located between markers M1 (SEQ ID NO: 1) and M34 (SEQ ID NO: 34), while markers M2 to M33 can also be used to select the chromosomal segment in subsequent germplasm. The specific selected markers used depends on the polymorphism between the male fertility locus donor ('Ganti' or 'Flame Fountain') and the non-donor parent (recurrent parent). Therefore, a combination of markers listed in Table 1 may be used for benefit in a first selection, while markers can be limited to a polymorphic subset in a given cross for further selections.

I. GENOMIC REGIONS, ALLELES, AND POLYMORPHISMS ASSOCIATED WITH MALE FERTILITY IN BCMS LINES

[0021] The inventors identified a novel chromosomal segment on chromosome 6 from *Capsicum annuum* that confers

male fertility in a *Baccatum* cytoplasmic male sterile plant, together with polymorphic nucleic acids and linked markers for tracking and introgressing the chromosomal segment into potentially any variety during plant breeding. The newly identified chromosomal segment on chromosome 6 from donor line 'Ganti' covers a region of 31.7 cM and is flanked by marker M1, a SNP change [A/G] at 213,924,156 bp on genome sequence version 1.55 of pepper line CM334, which can be found at solgenomics.net, and marker M34, a SNP change [G/C] at 21,133,217 bp. Interstitial markers, such as M2, a SNP change [C/T] at 213,907,080 bp, M3, a SNP change [A/G] at 213,907,920 bp, M4, a SNP change [T/C] at 427,239 bp, M6, a SNP change [T/C] at 87,022 bp, M8, a SNP change [C/T] at 89,795 bp, M12, a SNP change [A/C] at 3,009,771 bp, M13, a SNP change [C/T] at 2,999,718 bp, M14, a SNP change [G/T] at 3,504,248 bp, M18, a SNP change [A/G] at 3,475,770 bp, M19, a SNP change [G/T] at 3,422,765 bp, M20, a SNP change [A/T] at 4,276,008 bp, M23, a SNP change [A/G] at 70,994,266 bp, M27, a SNP change [T/G] at 8,522,176 bp, M28, a SNP change [A/G] at 9,799,932 bp, and M33, a SNP change [C/T] at 11,592,142 bp, can be used in any possible combination as flanking markers to select for the restorer locus on chromosome 6 from Ganti or another donor line.

[0022] The newly identified chromosomal segment on chromosome 6 from donor line 'Flame Fountain' covers a region of 24.9 cM and is flanked by marker M5, a SNP change [A/G] at 428,143 bp on genome sequence version 1.55 of pepper line CM334, which can be found at solgenomics.net, and marker M33, a SNP change [C/T] at 11,592,142 bp. Interstitial markers, such as M7, a SNP change [C/T] at 125,861 bp, M8, a SNP change [C/T] at 89,795 bp, M11, a SNP change [A/G] at 3,055,268 bp, M12, a SNP change [A/C] at 3,009,771 bp, M13, a SNP change [C/T] at 2,999,718 bp, M14, a SNP change [G/T] at 3,504,248 bp, M15, a SNP change [C/A] at 3,500,133 bp, M16, a SNP change [A/G] at 3,505,583 bp, M17, a SNP change [C/T] at 3,308,938 bp, M18, a SNP change [A/G] at 3,475,770 bp, M19, a SNP change [G/T] at 3,422,765 bp, M20, a SNP change [A/T] at 4,276,008 bp, M25, a SNP change [G/A] at 6,240,565 bp, and M26, a SNP change [C/T] at 6,241,544 bp, can be used in addition to the flanking markers to select for the restorer locus on chromosome 6 from Flame Fountain or another donor line.

[0023] Additionally, interstitial markers, such as M5, a SNP change [A/G] at 428,143 bp, M7, a SNP change [C/T] at 125,861 bp, M9, a SNP change [T/C] at 386,489 bp, M10, a SNP change [C/A] at 26,405 bp, M21, a SNP change [G/A] at 4,240,789 bp, M22, a SNP change [G/C] at 4,245,699 bp, M24, a SNP change [A/T] at 4,240,551 bp, M29, a SNP change [C/T] at 10,670,362 bp, M30, a SNP change [T/C] at 10,664,163 bp, M31, a SNP change [G/T] at 10,664,630 bp, and M32, a SNP change [T/C] at 11,108,817 bp, can be used in addition to the flanking markers to select for the restorer locus on chromosome 6 from either donor line 'Ganti' or 'Flame Fountain' or another donor line. Thus, the present disclosure provides a *Capsicum annuum* plant comprising a chromosomal segment on chromosome 6 of *Capsicum annuum* flanked by markers M1 and M34 that confers male fertility to BCMS pepper plants. In certain embodiments, one or both of the flanking markers are interstitial markers between M1 and M34, such as markers M2, M3, M4, M5, M6, M7, M8, M9, M10, M11, M12, M13, M14, M15, M16, M17, M18, M19, M20, M21, M22, M23, M24, M25, M26,

M27, M28, M29, M30, M31, M32, or M33 and comprise an allele from ‘Ganti’ or ‘Flame Fountain’ at said marker(s). In some embodiments, the chromosomal segment on chromosome 6 of *Capsicum annuum* comprising a plurality of the markers listed in Table 1, including any possible combination thereof. In some embodiments, the chromosomal segment on chromosome 6 of *Capsicum annuum* restoring fertility in plants with *Capsicum baccatum* cytoplasmic male sterility from ‘Ganti’ is flanked by markers M14 and M29, wherein interstitial markers M15, M16, M17, M18, M19, M20, M21, M22, M23, M24, M25, M26, M27, M28 or any combination thereof are used to select this region. In some embodiments, the chromosomal segment on chromosome 6 of *Capsicum annuum* restoring fertility in plants with *Capsicum baccatum* cytoplasmic male sterility from ‘Flame Fountain’ is flanked by markers M17 and M29, wherein interstitial markers M18, M19, M20, M21, M22, M23, M24, M25, M26, M27, M28 or any combination thereof are used to select this region.

II. INTROGRESSION OF GENOMIC REGIONS ASSOCIATED WITH RESTORATION OF MALE FERTILITY

[0024] Marker-assisted introgression involves the transfer of a chromosomal region defined by one or more markers from a first genetic background to a second. Offspring of a cross that contain the introgressed genomic region can be identified by the combination of markers characteristic of the desired introgressed genomic region from a first genetic background and both linked and unlinked markers characteristic of the second genetic background.

[0025] The invention provides a *Capsicum annuum* plant comprising an introgressed allele on chromosome 6 that confers male fertility in a pepper plant with BCMS, wherein said allele is located between marker M2 and marker M33, or preferably between marker M14 and marker M29. In addition, the invention provides a *Capsicum annuum* plant with a cytoplasm from *Capsicum baccatum*, in which male fertility is restored due to the presence of a male fertility restoration allele on chromosome 6, wherein said allele is located between marker M2 and marker M33, or preferably between marker M14 and marker M29. In some embodiments, the plant is a variety selected from the group consisting of Anaheim, Ancho/Poblano, Asian Long Slim, Asian Short, Blocky or Bell, Capia, Cascabel, Cayenne, Chiltepins or Small Hots, Corno di Toro, Cubanelle, ‘Fresno Chili’, Hungarian Wax/Banana/Hungarian White, Jalapeno, Ornamental, Pasilla, Pimiento, Santa Fe Grande, Serrano, and Waxy peppers. In certain embodiments, the plant has a blocky type fruit shape, a $\frac{3}{4}$ long type fruit shape, or a half long type fruit shape. For example, the fruit of the plant may have a length to width ratio less than 2.5:1, such as a length to width ratio of less than 2:1, or a length to width ratio of between 0.8 to 1.2. As used herein, blocky type pepper refers to a pepper wherein the length of the fruit is about the same as the width of the fruit. For example, the length of the fruit is about 0.8, about 0.9, about 1.0, about 1.1, or less than 1.2 of the width of the fruit. As used herein, $\frac{3}{4}$ long type pepper, often known as lamuyo, refers to a pepper wherein the length of the fruit is more than about 1.5 of the width of the fruit. These peppers can have a variety of different colors, for example white, purple, and green at the immature stage, and for example red, yellow, green, orange, and brown at the mature fruit stage. It is also well-known that definitions vary

regionally. For example, in the United States peppers with a length to width ratio of 1.2 to 1.4 are often referred to as “deep blocky”, while the terms half long and lamuyo are used interchangeably for sweet peppers with a length to width ratio over 1.4.

[0026] In certain embodiments, the genomic regions identified herein may be introgressed from any *Capsicum annuum* type into any *Capsicum annuum* type. Types of *Capsicum annuum* include, but are not limited to Anaheim, Ancho/Poblano, Asian long slim, Asian short, Blocky or Bell, Capia, Cascabel, Cayenne, Chiltepins or Small Hots, Corno di Toro, Cubanelle, ‘Fresno Chili’, Jalapeno, Ornamental, Pasilla, Pimiento, Santa Fe Grande, Serrano, and Waxy peppers, including Hungarian wax/Banana/Hungarian white.

[0027] In certain embodiments, the identified genomic regions are introduced into a jalapeno variety. Pepper fruit shapes are well-known to those skilled in the art of pepper breeding. Jalapeno peppers are a type of *Capsicum annuum* that have a characteristic fruit shape. Jalapeno fruits are typically bullet-shaped and have a length to width ratio of about 2.5 to 1. For example, a fruit having a length of about 10 cm would be expected to be about 4 cm wide. The fruit typically has thick walls of about 5-6 mm and the dry matter content is normally around 7%. The fruit of most plants develops from a medium green at the immature stage to red at the mature stage. As a commercial product, the fruits are harvested at the green stage. The pungency of jalapeno peppers varies from 0 units to over 5000 units on the Scoville scale.

[0028] The present invention provides novel markers for identifying and tracking introgression of one or more of the genomic regions from a donor plant comprising male fertility restoration alleles into *Baccatum* cytoplasmic male sterility lines. The invention further provides markers for identifying and tracking the novel introgressions disclosed herein during plant breeding, including the markers set forth in Table 1.

[0029] Markers within or linked to any of the genomic intervals of the present invention can be used in a variety of breeding efforts that include introgression of genomic regions associated with male fertility into a desired genetic background. For example, a marker within 30 cM, 25 cM, 20 cM, 16 cM, 15 cM, 10 cM, 5 cM, 2 cM, or 1 cM of a marker associated with male fertility described herein can be used for marker-assisted introgression of genomic regions associated with a male fertility phenotype.

[0030] Pepper plants comprising one or more introgressed regions associated with a desired phenotype wherein at least 10%, 25%, 50%, 75%, 90%, or 99% of the remaining genomic sequences carry markers characteristic of the germplasm are also provided. Pepper plants comprising an introgressed region comprising regions closely linked to or adjacent to the genomic regions and markers provided herein and associated with a male fertility phenotype are also provided.

III. DEVELOPMENT OF PEPPER PLANTS THAT PROVIDE MALE FERTILITY

[0031] For most breeding objectives, commercial breeders work within germplasm that is “cultivated type” or “elite.” This germplasm is easier to breed because it generally performs well when evaluated for horticultural performance. For example, *Capsicum annuum* is an agronomically elite,

cultivated pepper adapted to commercial use. However, the performance advantage a cultivated germplasm provides can be offset by a lack of allelic diversity. Breeders generally accept this tradeoff because progress is faster when working with cultivated material than when breeding with genetically diverse sources.

[0032] In contrast, when a breeder makes either intraspecific crosses, or interspecific crosses, a converse tradeoff occurs. In these examples, a breeder typically crosses germplasm of an economically important species with a non-cultivated or commercially unacceptable species. The breeder can gain access to novel alleles from the non-cultivated species, but may have to overcome genetic drag or interspecific hybridization barriers associated with such crosses. Because of the difficulty with this breeding strategy, this approach often fails because of fertility and fecundity problems. The difficulty with this breeding approach extends to many crops, and is exemplified with an important disease resistant phenotype that was first described in tomato in 1944 (Smith, *Proc. Am. Soc. Hort. Sci.* 44:413-16). In this cross, a nematode disease resistance was transferred from *L. peruvianum* (PI128657) into a cultivated tomato. Despite intensive breeding, it was not until the mid-1970's before breeders could overcome the genetic drag and release successful lines carrying this trait. Indeed, even today, tomato breeders deliver this disease resistance gene to a hybrid variety from only one parent. This allows the remaining genetic drag to be masked. The inventiveness of succeeding in this breeding approach has been recognized by the USPTO (U.S. Pat. Nos. 6,414,226, 6,096,944, 5,866,764, and 6,639,132).

[0033] The process of introgressing desirable genes from one species into another while avoiding problems with linkage drag or low heritability is a long and often arduous process. Success in deploying alleles derived from related species therefore strongly depends on minimal or truncated introgressions that lack detrimental effects and reliable marker assays that replace phenotypic screens. Success is further defined by simplifying genetics for key attributes to allow focus on genetic gain for quantitative traits. Moreover, the process of introgressing genomic regions from non-cultivated lines or different species can be greatly facilitated by the availability of informative markers.

[0034] One of skill in the art would therefore understand that the alleles, polymorphisms, and markers provided by the invention allow the tracking and introduction of any of the genomic regions identified herein into any genetic background. In addition, the genomic regions associated with male fertility disclosed herein can be introgressed from one genotype to another and tracked phenotypically or genetically. Thus, Applicants' discovery of accurate markers associated with male fertility restoration locus will facilitate the development of pepper plants having beneficial phenotypes. For example, plants and seeds can be genotyped using the markers of the present invention in order to develop varieties comprising desired male fertility. Moreover, marker-assisted selection (MAS) allows identification of plants which are homozygous or heterozygous for the desired introgression.

[0035] Meiotic recombination is essential for plant breeding because it enables the transfer of favorable alleles across genetic backgrounds, the removal of deleterious genomic fragments, and pyramiding traits that are genetically tightly linked. In the absence of accurate markers, limited recombination forces breeders to enlarge segregating populations

for progeny screens. Moreover, phenotypic evaluation is time-consuming, resource-intensive and not reproducible in every environment. The markers provided by the invention offer an effective alternative and therefore represent a significant advance in the art.

[0036] Phenotypic evaluation of large populations is time-consuming, resource-intensive and not reproducible in every environment. Marker-assisted selection offers a feasible alternative. Molecular assays designed to detect unique polymorphisms, such as SNPs, are versatile. However, they may fail to discriminate alleles within and among pepper species in a single assay. Structural rearrangements of chromosomes such as deletions impair hybridization and extension of synthetically labeled oligonucleotides. In the case of duplication events, multiple copies are amplified in a single reaction without distinction. The development and validation of accurate and highly predictive markers are therefore essential for successful MAS breeding programs.

[0037] Many desirable traits that are successfully introduced through introgression can also be introduced directly into a plant by the use of molecular techniques. One aspect of the invention includes plants with a genome that has been changed by any method using site-specific genome modification techniques. Techniques of site-specific genome modification include the use of enzymes such as, endonucleases, recombinases, transposases, helicases and any combination thereof. In one aspect, an endonuclease is selected from a meganuclease, a zinc-finger nuclease (ZFN), a transcription activator-like effector nucleases (TALEN), an Argonaute, and an RNA-guided nuclease, such as a CRISPR associated nuclease.

[0038] In another aspect, the endonuclease is a dCas9-recombinase fusion protein. As used herein, a "dCas9" refers to a Cas9 endonuclease protein with one or more amino acid mutations that result in a Cas9 protein without endonuclease activity, but retaining RNA-guided site-specific DNA binding. As used herein, a "dCas9-recombinase fusion protein" is a dCas9 with a protein fused to the dCas9 in such a manner that the recombinase is catalytically active on the DNA.

[0039] Non-limiting examples of recombinase include a tyrosine recombinase attached to a DNA recognition motif provided herein is selected from the group consisting of a Cre recombinase, a Gin recombinase a Flp recombinase, and a Tnp1 recombinase. In an aspect, a Cre recombinase or a Gin recombinase provided herein is tethered to a zinc-finger DNA-binding domain, or a TALE DNA-binding domain, or a Cas9 nuclease. In another aspect, a serine recombinase attached to a DNA recognition motif provided herein is selected from the group consisting of a PhiC31 integrase, an R4 integrase, and a TP-901 integrase. In another aspect, a DNA transposase attached to a DNA binding domain provided herein is selected from the group consisting of a TALE-piggyBac and TALE-Mutator.

[0040] Site-specific genome modification enzymes, induce a genome modification such as a double-stranded DNA break (DSB) or single-strand DNA break at the target site of a genomic sequence that is then repaired by the natural processes of homologous recombination (HR) or non-homologous end-joining (NHEJ). Sequence modifications then occur at the cleaved sites, which can include deletions or insertions that result in gene disruption in the case of NHEJ, or integration of exogenous sequences by homologous recombination.

[0041] Another aspect of the invention includes transgenic plant cells, transgenic plant tissues, transgenic plants, and transgenic seeds that comprise the recombinant DNA molecules and engineered proteins provided by the invention. These cells, tissues, plants, and seeds comprising the recombinant DNA molecules and engineered proteins exhibit resistance to *P. capsici*. Suitable methods for transformation of host plant cells for use with the current invention include virtually any method by which DNA can be introduced into a cell (for example, where a recombinant DNA construct is stably integrated into a plant chromosome) and are well known in the art. An exemplary and widely utilized method for introducing a recombinant DNA construct into plants is the *Agrobacterium* transformation system, which is well known to those of skill in the art. Another exemplary method for introducing a recombinant DNA construct into plants is insertion of a recombinant DNA construct into a plant genome at a pre-determined site by methods of site-directed integration. Transgenic plants can be regenerated from a transformed plant cell by the methods of plant cell culture. A transgenic plant homozygous with respect to a transgene (that is, two allelic copies of the transgene) can be obtained by self-pollinating (selfing) a transgenic plant that contains a single transgene allele with itself, for example an R0 plant, to produce R1 seed. One fourth of the R1 seed produced will be homozygous with respect to the transgene. Plants grown from germinating R1 seed can be tested for zygosity, using a SNP assay, DNA sequencing, or a thermal amplification assay that allows for the distinction between heterozygotes and homozygotes, referred to as a zygosity assay.

IV. MOLECULAR ASSISTED BREEDING TECHNIQUES

[0042] Genetic markers that can be used in the practice of the present invention include, but are not limited to, restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs), simple sequence repeats (SSRs), simple sequence length polymorphisms (SSLPs), single nucleotide polymorphisms (SNPs), insertion/deletion polymorphisms (Indels), variable number tandem repeats (VNTRs), and random amplified polymorphic DNA (RAPD), isozymes, and other markers known to those skilled in the art. Vegetable breeders use molecular markers to interrogate a crop's genome and classify material based on genetic, rather than phenotypic, differences. Advanced marker technologies are based on genome sequences, the nucleotide order of distinct, polymorphic genotypes within a species. Such platforms enable selection for horticultural traits with markers linked to favorable alleles, in addition to the organization of germplasm using markers randomly distributed throughout the genome. In the past, a priori knowledge of the genome lacked for major vegetable crops that now have been sequenced. Scientists exploited sequence homology, rather than known polymorphisms, to develop marker platforms. Man-made DNA molecules are used to prime replication of genome fragments when hybridized pair-wise in the presence of a DNA polymerase enzyme. This synthesis, regulated by thermal cycling conditions that control hybridization and replication of DNA strands in the polymerase chain reaction (PCR) to amplify DNA fragments of a length dependent on the distance between each primer pair. These fragments are then detected as markers and commonly known examples include AFLP and RAPD. A third technique, RFLP does not include a

DNA amplification step. Amplified fragment length polymorphism (AFLP) technology reduces the complexity of the genome. First, through digestive enzymes cleaving DNA strands in a sequence-specific manner. Fragments are then selected for their size and finally replicated using selective oligonucleotides, each homologous to a subset of genome fragments. As a result, AFLP technology consistently amplifies DNA fragments across genotypes, experiments and laboratories.

[0043] Polymorphisms comprising as little as a single nucleotide change can be assayed in a number of ways. For example, detection can be made by electrophoretic techniques including a single strand conformational polymorphism, denaturing gradient gel electrophoresis, or cleavage fragment length polymorphisms, but the widespread availability of DNA sequencing often makes it easier to simply sequence amplified products directly. Once the polymorphic sequence difference is known, rapid assays can be designed for progeny testing, typically involving some version of PCR amplification of specific alleles or PCR amplification of multiple specific alleles.

[0044] Polymorphic markers serve as useful tools for assaying plants for determining the degree of identity of lines or varieties. These markers form the basis for determining associations with phenotypes and can be used to drive genetic gain. In certain embodiments of methods of the invention, polymorphic nucleic acids can be used to detect in a *Capsicum annuum* plant a genotype associated with male fertility, identify a *Capsicum annuum* plant with a genotype associated with male fertility, and to select a *Capsicum annuum* plant with a genotype associated with male fertility. In certain embodiments of methods of the invention, polymorphic nucleic acids can be used to produce a *Capsicum annuum* plant that comprises in its genome an introgressed locus associated with male fertility. In certain embodiments of the invention, polymorphic nucleic acids can be used to breed progeny *Capsicum annuum* plants comprising a locus associated with male fertility.

[0045] Genetic markers may include "dominant" or "codominant" markers. "Codominant" markers reveal the presence of two or more alleles (two per diploid individual). "Dominant" markers reveal the presence of only a single allele. Markers are preferably inherited in codominant fashion so that the presence of both alleles at a diploid locus, or multiple alleles in triploid or tetraploid loci, are readily detectable, and they are free of environmental variation, i.e., their heritability is 1. A marker genotype typically comprises two marker alleles at each locus in a diploid organism. The marker allelic composition of each locus can be either homozygous or heterozygous. Homozygosity is a condition where both alleles at a locus are characterized by the same nucleotide sequence. Heterozygosity refers to different conditions of the allele at a locus.

[0046] Nucleic acid-based analyses for determining the presence or absence of the genetic polymorphism (i.e., for genotyping) can be used in breeding programs for identification, selection, introgression, and the like. A wide variety of genetic markers for the analysis of genetic polymorphisms are available and known to those of skill in the art. The analysis may be used to select for genes, portions of genes, QTL, alleles, or genomic regions that comprise or are linked to a genetic marker that is linked to or associated with male fertility in BCMS plants.

[0047] As used herein, nucleic acid analysis methods include, but are not limited to, PCR-based detection methods (for example, TaqMan assays), microarray methods, mass spectrometry-based methods and/or nucleic acid sequencing methods, including whole genome sequencing. In certain embodiments, the detection of polymorphic sites in a sample of DNA, RNA, or cDNA may be facilitated through the use of nucleic acid amplification methods. Such methods specifically increase the concentration of polynucleotides that span the polymorphic site, or include that site and sequences located either distal or proximal to it. Such amplified molecules can be readily detected by gel electrophoresis, fluorescence detection methods, or other means.

[0048] One method of achieving such amplification employs the polymerase chain reaction (PCR) using primer pairs that are capable of hybridizing to the proximal sequences that define a polymorphism in its double-stranded form. Methods for typing DNA based on mass spectrometry can also be used. Such methods are well known in the art.

[0049] Polymorphisms in DNA sequences can be detected or typed by a variety of effective methods well known in the art. The compositions and methods of the present invention can be used in conjunction with any polymorphism typing method to type polymorphisms in genomic DNA samples. These genomic DNA samples used include but are not limited to, genomic DNA isolated directly from a plant, cloned genomic DNA, or amplified genomic DNA.

[0050] For instance, polymorphisms in DNA sequences can be detected by hybridization to allele-specific oligonucleotide (ASO) probes. U.S. Pat. No. 5,468,613 discloses allele specific oligonucleotide hybridizations where single or multiple nucleotide variations in nucleic acid sequence can be detected in nucleic acids by a process in which the sequence containing the nucleotide variation is amplified, spotted on a membrane and treated with a labeled sequence-specific oligonucleotide probe. Target nucleic acid sequence can also be detected by probe ligation methods where sequence of interest is amplified and hybridized to probes followed by ligation to detect a labeled part of the probe.

[0051] Microarrays can also be used for polymorphism detection, wherein oligonucleotide probe sets are assembled in an overlapping fashion to represent a single sequence such that a difference in the target sequence at one point would result in partial probe hybridization. On any one microarray, it is expected there will be a plurality of target sequences, which may represent genes and/or noncoding regions wherein each target sequence is represented by a series of overlapping oligonucleotides, rather than by a single probe. This platform provides for high throughput screening of a plurality of polymorphisms.

[0052] Other well-known methods for detecting SNPs and Indels include single base extension (SBE) methods. In another method for detecting polymorphisms, SNPs and Indels can be detected by methods in which an oligonucleotide probe having a 5' fluorescent reporter dye and a 3' quencher dye covalently linked to the 5' and 3' ends of the probe. When the probe is intact, the proximity of the reporter dye to the quencher dye results in the suppression of the reporter dye fluorescence, e.g. by Förster-type energy transfer. During PCR forward and reverse primers hybridize to a specific sequence of the target DNA flanking a polymorphism while the hybridization probe hybridizes to polymorphism-containing sequence within the amplified PCR product. In the subsequent PCR cycle DNA polymerase with 5'

4 3' exonuclease activity cleaves the probe and separates the reporter dye from the quencher dye resulting in increased fluorescence of the reporter.

[0053] In another embodiment, a locus or loci of interest can be directly sequenced using nucleic acid sequencing technologies. Methods for nucleic acid sequencing are known in the art and include technologies provided by 454 Life Sciences (Branford, Conn.), Agencourt Bioscience (Beverly, Mass.), Applied Biosystems (Foster City, Calif.), LI-COR Biosciences (Lincoln, Nebr.), NimbleGen Systems (Madison, Wis.), Illumina (San Diego, Calif.), and VisiGen Biotechnologies (Houston, Tex.). Such nucleic acid sequencing technologies comprise formats such as parallel bead arrays, sequencing by ligation, capillary electrophoresis, electronic microchips, "biochips," microarrays, parallel microchips, and single-molecule arrays.

V. DEFINITIONS

[0054] The following definitions are provided to better define the present invention and to guide those of ordinary skill in the art in the practice of the present invention. Unless otherwise noted, terms are to be understood according to conventional usage by those of ordinary skill in the relevant art.

[0055] As used herein, the term "plant" includes plant cells, plant protoplasts, plant cells of tissue culture from which *Capsicum annuum* plants can be regenerated, plant calli, plant clumps and plant cells that are intact in plants or parts of plants such as pollen, flowers, seeds, leaves, stems, and the like.

[0056] As used herein, "blocky" type pepper refers to a pepper wherein the length of the fruit is about the same as the width of the fruit. For example, the length of the fruit is about 0.8, about 0.9, about 1.0, about 1.1, or less than 1.2 of the width of the fruit.

[0057] As used herein, "sweet" pepper refers to the fruit and the plant of the non-pungent chili pepper varieties. Sweet peppers belong to the genus *Capsicum*, of the nightshade family, Solanaceae. The term "sweet pepper" therefore includes bell peppers (*Capsicum annuum*), the "Thai sweet"—also a cultivar of *Capsicum annuum*, the "dulce"—a popular cultivar of *Capsicum baccatum*, as well as Numex Suave Orange (*Capsicum chinense*), an unusually sweet habanero-type pepper.

[0058] As used herein, the term "population" means a genetically heterogeneous collection of plants that share a common parental derivation.

[0059] As used herein, the terms "variety" and "cultivar" mean a group of similar plants that by their genetic pedigrees and performance can be identified from other varieties within the same species.

[0060] As used herein, an "allele" refers to one of two or more alternative forms of a genomic sequence at a given locus on a chromosome.

[0061] A "Quantitative Trait Locus (QTL)" is a chromosomal location that encodes for at least a first allele that affects the expressivity of a phenotype.

[0062] As used herein, a "marker" means a detectable characteristic that can be used to discriminate between organisms. Examples of such characteristics include, but are not limited to, genetic markers, biochemical markers, metabolites, morphological characteristics, and agronomic characteristics.

[0063] As used herein, the term “phenotype” means the detectable characteristics of a cell or organism that can be influenced by gene expression.

[0064] As used herein, the term “genotype” means the specific allelic makeup of a plant.

[0065] As used herein, the term “haplotype” means a chromosomal segment defined by the combination of alleles it carries.

[0066] As used herein, the term “introgressed,” when used in reference to a genetic locus, refers to a genetic locus that has been introduced into a new genetic background, such as through backcrossing. Introgression of a genetic locus can be achieved through plant breeding methods and/or by molecular genetic methods. Such molecular genetic methods include, but are not limited to, marker assisted selection, various plant transformation techniques and/or methods that provide for homologous recombination, non-homologous recombination, site-specific recombination, and/or genomic modifications that provide for locus substitution or locus conversion.

[0067] As used herein, the term “linked,” when used in the context of nucleic acid markers and/or genomic regions, means that the markers and/or genomic regions are located on the same linkage group or chromosome such that they tend to segregate together at meiosis.

[0068] As used herein, “cytoplasmic male sterility” refers to plants that are not usually capable of breeding from self-pollination due to the failure of the plant to produce functional anthers, pollen, or male gametes in absence of a male fertility restorer locus, but are capable of breeding from being cross-pollinated when used as the female parent. Furthermore, the male sterility is the result of an incompatibility between the cytoplasm and the nuclear genome.

[0069] As used herein, “*Baccatum* cytoplasmic male sterility” or “BCMS” refers to cytoplasmic male sterile plants wherein the cytoplasm is from a *Capsicum baccatum* plant and the nuclear genome from a *Capsicum annuum* plant.

[0070] As used herein, a “female parent” refers to a pepper plant that is the recipient of pollen from a male donor line, which pollen successfully pollinates an egg. A female parent can be any pepper plant that is the recipient of pollen. Such female parents can be male sterile, for example, because of genic male sterility, cytoplasmic male sterility, or because they have been subject to physical emasculation of the stamens. Genic or cytoplasmic male sterility can be manifested in different manners, such as sterile pollen, malformed or stamenless flowers, positional sterility, and functional sterility.

[0071] As used herein, “uniform female fertility” refers to the production of male sterile flowers that are otherwise developmentally normal (i.e. female fertile) and produce viable fruit and seed if fertilized with a male fertile pollen source. A locus that confers uniform female fertility means that all flowers of a *Baccatum* cytoplasmic male sterile plant carrying the locus will comprise functioning female organs and non-functioning male organs, in absence of a male fertility restoration locus.

[0072] As used herein, “good flower” refers to pepper plants comprising a flower that is female fertile and developmentally normal. Plants with good flowers can be male fertile or male sterile.

[0073] As used herein, “male parent plant” refers to a parent plant that provides pollen to (i.e. is a pollinator for)

a female line. They may be useful for breeding of progeny pepper plants, such as parthenocarpic seedless progeny plants.

[0074] As used herein, “resistance allele” means the nucleic acid sequence associated with resistance or tolerance to disease.

[0075] As used herein “resistance” or “improved resistance” in a plant to disease conditions is an indication that the plant is less affected by disease conditions with respect to yield, survivability and/or other relevant agronomic measures, compared to a less resistant, more “susceptible” plant. Resistance is a relative term, indicating that a “resistant” plant survives and/or produces better yields in disease conditions compared to a different (less resistant) plant grown in similar disease conditions. As used in the art, disease “tolerance” is sometimes used interchangeably with disease “resistance.” One of skill will appreciate that plant resistance to disease conditions varies widely, and can represent a spectrum of more-resistant or less-resistant phenotypes. However, by simple observation, one of skill can generally determine the relative resistance or susceptibility of different plants, plant lines or plant families under disease conditions, and furthermore, will also recognize the phenotypic gradations of “resistant.”

[0076] The term “about” is used to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value. The use of the term “or” in the claims is used to mean “and/or” unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and to “and/or.” When used in conjunction with the word “comprising” or other open language in the claims, the words “a” and “an” denote “one or more,” unless specifically noted. The terms “comprise,” “have” and “include” are open-ended linking verbs. Any forms or tenses of one or more of these verbs, such as “comprises,” “comprising,” “has,” “having,” “includes” and “including,” are also open-ended. For example, any method that “comprises,” “has” or “includes” one or more steps is not limited to possessing only those one or more steps and also covers other unlisted steps. Similarly, any plant that “comprises,” “has” or “includes” one or more traits is not limited to possessing only those one or more traits and covers other unlisted traits.

VI. DEPOSIT INFORMATION

[0077] Deposits of seeds of *Capsicum annuum* lines designated ‘Flame Fountain’ and ‘Ganti,’ which are disclosed herein above and referenced in the claims, were made with NCIMB, Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen, AB21 9YA, Scotland, U.K. The date of deposit was May 29, 2018 and the accession numbers for those deposited seeds of lines ‘Flame Fountain’ and ‘Ganti’ are NCIMB Accession Nos. 43054 and 43055, respectively. All restrictions upon the deposits have been removed, and the deposits are intended to meet all of the requirements of 37 C.F.R. § 1.801-1.809. The deposits will be maintained in the depository for a period of 30 years, or 5 years after the last request, or for the effective life of the patent, whichever is longer, and will be replaced if necessary during that period.

VII. EXAMPLES

[0078] The following examples are included to illustrate embodiments of the invention. It should be appreciated by

those of skill in the art that the techniques disclosed in the examples that follow represent techniques discovered by the inventor to function well in the practice of the invention. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

Example 1. Creation of a BCMS *Capsicum annuum* Plant

[0079] The compatibility between *Capsicum annuum* and *Capsicum baccatum* for interspecific crosses is very low. To transfer genetic information between these two species, intermediate pepper species, such as *Capsicum chinense* or *Capsicum frutescens*, have been used as a ‘genetic bridge’. However, this method only allows for the transfer of nuclear traits. To develop a BCMS plant where a *Capsicum annuum* genome is introduced into a *Capsicum baccatum* cytoplasm, it is necessary to pollenate a *Capsicum baccatum* flower with *Capsicum annuum* pollen. These combinations never led to viable hybrid seed and it was therefore necessary to use embryo rescue techniques to recover a *Capsicum baccatum*×*Capsicum annuum* hybrid. The method described herein was used to develop *Capsicum baccatum*×*Capsicum annuum* hybrids from several *Capsicum baccatum* accessions, such as PI497974, PI159242, and PI640880.

[0080] Plants of these *Capsicum baccatum* accessions were grown simultaneously with *Capsicum annuum* lines that served as pollen donors. Flowers on the *Capsicum baccatum* plants were emasculated before the anthers shed and subsequently pollinated with the *Capsicum annuum* pollen. One day after the first pollination, the pollinated flowers were dipped in 200 mg/L NAA (1-naphthylacetic acid) followed by a second pollination after the NAA solution (growth regulator) had dried. The flowers were then left to develop fruit. Ripe fruit were harvested and seeds were extracted from the fruit for embryo rescue. The embryo rescue was performed under aseptic conditions by dissecting the embryos from endosperms. The extracted embryos were cultured on MS media until seedlings had fully developed. These F₁ seedlings were checked for *Capsicum baccatum*×*Capsicum annuum* hybridization using polymorphic DNA markers. Seedlings that were true hybrids were selected for further backcrossing to the *Capsicum annuum* parent. Early

backcross generations were selected phenotypically for male sterility and the *Capsicum annuum* recurrent parent phenotype and the genome was evaluated for *Capsicum annuum* percentage using polymorphic DNA markers. In later generations, additional horticultural traits were used to select plants for advancement.

Example 2. Mapping of the Male Fertility Restorer Locus

[0081] After extensive crossing and evaluation of F₁ progeny, two *Capsicum annuum* accessions were identified that can restore male fertility in BCMS lines: ‘Flame Fountain’ and ‘Ganti’. The ability of ‘Flame Fountain’ and ‘Ganti’ to restore male fertility in BCMS lines was later validated by remaking the F₁ populations. Two F₂ populations, BCMS Donor Line 1×‘Flame Fountain’ and BCMS Donor Line 1×‘Ganti’, were developed by selfing male fertile lines from these F₁ crosses. These F₂ populations were used to map QTL associated with male fertility restoration.

[0082] Individual F₂ plants from both populations were genotyped and evaluated for fertility in the greenhouse. Fertility was determined by the presence or absence of normal anthers and visible pollen in the flowers, although flower formation, fruit set, and presence of seed in the selfed fruit were also noted. QTL Cartographer was used to run a single marker analysis on both mapping populations using the presence or absence of visible pollen to determine whether fertility was restored in individual F₂ plants.

[0083] The BCMS restorer locus (Rf) from the two *Capsicum annuum* lines mapped to the short arm of chromosome 6 in both populations, between the consensus positions 24.2-32.9 cM (2-LOD interval) in the BCMS Donor Line 1×‘Flame Fountain’ population and the consensus positions 17.2-45 cM (2-LOD interval) in the BCMS Donor Line 1×‘Ganti’ population. Rf was found to be completely dominant since the Rf/rf genotype provided male fertility and recombination was suppressed on the short arm of chromosome 6. The Rf locus can be tracked in the population via marker assisted selection by detecting the haplotype associated with the Rf locus (Table 1). Further fine mapping reduced the genomic interval restoring fertility in BCMS lines to a region flanked by M14 and M29. It was determined that markers M18, M19, M20, M21, M22, M23, M24, M25, M26, M27, and M28 are comprised within the region where the restorer factor is located. These markers should therefore comprise a haplotype corresponding to the Rf locus donor line. Markers M14/M15/M16/M17 and M29, which flank the chromosomal interval, may be used to select the recurrent parent allele. Depending on the Rf locus donor line used, M14 may be used if ‘Ganti’ is used as the Rf locus donor and M15, M16, or M17 may be used if ‘Flame Fountain’ is used as the Rf locus donor.

TABLE 1

List of markers and favorable alleles at each marker for tracking the Rf locus							
Marker Name	Marker Sequence (SEQ ID NO)	Favorable Allele ‘Flame Fountain’	Favorable Allele ‘Ganti’	C. <i>annuum</i> Genetic Position (cM)	SNP Public Position CM334v1.55 (bp)	SNP Position in	
						Marker	SNP Change
M1	1	—	G	17.2	213,924,156	93	A/G
M2	2	—	T	17.2	213,907,080	243	C/T
M3	3	—	G	17.9	213,907,920	2353	A/G

TABLE 1-continued

List of markers and favorable alleles at each marker for tracking the Rf locus							
Marker Name	Marker Sequence (SEQ ID NO)	Favorable Allele 'Flame Fountain'	Favorable Allele 'Ganti'	C. <i>annuum</i> Genetic Position (cM)	SNP Public Position CM334v1.55 (bp)	SNP Position in Marker (bp)	SNP Change
M4	4	—	C	18.8	427,239	216	T/C
M5	5	G	G	19.1	428,143	287	A/G
M6	6	—	C	20.8	87,022	117	T/C
M7	7	T	T	20.8	125,861	93	C/T
M8	8	T	C	20.8	89,795	484	C/T
M9	9	C	C	21.8	386,489	313	T/C
M10	10	A	A	22.8	26,405	1149	C/A
M11	11	G	—	24.2	3,055,268	188	A/G
M12	12	A	C	24.2	3,009,771	387	A/C
M13	13	T	C	24.9	2,999,718	330	C/T
M14	14	T	G	25.7	3,504,248	294	G/T
M15	15	A	—	25.7	3,500,133	41	C/A
M16	16	G	—	25.7	3,505,583	98	A/G
M17	17	T	—	26.3	3,308,938	523	C/T
M18	18	G	A	26.4	3,475,770	779	A/G
M19	19	T	G	26.4	3,422,765	592	G/T
M20	20	A	T	30.2	4,276,008	279	A/T
M21	21	A	A	30.2	4,240,789	534	G/A
M22	22	C	C	30.2	4,245,699	279	G/C
M23	23	—	G	31.8	70,994,266	49	A/G
M24	24	T	T	32.4	4,240,551	287	A/T
M25	25	A	—	34.7	6,240,565	275	G/A
M26	26	T	—	34.7	6,241,544	30	C/T
M27	27	—	G	35.0	8,522,176	1140	T/G
M28	28	G	G	35.0	9,799,932	182	A/G
M29	29	T	T	40.8	10,670,362	246	C/T
M30	30	C	C	40.8	10,664,163	33	T/C
M31	31	T	T	42.2	10,664,630	142	G/T
M32	32	C	C	43.0	11,108,817	1613	T/C
M33	33	C	T	44.0	11,592,142	146	C/T
M34	34	—	C	48.9	21,133,217	285	G/C

[0084] In addition, to have uniform female fertile flowers, BCMS plants should have the Good Flowering (Gf) locus, which is a dominant *Capsicum baccatum* allele flanked by marker sequence SEQ ID NO: 35 and marker sequence SEQ ID NO: 36 on the short arm of chromosome 6. The Gf locus can be introgressed into BCMS plants from any *Capsicum baccatum* line and detected by using markers that flank the Gf locus (Table 2).

TABLE 2

Flanking markers and favorable alleles at each marker for tracking the Gf locus					
Marker Sequence (SEQ ID NO)	Favorable Allele	C. <i>annuum</i> Genetic Position (cM)	SNP Public Position CM334v1.55 (bp)	SNP Position in Marker	SNP Change
35	G	1.9	3,064,350	358	A/G
36	G	35.7	21,133,217	285	C/G

[0085] The identification of the Gf locus and genetic markers associated with the locus is described in U.S. Provisional Appln. Ser. No. 62/690,722, filed concurrently herewith, the disclosure of which is incorporated herein by reference in its entirety.

[0086] F₁ progeny from the crosses BCMS Donor Line 1×'Flame Fountain' and BCMS Donor Line 1×'Ganti' have genotypic segregation of ½(S)Gfrf/gfRf+½(S)gfrf/gfRf and phenotypic segregation of ½ completely fertile+½ com-

pletely sterile. This segregation occurs in the F₁ because the plants of BCMS Donor Line 1 (or any other BCMS line) that have female fertile flowers to facilitate crossing have the heterozygous genotype ½(S)Gfrf/gfRf. The F₂ progeny are created by selfing F₁ plants with complete fertile flowers, (S)Gfrf/gfRf. Thus, the expected genotypic segregation in the F₂ progeny would be ¼(S)Gfrf/Gfrf+½(S)Gfrf/gfRf+¼(S)gfrf/gfRf and the expected phenotypic segregation would be ¼ male sterile+½ complete fertile+¼ complete sterile. However, the observed genotypic segregation in the F₂ progeny is ½(S)Gfrf/Gfrf+½(S)Gfrf/gfRf and the observed phenotypic segregation is ½ male sterile+½ completely fertile. The completely sterile plants are missing from the F₂ progeny both phenotypically and genotypically, indicating that the gfrf genotype is not transmissible via pollen (male gamete) from BCMS plants. The F₂ progeny in both mapping populations had normal germination rates of 92% which suggests that the underlying failure of gfrf transmission occurs early in the reproductive process, before seed formation.

Example 3. Genetic Model of the Male Fertility Restorer Locus for BCMS

[0087] There is a dominant nuclear allele present in some *Capsicum annuum* accessions, such as "Flame Fountain" and "Ganti", that restores male fertility in the presence of the *Capsicum baccatum* cytoplasm. The dominant *Capsicum annuum* allele is denoted as Rf, and the recessive allele is

denoted as rf. Gf functions upstream of Rf, such that the gfgf genotype masks the male fertility restoration effect of Rf (i.e. recessive epistasis). This is consistent with the observation, represented by formula (I) below, where (S) denotes a *Capsicum baccatum* cytoplasm and (N) denotes a *Capsicum annuum* cytoplasm, that restored F₁ progeny from the BCMS Donor Line 1×'Flame Fountain' and BCMS Donor Line 1×'Ganti' crosses segregate for 50% completely fertile flowers and 50% completely sterile flowers, even though all of the F₁ progeny are heterozygous at the Rf locus:

$$\begin{array}{l} \text{Parental cross: BCMS Donor Line 1: (S)Gfrf/gfrfx} \\ \text{'Flame Fountain' (N)gfrf/gfrf} \rightarrow F_1: 1/2(S)Gfrf/ \\ \text{gfrf (completely fertile)} + 1/2(S)gfrf/gfrf(\text{com-} \\ \text{pletely sterile}) \end{array} \quad (I)$$

Example 4. Utilization of Uniform Female Fertility and the Male Fertility Restorer Locus for BCMS to Produce Hybrid Pepper Plants

[0088] Gf and Rf are tightly linked in repulsion on the short arm of chromosome 6. Therefore, the gametes Gfrf (*Capsicum baccatum* haplotype), gfrf (*Capsicum annuum* non-restorer haplotype), and gfrf (*Capsicum annuum* restorer haplotype) exist, but the recombinant gamete Gfrf does not exist and cannot be recovered readily in segregating populations. Furthermore, the gfrf gamete is not pollen

transmissible from plants that have the *Capsicum baccatum* cytoplasm, as these gametes are generally not viable. This is consistent with the observation, represented in FIG. 3, where (S) denotes a *Capsicum baccatum* cytoplasm and (N) denotes a *Capsicum annuum* cytoplasm, that F_n progeny derived from restored F₁ plants (e.g. BCMS Donor Line 1×'Flame Fountain') segregate for 50% completely fertile and 50% male sterile plants.

[0089] Under this genetic model, the possible genotypic combinations and associated flowering phenotypes that can be observed are shown in FIG. 1.

[0090] Using the genetic model of BCMS described herein, it is possible to design a breeding system that allows easier production of pepper hybrids. In the female pool, the breeder maintains two types of germplasm: an "A-line", which is a line that carries the *Baccatum* CMS trait and a "B-line", which is in the same breeding pool as the A-line, but comprises *Capsicum annuum* cytoplasm. The B-line is the backcross parent for the A-line. The male pool contains *Capsicum annuum* germplasm lines that are homozygous for the Rf allele. Fully fertile hybrids may be produced by crossing the A-line as the female parent and the C-line as male parent (FIG. 2). Due to the male sterile nature of the A-line, it is possible to produce hybrids that do not contain off-types due to selfings. Furthermore, labor is reduced because the female plants do not need to be emasculated.

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acacaagtgc atcaaagagt taccctcttg ctcagaacca tcgaccagat gaatgtcagc 1680
ctcaaaaagc ttccttctga gatagtcaat ctttacctca ctcacggttt tctagcaaaa 1740
gcaaggatta aactaagaaa tcaaaactgaa tcagaaataa cagaaggaag caaaagactg 1800
ttacatgtga ttcataatctt aaaagcaact cttcatacac ctttgaagt tccaggaaat 1860
cactgtcttc ctgccaaaat taaaaaataa taacacaaca gtggtataat acaattttga 1920
aagaaagctg aaatagcagc gcaaggtgtg aaccattgag aagctttttt tcttagaggc 1980
tccttttctc ctgctagtga catgcaacaa agcccgcggg tcaggagag tacagtcttc 2040
agcttgatta ctccttgcag tgtctctctg cttgccagag tctaaattga tgtcaacatc 2100
taatagttct tcaaaaggaa gtaagggtcc aatgtcacgt tcaagccttt tggctttgac 2160

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<210> SEQ ID NO 11
<211> LENGTH: 493
<212> TYPE: DNA
<213> ORGANISM: Capsicum annuum

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<400> SEQUENCE: 11

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accgtgaggg atgcttttgt atatactgta aagccagtct taagtccaaa tatctgaagt 60
tggtttcggc acttgctatg cttattggtt ccaagtaatt ttgttctgaa tggttgtttt 120
tagtgttgta atttgtaaat gatgatatgt atgttcttct acagaccgaa agttctggag 180
gttcaccaga gcaggatttt gtccagaagg catttgagat tcatgacaag tatatggtgt 240
atgtgaaagg ctgttttggc gataacacta tctttcacia ggtattccga ctgttaactg 300
ctgtacgctg atccctttta cgatgaatta cttgagacgt tatatagtta tgttcagtgt 360
tgtcgaaggt gcacataaga cctgaatcga ggctcaaagc tgtttgagag tttagcgtcg 420
cttaatgtgc acatattcct ctgaatgggt ctcttaggct gatgtataaa ctctttgact 480
ctcataggct ctg 493

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<210> SEQ ID NO 12
<211> LENGTH: 516
<212> TYPE: DNA
<213> ORGANISM: Capsicum annuum

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<400> SEQUENCE: 12

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tgctattgag gatacattgg ataaggatg cacaaaatctt acatctccga tcaatgttgg 60
ggtcgaactt catatctttg ctcaaacatc gtcaaaaattg tgtacttttg ttaggtggtc 120
aagcttgtga tgtacatcag cgacaaagat gtgtttgctg agttctacag gttggaatc 180
agtgccagca gttgttttga catctttaa ctttgatg gctaactgtc ttaatactta 240
taggaagaag ctttctcgcc gattgctttt tgatagaagt ggcaatgaag aacatgaaag 300

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gcttatctta tcaaagctaa aacagcagtg tggtaggacag tttacatcca agatggaggg	360
aatggtaagg attgttcaat ggacttagaa agttttgtct ctttaccgcg cttgagatat	420
ctgaaaacga atgactggaa atgtaaaaca ggtaacagat ttgtcattgg tgaaggaaaa	480
tcagactcac ttacaggaat atatcagtaa caaccc	516

<210> SEQ ID NO 13
 <211> LENGTH: 1880
 <212> TYPE: DNA
 <213> ORGANISM: Capsicum annuum

<400> SEQUENCE: 13

gttccatgta tttctggaaa acagatggta caagggtagg ttaaggaaa gggtaatttg	60
gtaaacaaac atttttttac aaaatttata taaatatata ttatctttaa tacatcaaac	120
caacaatgt gtaaaaacaa atgtatgtgt aactaatact agcattacta ctacttgcac	180
taataatata agcattacaa atcccccta tttagcatta ttcttatata ctctacaaa	240
cgacacccta agcactttgg catgtacaaa tactacctac catacgatct tttttaacac	300
aaaactttca tgcttgcaac agttttttgc atgtcgtctc aatgaatgaa ccaagttcaa	360
attaanaatgg gaactcactg gcagagtttt gcagtgtctc tatagattct ccttgactgg	420
ttccgttcaac catgcttggg gctgtctaat tctagtaagg aggcaccatg gagaaacttt	480
aatgcccgtt ggaatagat acccgtactt tcaactgatc cagaactctg aaccnaatag	540
ccaaagtcaa cataaaataa ataaatgaaa caaataagaa agcatatcca ccatattttg	600
tccccctctt tcttaggaca taagtgtaaa ttcttttaac cttgagacga tctgccagat	660
gttttaggtt tgtggcttct tttatggcat ttgtagcagc ttgacttgat gaatccttct	720
gagcagggct gggaacatct tgatccctga ccttattagc agtattatta ggtaggtttc	780
catggtggct tttagctggt ttaccctgtc ttgaagcatt tgacttttcc ctttcagaag	840
catcaactat aacacaattt gcctcgtttg cccgtccgga tccaggaact ggctctttaa	900
atacaaccgt ctcaattctga actcctgtta atggaaactt ggaagagact tcaatctgct	960
cacttctggt ggaacaacgc ttcttagatg atctctgatc atttcgatct ggtagtgcag	1020
cctggccttt atccaggcgg gatgcaacat ccgatcgagc ttctgcacca tcatgatgac	1080
caactcgtgg ttgattatca cctttaacat tttgtccaa taattttcca gatgaatcct	1140
tcttagaact cttctttaat ctatctgaac caacaacatt ctctgaaac ttgttttttc	1200
ctggtgccgt cttctcctcg tctaattggca tttgatctga agataaattt aaaatatcag	1260
gatttttggg gttacattgg tcagaatcag attctggagc ccgattcttc tctttgcgcc	1320
gtgacaaaga acctctttta ccatttgaga caaactcact atcctgacac tgatagtcat	1380
tctttctctc cttatcagag ccttggctctg agttttctgt cttgcaagaa aattgaccaa	1440
cttgacgtaa tggatcggca tttacatcag catccagatg agttgcataa ccagagacca	1500
tggctcttgc ttaactggtt gtatctgatt cacgatggac atctttctcc tctaaatcag	1560
cttcagctaa cacaaacataa ctctgtccat tgcctgcact atccttctta acaaaaactgg	1620
attcatttga gaaccatca ttttcccat cagaagacct cttgggactt gaagtgggaa	1680
cagctgatga ggcagcagca gaaggccgtg aattgcctag atcctttttg aagaatctgc	1740
agcttttaca ctctgcaaag atacagttag atcttgccca gcctgctgtt cctttttaac	1800

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tgcacatggt ccagcactac ctttgcttgc gctagtgtct ttccggtcag acttggagac 1860
ccttgcttcc ttttccttcc 1880

<210> SEQ ID NO 14
<211> LENGTH: 2286
<212> TYPE: DNA
<213> ORGANISM: Capsicum annuum

<400> SEQUENCE: 14
gttaggggaa gtacaaataa atcagcaagg aaaagacaag tccatttcat attgcagaaa 60
acatgttttc cacttcataa gtaggccatc atagatctat aagggattat tgatcattct 120
agtgatagtg ttcttaaccg ttactaacia agatgaagga tatgtatagt tcttgggtgt 180
agtatgcccc tgagaattgt aagatgggat tttagtttca ctataagctg cttttttgtt 240
tcacctgtaa catcttctgc tatctagatt ggacattggt ttggtctaca gctgatgaac 300
tttgagcagt ggactacgct tgaatttta aaattatcca ctatgctgat ttttacgcat 360
taccataact aactgcattg cggtgaaaac tcaatgcatg tccttaagcc gtaggttget 420
ttctaatacag caaacaagtt tgggaaacia gttctctgaa tctatccatt ggttatoctt 480
gtctcgacia gctagtctct tttccttttc tattctgttt gaatttatag agtcagcaaa 540
gtatagattt tgaaggagtt caagtaggaa atattaccgc tttggaaatt tacagatttg 600
cttttaaaac ttttaattatg tgccacaagt ctagcttttg gaaatattta acatgcttct 660
tgaactgggt ttcataatgg tcaagtgcc c atgaatttta tttttctct tccgctgaat 720
acattatcaa acttcgaact tgaatcttca ttactttgca gacaactaga gtatttgctc 780
cttggtctca taatgcaagc ttagagaatg aagaatcttg gcatgaaatt gctcgccctc 840
aagttcatgg tcatgacata aattgtgtga cagtaataca aggaaaagga aaccatcggt 900
ttgttggcgg ggctgacgag aaagttgcca gagtttttga atctctctca tcctttctga 960
agacattgag ccatgttact tcagacagct ctagttttcc tgccgacatt caagctgatg 1020
tgcagatatt aggggcaaat atgtctgctc taggtctatc gcagaaacct atatatgttc 1080
agggtgagtt ttccagctca ttgtgtggta tcaattttgc ataataacat ataagatact 1140
cctattcatt tatgtttcca tcatcacttt tgatgttaca gggatgata taactgagtt 1200
gttgacttgt caaaattaac tgtaaagacc ttctctaac catgtattga attagaatta 1260
atgtgatgtg ttaccgaact tttctctaag ttgcttaaa gtgctgtgat atctgatgca 1320
aagtgttctg actttgatc aaacagcgat taccctgaat atttcgttg aggactctta 1380
tatgttataa tatctcatic atctaagcct atcttttga gcagcatcga caccaacaga 1440
cagaagcaat acggaagggt ttgatacact agaaaactgtt cctgaagcag ttccagttgt 1500
cttgacagag ccacctattg aagagcagtt ggcatggcat actctatggc ccgagtcgca 1560
caaaacttac ggtcatggga atgagatttt tgctctatgt tgtgatcagg agggaaagct 1620
tgtagcttca tcctgcaagg tttttctctc tttccctttt ctctctctt cttttgggtg 1680
ttggcatggg tcttccttgt ctgtcttgaa tcttctgtta atagcattca acttgattgt 1740
acttttgcta agtgccctga taaagttgg ttttagtttc tggctttaat ataactogat 1800
agaaaaaaaa ggtagtactc cattccatc atgggtgcaa tttttttcaa tatcttgacc 1860
attagacgoc cctattttat tgggtgatgg ggttagttaa ggaatagctg ggtttgatcc 1920

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tgccgcattt tctctcttat gagaaatcaa acgccaatct gctcatttta tataacttac	1980
tggtgatgct cactacctcg gacttttacg ttgtagaga cctctatgaa ctatagcaac	2040
acatgatgga attcacaata ttctctctaga tactcctttc aacttctaata gatttttttg	2100
ggggaagtct ttattttctt aattagttgt gtctatcaga aacactctct ctacctccga	2160
ggtaggggtg acgtgtgogt gcgtacactc tacccttctc agacctcact ttgtgtgatt	2220
acactggaga tgtttttctg ttcttattta attgtgaata ctaattatgc tggggctata	2280
taatcc	2286

<210> SEQ ID NO 15
 <211> LENGTH: 358
 <212> TYPE: DNA
 <213> ORGANISM: Capsicum annuum

<400> SEQUENCE: 15

gattccgtaa tcactctaac ggctgaaata tatctgattt agtgcttttc ggttcattat	60
aatcagtaat ccagccagta tgggtgacgg tcatgtcaat aatctgaaat gttatctgcc	120
ttttctgta atactatagt ctatagtatc atttatgtgc accaagagag cgaactaagc	180
atcaatccac gcagctatct ctgtttaaga atttaggcta agtagatttt tgtgccagta	240
aatgttttac tcttttagtt tctaagttga aggtgattca ttaattactc ttttagtttt	300
taggttggag gtaacctcta tgcagattta gttgggttta taaggatgca attatatg	358

<210> SEQ ID NO 16
 <211> LENGTH: 1273
 <212> TYPE: DNA
 <213> ORGANISM: Capsicum annuum
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (470)..(470)
 <223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 16

tgggggcatc tctatggccc gagtcgcaca aactttacgg tcatgggaat gagatttttg	60
ctctatgttg tgatcaggag ggaagccttg tagcttcacg ctgcaagggt tttctctctt	120
tccctttctc cctcctttct tttgggtggt ggcattggtc ttccttgtct gtcttgaatc	180
ttctgttaat agcattcaac ttgattgtac ttttgctaag tgccctgata aagttgggtt	240
ttagtttctg gtcttaatat acttcgatag aaaaaaaagg tagtactcca ttccatatat	300
ggtgtcaatt tttttcaata tcttgacct tagacgcccc tattttattg gtggatgggg	360
ttagttaagg aatagctggg tttgatcctg eggcattttc tctcttatga gaaatcaaac	420
gccaatctgc tcattttata taacttactg gtgatgctca ctacctcggg acttttacgt	480
tgtagagac ctctatgaac tatagcaaca catgatggaa ttcacaatat tcttctagat	540
actcctttca acttctaata atttttttgg ggaagtctt tattttotta attagttgtg	600
tctatcaaaa acactctctc tacctcogag gtaggggtga cgtgtgcgtg cgtacactct	660
acccttccaa aacctcattt tgtgtgatta acactgggga tgtttttctc gtttctattt	720
aattgtgaat actaattatg ctggggctat tatatcctgc aggtcaatca ccaccattgt	780
tgaaatatgt tatgggcagt tgggttctgg aaaatcattt ggtcttttgg cattccaaat	840
ttaaaagtga cccaaggga tttcccatg aaaccaattt ctcttgacct ttaaaaaaac	900

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cccttttttg ttttttaaat taatcaaaag gtttgaacat ttaccacct cctttgocaa 960
aaattttaaa aaatacaaaa ccaaaaattt tttttcccc accccaatgt ttatatatac 1020
tgtaaatata aaacttttct cccttttgga agtttttttt agaaaaacct ttctactttg 1080
gagaggggaag agcctggcca actccccccc aaaaccccca cggtgtaaaa ttttattttc 1140
tttctcccc ccctccacc cccctctctc taccaccccc ctaaaataat ctcccttttt 1200
ttaatataaa aaacaaaaaa aaaaaaccct cctcttttta accccttccc cttaaatfff 1260
ttatttcccg cca 1273

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<210> SEQ ID NO 17
<211> LENGTH: 1253
<212> TYPE: DNA
<213> ORGANISM: Capsicum annuum

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<400> SEQUENCE: 17

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gcagaaatta aaatacacag gtaagataag tgggaagcaa catctaattg ggatttttct 60
tttcttttca tatttacaga ttgttttgta tttagacaaa ggagaaaaaa atctaaaaat 120
aacagatagt ggcggagcca cctttgctcc aggggttcat ctgaatccct cgatggaaaa 180
ttttaccggt tttataatat ttttacatgg ttaaaaaat ttttattcat gtatagtaga 240
tgttgaacce cgttcgacta gttcgtattt atacttccgt acccctcaa tgaaaaattt 300
ggctccgaca ctgataacag aatgtgatat agacaaacaa caccgtcaga tttctctcta 360
tttataatgg acttgttggtt atttactgtg caatcaaaact aacctttcct ggtagctct 420
tttcatgaaa cttatccaca tgcaagacta cagtacttga aacagataaa agtggtagac 480
ttgtcttagc agatcaacta atgttgact ctggatacga ctccagtggc gggcttgta 540
aaactgcag gaataacatg tgaatttca aaactttcaa gcattcaaca aatatcatct 600
caagtgttga aattccaaag tatatgtcct gaatcttgc ctaatatcca agaaatttaa 660
ataataaaaa aaaaactacc ctaggacgat atatgtagtc tgccgatatg atcttcttta 720
gagcagctct ccaaggatgt gataggggat tgaaaatggg aacatagaag aattgatgaa 780
aaaatttcca agagttcaaa ttatttctac tgggtgaggct gttcatagtt cagataagaa 840
gtgtaccaac aatgcaagtt gcagcaatat ctttctgtg cctgactca atattgagac 900
atccttcttt cgaatacatc tacgcattca caatgaaccg ataaataaaa gattttgtaa 960
atactgatca ttttcagatc aagattatcg aaagatcact tgaatggttg ttaattttaa 1020
aatggtaaca aaaggagat agcaagtcca tttcatgaaa ctccacaatt acagcgtatt 1080
tgacattgaa aaggcaacat atatgcaatt aaaaaaaaa agaaatgagt ctacatcttt 1140
tcaaatacaa aagaaaatca aactcaccat ataaagtcta ccaaaagaaa gtgacatagc 1200
accgaagata tatggactcc ataccacct ttactctgat aaagaagcag tcc 1253

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<210> SEQ ID NO 18
<211> LENGTH: 1323
<212> TYPE: DNA
<213> ORGANISM: Capsicum annuum

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<400> SEQUENCE: 18

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ctaaaatfff agcgtatfff tgtcaacctt ttttaactgac atagcacctt tttagctaac 60
gtggcacctt tgacgtggcc cccattttta tgtaataaag gtgccacgct agcacaaaag 120

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agtgacaaaa atacgctaaa attgagttcg ggggataata ggaccccggtg aagttggagt 180
gtgtcatagc aactttggtc ataatacaag ggggtacaag atgcttatct cgaagaaaaa 240
tgaaaaatca caagcgcaac gtactttata tgtcagagtg tagcgagtgc acttcaagat 300
gtataataat ttgctcgagg atacagtact aattgaagcg aattacatcg tttccctat 360
ctatttaata taagaaaggt tttgccgaag aattgggtga aggtgattag acatgacatg 420
acacaacttc aactgaccga ggacatgac ctatatatga aggcattggag gtcgaggatt 480
agcgtcgaac gttaataggt agacacaagt tgtcatactt ctagtaggat gatttaggta 540
tcacgtagtt tttcccgct atgttcatta ataactgttg ctatacttgt tttgtgtatt 600
gtttttctgt agtctctctt ttttctctta cttccttctt tttttgccc gagggctat 660
cggaaaaaac ctctctaccc cacaaagata gaggtaaggt ctgtttacac tcgatttact 720
gagctcggtc gaggttaagc tgatagctca gaactgaaat gtatattcgt tggtcataat 780
caatctccga accagatcaa cagctccgac accaaggtag ttgagttaag actagcacia 840
ctttaactaa gctcgcgaact tctagtaaag gacgcgtggt tgtgaaacat ggctgttagg 900
ttctttgttt ttctttgaat aaccgagaaa tatgttgttt ttatcttcaa aactcgggtg 960
atgtttaagc ccctttctc tatctttctc cgtttaaata ccaaacttaa ttcaccgata 1020
gaatttgagc tcattgatgtg cgctactac acattctct tctaacaagc atgagagtga 1080
caggaaaggg cagtaaggaa acaaaaga tcatatcaaa agaaaatgtg ggcgaactg 1140
gttgattcct cggtcctttt tccagcaatt ttgcccttgg gagccgctga caactgtaaa 1200
gattagaaca cgggatcttc aactcctct ctccttgtaa gtaagaagac tagttgtttc 1260
actgtaacta ataatgaaac actaacctgt gaggcaatat cgattccaat ttcacgagg 1320
acc 1323

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<210> SEQ ID NO 19

<211> LENGTH: 786

<212> TYPE: DNA

<213> ORGANISM: Capsicum annuum

<400> SEQUENCE: 19

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actccatttg aactcatctc gttccaatgt tatgtcaata cagataaaag cttccacctt 60
gagtaggggc aattttctga aacatatccc ccgctacaaa gtccaaattt tcacttcctt 120
gcagattagc cacgacaggc ggaagatcaa gaacaataca tttcatgtca ggggaatttt 180
ttgttatggc cattgcaaca gtaccatttc tcctccaaca tccactaaag atgtcaatcc 240
tctgaaaaca tccttaaact cgttgccaat cagtacgtta atgaatgatt gcgaatctct 300
agccatgttc gcgtgaaacc agttaccact actcgtatct ctcgaaagtt tactccagaa 360
taaatctcca tatgctgtgt aaaatgcatt tggatcctca tttctgaacc aatcacctaa 420
acagttcgat ccattgctta aagaaaaatg aatcttgatc tccatcaag ttccaaggcc 480
catccttcat tataatocgg tcagctagtg caaccgaata ataccttta ccatcatcac 540
catcgtccac attgtgtctc tcattgtttt gtagaatcaa caagccataa cgaactacta 600
ttggtgtaag gcggtgaaag tcgggattat ttgaagggtc aatagacagg gaagacatga 660
gttttgatag tgtcattggt tttccttgtt tgggtaagtc attaggtatg cctaattgaa 720
gtgcacattt caatgttatg gtaaaagata tgatgaattc acaaacggtt gacacttatt 780

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attttc 786

<210> SEQ ID NO 20
<211> LENGTH: 597
<212> TYPE: DNA
<213> ORGANISM: Capsicum annuum

<400> SEQUENCE: 20

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gaagtagatg tttgagaagc aagtgagaag tgtgaaactcc tctctcgaac agagggagaa 120
gtgtgtcgtt tatgaatact accatcttcc tcatttatga tctgcacaaa tcacaatagt 180
tccacacttt actccacaac atcggcctta aagactatct cggaaaagat gccattatc 240
tgtcttgtaa ggtgctggc agtaatttat aagagaaaag agagtacctt ttggatagca 300
ggatccaagg acgaaagcaa acgcctagaa cgttctggcc aagttcttgc aaacattctg 360
tacaanaatc ttgcagtga tcggacctgt tgttaaatcc aagggatatg aattgccaac 420
agatcttgaa tcgaattaaa ggggggtatac atgcagataa ctatgccaaa atatgatact 480
cagagttcaa caagcatacc tcaccattg catcagagac acaacacttt ataaggtctt 540
catagagctc tgctgaacgt tgtacctcag atgcatcagg ccaatgttca aggatca 597

<210> SEQ ID NO 21
<211> LENGTH: 1085
<212> TYPE: DNA
<213> ORGANISM: Capsicum annuum

<400> SEQUENCE: 21

ggatccacga acctaataag caccctccat ggatacctcc acaaccgctt cctcccattcc 60
acgcaacaac atgcctagtt caatcgccaca aaaggggtct agggagagta gagtatacgc 120
agaccttacc cctaccttgt agagataacc aaaaccctaa aacaatggat caaaactcct 180
gtttcatata gcctctaaaa ccccgcttag cccagtttct gccctctga cctctggctc 240
actgggacct tgccatccca tatagccact tgccaatgga aaactggcta ctcagatgga 300
tgcaagacca atttgataat ggaaacctca tagcacgaaa ggcaaaagttg ttggttatcc 360
taaaaataaa agcgattata ctcaaacgaa aaatgtcatg actgatatct tcataaaaat 420
cttggttcat ttacacattc atagtatact tacaacgaaa gtaataaaaa caaaggtaat 480
atactacctc aacagaaatg gggttgagtt tgagacagtc gaaactacaa tgaattttca 540
ccttcttgc tcttttata gatttcttaa ctttttaaat gaagatgac tagatacttg 600
tactaaatag tccaatagca agatgtatca ggagggactt tggtagcaga tctaacttag 660
ctgaggatgc tacattatcg aagttgcaag aggcgctaata acaataacaa cgacacacgt 720
atagcaaatc tggtttctcc gaaagagagg aggagccttg aaacaacgat aaagttgtct 780
ccatggttca agccgtggaa ttaaccgctg atgcttgta gagtaggctg cctacattac 840
acccctcagt ttgcgacct actcggacta tgcgtgaatg caggaagctt catgcaccga 900
actagctttt ttagtcttcc ataaacctat acgtcaaaga gaaggcttgt tgagtatcaa 960
agttatcacc acaaatgcaa atggcaattg aatgcaatgc accatcacca gcaaaagaat 1020
agacaagcga caaccaata cggcagcata aaacaccaag gcaataaact tcaatctaca 1080
gttgc 1085

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<210> SEQ ID NO 22
<211> LENGTH: 756
<212> TYPE: DNA
<213> ORGANISM: Capsicum annuum
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (756)..(756)
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 22

gatgatgtcc acaggatgca gcatggtecc tatgatgtat cctggattcc cgcaatatat    60
gccacaatg ggaatgaaca tggggatggg gatgagtatg gaaatgggca tgaatcggcc    120
aatagtctct tatcagcogt taatgccaag tccagcaatg cagaatgcag ctgcagtagc    180
acaaatggct cctagatata ctcttcgggc atatacattg ccaccatttc ttgcacctga    240
ttcatccaga atccctgttg cgaatcagcc agatcctccg aggctaaact cacttgttgg    300
acataatacc aatcagccaa aacttcogca ttttgctgat tcatatgatc aatattttgg    360
tctccagcag gcacgactga tgttacocca ggcaagtcct tgttctctc acacactata    420
tttatttatg tccttcaccc attgatattg caccttaacc tcattactgt tcacaccac    480
ctaattgtga gatactgatg aaactcacta acctgactaa tccaaattaa cgcatattta    540
tattatcgtc atgataacta cgttcatgta gaaattatca tctagaatat catcttgcaa    600
cataaactaa actctaaact gtggtcagct gcctacaaca gcttgtttca tcacctcaat    660
ttattgcacg ttcttccaag aaatcttttg acaattcatt gtattgaaat acaggataag    720
ggagtggaac agctgaactg cagtaaaccg aacagn                                756

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<210> SEQ ID NO 23
<211> LENGTH: 481
<212> TYPE: DNA
<213> ORGANISM: Capsicum annuum

<400> SEQUENCE: 23

ggtgtcaaaag aggcacctct ttgtccattt gttgtatccc ccgacacaac atgagtagaa    60
gttacttctc gatctccggt ctcaccttgt ggtgcagcat ttagatccac atttagcacg    120
gtatttcttc tcgagttgga ccttgtagc ttttttggtt gtgcocttct atctcgggta    180
ctcatgttat gctcagttgt accctaatta aaacaggaaa agacggaaaa atttgaagtc    240
atgatagggt ttatattctt aataatctat tacctcaaaa agcaagaata aaaagaagta    300
gtagtaatgc aagtatctca aatttactaa atatacttcc gcatgagcta ttacagtgga    360
ccttaacata caccacggcg atcaatgaat agacttgcaa attgtatggt ttcaagaatt    420
ttagcaagtt gtatttacat tgggctgcca tctggaaaagc aaagcatgga catcggaagt    480
g                                                                    481

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<210> SEQ ID NO 24
<211> LENGTH: 554
<212> TYPE: DNA
<213> ORGANISM: Capsicum annuum
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (154)..(154)
<223> OTHER INFORMATION: n is a, c, g, or t
<220> FEATURE:
<221> NAME/KEY: misc_feature

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<222> LOCATION: (195)..(195)
<223> OTHER INFORMATION: n is a, c, g, or t
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (217)..(217)
<223> OTHER INFORMATION: n is a, c, g, or t
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (469)..(469)
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 24

ggtgttttat gctgccgtat ttggttgcg cttgtctatt cttttgctgg tgatgggtgca    60
ttgcattcaa ttgccatttg cattttggtt gataactttg atactcaaca agccttctct    120
ttgacgtatg ggtttatgga gaactaaaaa aagntagttc ggtgcatgaa gcttcctgca    180
ttcacgcata gtcnagtag ggtcgcaaac tgagggntgt aatgtaggca gcctactcta    240
acaagcatca gcggttaatt ccacggcttg aaccatggag acaactatat cgttgtttca    300
aggctcctcc tctctttcgg agaaacgaga ttgctatac gtgtgctggt gttattgtat    360
tagcgctctt tgcaacttcg ataatgtagc atcctcagct agattagatc tgctacccaa    420
gtccctcttg atacatcttg ctattggact atttagtaca agtatctant gcatcttcat    480
ttaaaaagtt aagaaatcta taaaagaaag caagaagggtg aaaattcatt gtagtttcga    540
ctgtctcaaa ctca                                         554

<210> SEQ ID NO 25
<211> LENGTH: 1216
<212> TYPE: DNA
<213> ORGANISM: Capsicum annuum

<400> SEQUENCE: 25

gacaaaagacc acaagaaagc tctactcadc ttatcttttg ctcttcttgc aatccttttc    60
catcatcctt gtatattctg atccaccatt ttgatcttca atcaattcat aataacatcc    120
aggcactcct tagagaattc acggagaaaa tatcgatggt atagagtttc agattgagta    180
ccacataata gggagccatc atcatcacta agtccaatat gcttcattct tgccttggtc    240
atgagtcggt tatgcatggt tatctaacat ataaagctat gcgttggtat attggattta    300
ttccacactc ccctccattc aggccagcaa gagccaatcc ctatttacag atatagccac    360
tcagaatagt atattgtcat tccttttaag ccatccattg tgaacatgcc ctgccacaaa    420
tttagccttt agtccacaaa ttttcttcca gtggcaacat gaataagtag gggcatttaa    480
actttaccaa gacaaaata gaagtcaatc ctcaacccca gatagctatg gtatagatgt    540
ctacctcaag tagctatggt ttctttctat caacagtgac aaaattaaaa tatacatctc    600
gtaatagagt tacttttttag taaggatat attacaaaaa gtggaagaag tcacgtaaca    660
aactatgatc agttagtgtg ctcaatcgtc tactgttgta ccgactagct ctgatacaag    720
gcttttttgt gtcactaatc tagatgcact agaaatacaa taggcagctc ctatcatcat    780
atccagcagc attaggcacc gaagtgccaa acccattgga gacgacaata actatcggca    840
tgaaacattc atggagaacc ctctttttct caatagtctc ccccacgtct ctctctctct    900
ctctctttgg tgtgatgta ataggtactc ttggaaaaag acaagttaac tctaagagaa    960
aggagtcatt caaaaactca aaccagaact tgcagcacac ggggctctcc tattatcctc    1020
agttaaagaa tgggtatcat caatctcaag tataaaatgc tcagcacata cgacatgtca    1080

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agtatccttg ttttggatoga cctgaacact atattattaa actcactagg gaaagagcca 1140
atccttttga caactcaaag cagcaagtaa aaaataagta atctacacct atcgactatc 1200
cttctageta ttcctc 1216

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<210> SEQ ID NO 26
<211> LENGTH: 893
<212> TYPE: DNA
<213> ORGANISM: Capsicum annuum

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<400> SEQUENCE: 26

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gaactcctct tgtgattaaa tcttctagcc atgatctgtc tttacacggt ggctccttca 60
tgagctctct agtatgcaag catttgttcc cattttaatt ctttgggtca ctatctgtgg 120
gcatgggaca ttgtattca atagggcctc attcctttgcc atccaaatgt ggtatattag 180
cgctgcggtc tctgctcata tgggttcctc gctaaccat ccatttgttc gtctcactat 240
cctagtccag aaccctcoga tgggttttatt ttttaccctt attttttagca attggagggt 300
ctcttctaag ctgacttttg aaaaaccgct ctcgaaagat aaagatcatt agtttcattc 360
tcccttcac atagtaggca gacttcgtct tggcatatcc ccattcagta taatcgatct 420
cttgtaata gtctccctcg tgcattagta aacaacaaat gaggctgtgc tttggaacat 480
ttactctatt ccatacacct ctccaagtgg tccataggcc tcttctctcc tttgtccaag 540
tgcagcaact tctgattgtg tatttgctg aacttgtgag ccagccattg tgtacatagc 600
ctggagcaaa cactgttttc actctgcaaa tcattttcca gtaccagcag atattaatgg 660
aggtctata tccccaccag ttgtcacctc ttaagtaaag tggttcaccc atgtaacca 720
aaggttatca gcttttcttg ctttgttcca tacttatttc gctactgctg cttcattcca 780
agatacagtc tctcaccctc aaacttcgct ctttctttgt tctacaaatc atateccaat 840
atatatagga ggtagttagc ctgtgttaac atttccactc catataaagt tcc 893

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<210> SEQ ID NO 27
<211> LENGTH: 1622
<212> TYPE: DNA
<213> ORGANISM: Capsicum annuum

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<400> SEQUENCE: 27

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cgttgataaa cactagaaga gaatatgtga atggttcctg cacataaaat agattttaca 60
tatgaagttg tctcattat ttgtatttgt gatgatgcag gacatgtctg aagaaaacta 120
tacaatcatg ttaatctcag tggttgttat aacaggagtt gtatcacctc ttgttaaggt 180
tctatatgac ccttcaagta agtacattgc ctacaagagg aggacaataa tgcattagcag 240
agaaaaagat gaatttcgta tacttgcttg tatacacagc caagaaaatg ttcgcgagct 300
catcagttta cttcaagtct cgaatcctac aaaagaaagt tgtattaatc tagtagttct 360
tcatcttact aagctaacgg gccgtgctc ctcagttctc atagcccac aaagcgtgta 420
caggccatca acaaatccaa cacaatctga aagaatcttc aatattttcg aaaaacttga 480
gcaacaaaac agtgacctca ttatggtaca ttgctacaaa ggagtctcac catatgtaac 540
aatgcacaat gatgtttgtt ccttgcttt agaaaagaga acaactetta tcattgtccc 600
ttttcacaag cattggatgt gtgagaaaag aatcgaaaca tcctatgcat atcgacatct 660
aaacaagaat gtacttgaga aatctccttg ttcagtcggg atactgattg atcgaggcaa 720

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caaaaaaaaa ctcgttatgc aatcacagta ccatcattat atagagtcgt ggtactattt 780
tttgccggag cagatgatcg cgaagctttg tcatatgcag aaagaatgtc caaacatcct 840
agtgtgaaaa tgacattcat acgtttcacc agatcaggta atgcatttga aaatgttgtt 900
ggtggatcgg agagaagtaa ggttcttgat tcacaaatct tgaatgaatt caatctocaa 960
taccttcatt cagaacaagt ttcttatcaa gaagcagagg tgaaaagagg cgcggacgtg 1020
ttagaagtta ttaaatcttt gggacgtttt tatgatcttg ttatgggttg taaacgtcac 1080
gtagactcac caatattggt gcaattgaca aagaggactg atgaggatgg tgaattaggg 1140
attgtaggag gcattcttgt cgcctcagat ttgaaagtg agacttcagt ttggtggtg 1200
cagcaacaaa ccagattatg gggacttcat gatcctgaag agtctacaca ttgagaagg 1260
ataaactaat tatagttgta tagacgtatg tcgctctgac tcaatcaaaa atgtcaatgg 1320
atgtgtgtta catcctgaag tagtctacac atttgagaag gataaactaa taattgtagt 1380
tgtatagaca tgttgctctg actcatcaaa aatatcaacg agtacgtgtt agatcttgaa 1440
gagtcttaac acatttgaga aggatcaact tatagttgta tagacctata tcgctcgaa 1500
acttcaaaaa atgttgatgg tgcgtgcaag accttcaaaa agaagtgctt ttttcgagaa 1560
tctaacacag gtgctgcact atagtgtcac ctaaactcgt tacgatttag gtgacactat 1620
ag 1622

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<210> SEQ ID NO 28
<211> LENGTH: 393
<212> TYPE: DNA
<213> ORGANISM: Capsicum annuum

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<400> SEQUENCE: 28

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atcttgttca cttatgagga catctgtcac aggctacatg attaaagtgt gtaattcttt 60
ggtctcatga aaagcaaaga agaaaactac atttttaagt agttcagctt aagctgaata 120
cagaagcctt gcttgactg tgcagagtt agtatggta cttagaatac tgaagaagt 180
aaatgctgaa gttcagttac cagttcaagt ttatagtac aacaaggttg ttattcaaat 240
tgcagtcaat ccagtgttat catgaaagga acaaaccata ttgaaattga ttgtcatttc 300
acaagagaaa aattgcaaca aggcattgatc aaagttaact atcattctac tcaagaacaa 360
ccagttgatg tgttgacaaa aggccttatct aga 393

```

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<210> SEQ ID NO 29
<211> LENGTH: 733
<212> TYPE: DNA
<213> ORGANISM: Capsicum annuum

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<400> SEQUENCE: 29

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gcaagagtta tcaactaaag gaaagaactt gataggcaga aagccaagtt tcacaagcta 60
gattttctgc actaactctg attttgaaat cttgtactac ttttaagtat cgattacaag 120
cacgacatgt tcattagctt cctacttggt tatcgtaatt atcaaaactt gatttagatg 180
gagtcttggg acagacttta ttgtgataaa aagttgcaga ctttttgtga gagatgtagt 240
tacaacgata gctactgatt gactcgtgaa aaataattga actggatgct ccaatgggac 300
gacctagcca atgatcgagt tggagtatag accttgaac caagattcta attccagtag 360
aggtgataca aagtgaaatt ttccatcta cctgggcata gttagctgat atcttttctg 420

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gtgggaggt acaagtacac ttaagttagt tgaggagtgc gtgacctcc cgctagctca 480
ggagtgtgca agcttccacg aataccatga tatctttaa aggaggagt gatatggta 540
caggctcttc caagtcttgc ttcttataca ttttgtactg gcaacaagt tgctgaaggg 600
tagatttgat tacaaaaag ctatttacga caaagatatt gctgtcatgt acttgtacat 660
tgtatttctc tttgttttat tattctttgc tagccgaaca aatttgcttt actctgtgat 720
ttgcagtggt gat 733

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<210> SEQ ID NO 30
<211> LENGTH: 730
<212> TYPE: DNA
<213> ORGANISM: Capsicum annuum

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<400> SEQUENCE: 30
gcaaagaact gcagaattta ctcccacact aactgacat aattacactt caatgtatac 60
atatttgcaa ctttgtcatc atctctacat tctcttttcc cgctacgcca gctggccttt 120
ttatagggtt tccacctaa gatcttggtt tggtaaatgt attttttatt gagcatcggt 180
tcaaagatta gaatcttaga aaaggaaaag cagccataag ggacgtgaaa tttgggtaac 240
agcttaatta ggctaaacgc ttactacaac aacaacaaca acataccaag tgtaatccca 300
gtattgataa agaaaccocag acgataacat aatatgtacc tggaaatgca aatcacagta 360
ttgatacacc atcataagat attgacgaac acattatagt atagtacaac tgtgacaaat 420
ttaaacctag taaaatttgt tgtgggaact caaacaattt ggattcaaga agaaaccaag 480
gaactagata attttagtaa tttttaggac caaagaaaaa aaagggtgtt ttaaaaaaat 540
catttctttg atttcttga atcttctcct attttccttt tgcttcttct cgagatgctg 600
aagtgatctt ctctgttaaa gtcacaaaat gtgtttaata aaagggtggg gaaatacata 660
tagagctcog taaacttggc aacaattttc acttgaatac ctctacttga ggcttgtacc 720
tattagatac 730

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<210> SEQ ID NO 31
<211> LENGTH: 430
<212> TYPE: DNA
<213> ORGANISM: Capsicum annuum
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (39)..(39)
<223> OTHER INFORMATION: n is a, c, g, or t
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (45)..(45)
<223> OTHER INFORMATION: n is a, c, g, or t

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```

<400> SEQUENCE: 31
tttattaaca cattttgtga ctttaacaga gaagatcant tcagnatctc cagtagaagc 60
aaaaggaaaa taggagaaga ttcaaagaaa tcaaagaaat gattttttta aaaacacctt 120
ttttttcttt ggtcctaaaa agtctacaaa ttatctagtt ccttgggttc ttcttgaatc 180
caaattgttt gagttcccac aacaaatttt actagggtta aatttgtcac agttgtacta 240
tactataatg tgttcgcoaa tatcttatga tgggtgatca atactgtgat ttgcatttcc 300
aggtacatat tatgttatcg tctgggtttc tttatcaata ctgggattac acttgggatg 360
ttgttgtgtg tgtttagta agcgttttagc ctaattaagc tgttaccxaa atttcaogtc 420

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ccttatggct 430

<210> SEQ ID NO 32
 <211> LENGTH: 1887
 <212> TYPE: DNA
 <213> ORGANISM: Capsicum annuum

<400> SEQUENCE: 32

tatagtgcag caggaataact tttgaatcag agaaagtatg tgmtggaact tatatctaag 60
 ttaggtttga gtgggtcaaa acctgctccc acacctttgg aacttaataca caagctcacc 120
 acaatggagt atgatgagat tacagataat actgctggag atgaactatt gagtgatgta 180
 agttcatatc aaaagttgat tggaaagttg ttgtatgta ccatcacaag gcctgatatc 240
 agcttctcag tgcaaacctc gagtcagttc atgcagcaac ccaagaaatc acattgggaa 300
 gctgcactca gaactgtaag atacctgaaa aatgcaccag gtcaaggaat ccttatgaaa 360
 tctggtcata ctcaacaact tacatgttgg tgtgactctg actgggcagc atgtccaaac 420
 accagaaaagt cggttactgg ttttgacgtg aaatttggag agtccttgat ttcttggaa 480
 caaagaagca acaaaactgtc ttaaggagct cagagtatag aagccttgca gcagcagtag 540
 cagagttgac atggttgcaa ggattatttc aggagctgga tgmtcacatt gacaaacct 600
 ttgcagtctt cagtgatagt aaactctgcca gattgcagag aatcccatat ttcacgaaa 660
 aacaaagcac atcgagatcg attgccattt tatcagagac gagattaaag aaggagtgg 720
 taaggctgtc tatgtgaata cgaaggaaca agaagctgac ttgctgacta aggccttgac 780
 tacttctcaa cacatgcatt tacttggcaa gcttggagtg ttcaacattt tggaccctcc 840
 agcttgaggg ggagtattaa agtcaattag agtcagttag ataggtgctt agctgaagtt 900
 agttcggtt gttagtggta gttgaagttt gttagtatg gaagttgta gccagctgct 960
 atgtccagct gtcaatgatt aagttagtgg ggcaacaatg agggaggtta ctagaagctt 1020
 atgagcttgt gtatatatat tctattccag attgaataat caattaattt cattaccaca 1080
 aaatatcttc tttctcaatt cctatcataa actagcaatt ctgtcacttg atgttgcttc 1140
 agttttgggt atgggattca ctggactctg gtgttttcga tttgaaatca tctattccat 1200
 aatgatgact catagactga tagtacagtc tattggcccc tgtagagtt tatgaccg 1260
 attttggtga tccggccctg aaaagttcac ggtgcgatcc atgtttgtgc tttttgtgg 1320
 gtctgtggtg gtgatgtttg tgagcttagg atttatttgt atgcacgtat tatataagt 1380
 catttagtgg gctatttcga gcagttttca catacacgta attgagacta tcgtctccac 1440
 cttgtattcc tctcttccat agtgaatttc ctctctctgc ccgtgggttt tcccattgg 1500
 ggtttccaog taaatatgtg tgtctctctt gtttttattt tgcttgggtt attgcctatt 1560
 ctgtccgatc ataacaactc caaatatatt tgttcaaac tgggaaggatg gtcgaaggag 1620
 tatggagagt ttttcgagcc ttgtgcatac ttagtogaac gaacttccaa gagatgttct 1680
 ttggtgagaa attgttaatt ttcttgaaca cctgaaatga aacaactacg agaaaatgga 1740
 ttggcaaaaca ctattgtttg acgaattgca tcacgtgttg ctgacagaag acaaaaggat 1800
 caattgata ataaatgta ccggtatgtt gggctctaac aacaacaatt ggattgatct 1860
 tttgctaatt gaggagtttc acacgtc 1887

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<210> SEQ ID NO 33
<211> LENGTH: 1024
<212> TYPE: DNA
<213> ORGANISM: Capsicum annuum

<400> SEQUENCE: 33
gaactcaata gcccttcttt tattccagct ctaacgggac cttcacatth tctttcgtac    60
aattgcacca ccttctcttc aacagagaaa gaagccagtg ttctgatact tccaactgct    120
tcactagcaa cttgactcgc atcctcgtat agtttctaca agaaaatctt acttaataaa    180
gcactgttag agtgtgtgcc ctactaattt taatatttta ctctactggt tctctatctg    240
tgcaacaagt cagtaaacgt ggttcacata attaaaagct gaacacagtg tttttaagtg    300
aaaaacaccc ggctcaagaa aggtgtaaag gctcaagaaa agtgtaaaaa accacgacct    360
atacctctac agtattttaa ccctactta ctataactct tgagcctcaa caatcaacaa    420
tattacaaga ctttttgtaa aactaggaat taaaacttcc aactcctata ctacaaaaac    480
acaaatcttc ccaagtcttc gagttcctta acttgaagct cctaactcag tttatagatt    540
actgagacta gaacaatgac tcattacaac tcaaagaacc tatctactac aaacagtcta    600
agactttgct aactaaagga ccaggttctt cttcaagtaa gtgaactggt ttgctgattg    660
ttgattatct tatcagtgca tgagtgaatg ctttgaaagt tgtttcttcc atttaagcca    720
acacctgatg tatatttota tctcactag gactcccagt tagtttctact cattctttac    780
tgccctctct ccctctgtgt gtttgaatag gactcttctt gattatctca taatttctgc    840
aagacttctt atcgaccga acttcttcaa gatttcttat tcaactcaac ttctgcaaga    900
cttctgttcc aaaccaaact tcttcaagat ttcttggtca actcagcttc tgcaagactt    960
cttgttgtga taagtgttat ccactcccat tttgcacgca gattcagttt ttcaataact   1020
tgcc                                                                                   1024

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<210> SEQ ID NO 34
<211> LENGTH: 782
<212> TYPE: DNA
<213> ORGANISM: Capsicum annuum

<400> SEQUENCE: 34
cacaatgctt agcagttccg ctgtttctct ctaattgtat ccggtcaaaa atacaagctt    60
gtgacagatg aatcttagac tctctgacta cttgtaattc tatctacaat ttagccatct    120
ttctctatca tttgcttttg aaatggagtg acatgaacaa tgaggattca ttcattcagt    180
cgatctccac ttgctttgca ttgaggagtt ttttctctat catttgcttg agagttcaag    240
tgtctgggat ggacaatgty gtcgttaaca ttgcctcttc tcatccaggt ttgttctttt    300
gcttcttaac tgaggggtat ttcttggttc cggagaatct tttttttggt aactaacata    360
ttcattttat taccaaagta atattacaaa tcaactacca gcctgatgac acaacctggt    420
agactcaaag aagaacccta ctgatcagac taataataat gaggatagta gtttagatca    480
ttgatcacag ctgctaactt atgcctaaca gtagcaatac aaataacatc ctgaaatact    540
agcctaacca ctttctccgg tgtagtactg attgtcctga caatcctaca attcctatca    600
tgccatatat ggtaaacgca tgcagcagta ccactcctgaa tatgtgttga ggctgatcga    660
cctttgacat tgttctcaat ccactcaagt tcaactggacc aagccaaagc aatcctttga    720
atcctttatcc agccgagcaa agcattocca cagtttagca gcatagetac acctaaagaa    780

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ct 782

<210> SEQ ID NO 35
 <211> LENGTH: 1641
 <212> TYPE: DNA
 <213> ORGANISM: Capsicum baccatum

<400> SEQUENCE: 35

gaaatttggt cttcttcttt ccttctcttt ttgttttgg attttcctca ttgcaagtag 60
 aatgtctct aacggctatg gttttaatag ttcacccgag aagagagtta ctgctgttgc 120
 catcagtaat gacggtcggt ttgtagcttt cgcggataaa ttgggtgtaa tttatgcagt 180
 tgaatatagaa ggttatcatg aaaatcaaac tctgcccgat aagaaggcag tcccgattct 240
 tgcccactat tgcagcatca ttactagtct ggtatgttcc tttcctttcc tcttcatgtg 300
 aatttgcttt agttttcgat gagtcaattt ttgtatccta tataatagag ggtagcagt 360
 ttactcagat attgttacga tactcatgat ctatgttgct cgaactcttt aaaaatgcta 420
 ttagtgcat gtcggattct tcaaaagtag ggcatttttg gaggatccga cacgagtatg 480
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What is claimed is:

1. A *Capsicum annuum* plant comprising a *Capsicum baccatum* cytoplasm, wherein said plant comprises a chromosomal segment on chromosome 6 that confers male fertility in a male sterile *Capsicum annuum* plant comprising a *Capsicum baccatum* cytoplasm relative to a plant lacking said chromosomal segment.

2. The plant of claim 1, wherein the chromosomal segment is further defined as:

(a) flanked by Marker M29 (SEQ ID NO: 29) and a marker selected from the group consisting of Marker M14 (SEQ ID NO: 14), Marker M15 (SEQ ID NO: 15), Marker M16 (SEQ ID NO: 16), and Marker M17 (SEQ ID NO: 17) in said plant; or

(b) located between 26,405 bp and 213,924,156 bp on chromosome 6 of public pepper genome sequence Pepper CM334 v.1.55.

3. The plant of claim 2, wherein said chromosomal segment comprises a marker locus selected from the group consisting of Marker M18 (SEQ ID NO: 18), Marker M19 (SEQ ID NO: 19), Marker M20 (SEQ ID NO: 20), Marker M21 (SEQ ID NO: 21), Marker M22 (SEQ ID NO: 22), Marker M23 (SEQ ID NO: 23), Marker M24 (SEQ ID NO: 24), Marker M25 (SEQ ID NO: 25), Marker M26 (SEQ ID NO: 26), Marker M27 (SEQ ID NO: 27), and Marker M28 (SEQ ID NO: 28) on chromosome 6.

4. The plant of claim 1, wherein said chromosomal segment comprises:

(a) the haplotype of variety Ganti, wherein a representative sample of seed of said variety has been deposited under NCIMB accession number 43055; or

(b) the haplotype of variety Flame Fountain, wherein a representative sample of seed of said variety has been deposited under NCIMB accession number 43054.

5. A seed that produces the plant of claim 1.

6. A plant part of the plant of claim 1.

7. The plant part of claim 6, wherein the plant part is a cell, a seed, a root, a stem, a leaf, a flower, a fruit, or pollen.

8. The plant of claim 1, wherein the plant is a sweet pepper variety.

9. The plant of claim 1, wherein the plant has a blocky type fruit shape.

10. The plant of claim 1, wherein said plant further comprises a chromosomal segment from *Capsicum baccatum* on chromosome 6 that confers uniform female fertility in a male sterile *Capsicum annuum* plant comprising a *Capsicum baccatum* cytoplasm relative to a plant lacking said chromosomal segment, wherein said chromosomal segment from *Capsicum baccatum* is flanked by Marker A12 (SEQ ID NO: 35) and Marker A35 (SEQ ID NO: 36) in said plant.

11. A method for producing a *Capsicum annuum* plant that confers male fertility in a male sterile *Capsicum annuum* plant comprising a *Capsicum baccatum* cytoplasm, comprising introgressing into said plant a chromosomal segment on chromosome 6 that confers male fertility in a male sterile *Capsicum annuum* plant comprising a *Capsicum baccatum* cytoplasm relative to a plant lacking said chromosomal segment.

12. The method of claim 11, wherein said introgressing comprises:

a) crossing a plant comprising said chromosomal segment with itself or with a second *Capsicum annuum* plant of a different genotype to produce one or more progeny plants; and

b) selecting a progeny plant comprising said chromosomal segment.

13. The method of claim 12, wherein selecting a progeny plant comprises detecting at least one allele on chromosome 6 flanked by Marker M29 (SEQ ID NO: 29) and a marker selected from the group consisting of Marker M14 (SEQ ID NO: 14), Marker M15 (SEQ ID NO: 15), Marker M16 (SEQ ID NO: 16), and Marker M17 (SEQ ID NO: 17).

14. The method of claim 12, wherein the progeny plant is an F₂-F₆ progeny plant.

15. The method of claim 12, wherein said crossing comprises backcrossing.

16. The method of claim 15, wherein said backcrossing comprises from 2-7 generations of backcrosses.

17. A *Capsicum annuum* plant produced by the method of claim 11.

18. A method of producing food or feed comprising obtaining a plant according to claim 1 or 17, or a part thereof, and producing said food or feed from said plant or part thereof.

19. A *Capsicum annuum* plant obtainable by a method comprising the step of introgressing into a plant a male fertility restoration locus allele for *Baccatum* cytoplasmic male sterility, wherein said male fertility restoration locus allele is defined as located in a chromosomal segment on chromosome 6 flanked by Marker M29 (SEQ ID NO: 29) and a marker selected from the group consisting of Marker M14 (SEQ ID NO: 14), Marker M15 (SEQ ID NO: 15), Marker M16 (SEQ ID NO: 16), and Marker M17 (SEQ ID NO: 17).

20. The *Capsicum annuum* plant of claim 19, wherein said introgressing comprises backcrossing or marker-assisted selection.

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