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(71) Applicant(s)
Enza Zaden Beheer B.V.

(72) Inventor(s)
Heidman, Iris Alke;Lindeman, Wouter

(74) Agent / Attorney
Fisher Adams Kelly, Level 29 12 Creek Street, Brisbane, QLD, 4000

(56) Related Art
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(71) Applicant *(for all designated States except US)*: **ENZA ZADEN BEHEER B.V.** [NL/NL]; Haling 1E, NL-1602 DB Enkhuizen (NL).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **LINDEMAN, Wouter** [NL/NL]; Kruideel 79, NL-1602 GK Enkhuizen (NL). **HEIDMAN, Iris Alke** [DE/NL]; Seyndersloot 20, NL-1602 HA Enkhuizen (NL).

(74) Agent: **HOOIVELD, Arjen J.W.**; Arnold & Siedsma, Sweelinckplein 1, NL-2514 GK The Hague (NL).

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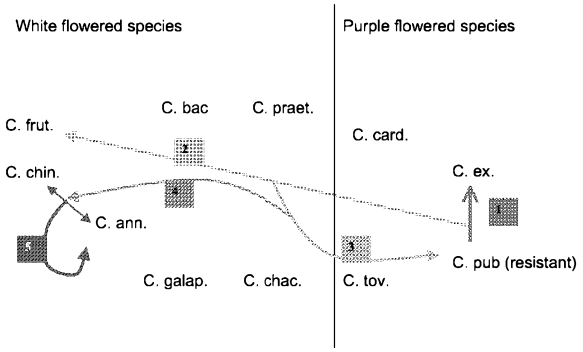
(54) Title: METHOD FOR TRANSFERRING ONE OR MORE GENETIC TRAITS FROM A PLANT OF THE PURPLE-FLOWERED *CAPSICUM* SPECIES TO A PLANT OF THE WHITE-FLOWERED *CAPSICUM* SPECIES



(57) Abstract: The present invention relates to a method for transferring one or more genetic traits from a plant of the 5 purple-flowered *Capsicum* species comprising said genetic trait, to a plant of the white-flowered *Capsicum* species, said method comprising the steps: (a) preparing a first hybrid plant comprising said genetic trait by crossing a first plant of the purple- 10 flowered *Capsicum* species comprising said genetic trait with a second plant of another purple-flowered *Capsicum* species, and selecting plants comprising said genetic trait; (b) preparing a second hybrid plant by crossing said first hybrid plant comprising said genetic trait, with a 15 first plant of the white-flowered *Capsicum* species; (c) crossing said second hybrid plant with said first plant of the purple-flowered *Capsicum* species comprising said genetic trait, and selecting from the progeny thereof plants comprising the genetic trait; 20 (d) preparing a third hybrid plant by crossing the plants obtained step c) with a second plant of the white- flowered *Capsicum* species, and selecting from the progeny thereof plants comprising the genetic trait. The invention further relates to the plants per se, as well as to the 25 fruits, seeds and other plant parts derived from said plants.

WO 2007/038980 A1

METHOD FOR TRANSFERRING ONE OR MORE GENETIC TRAITS FROM A PLANT OF THE PURPLE-FLOWERED CAPSICUM SPECIES TO A PLANT OF THE WHITE-FLOWERED CAPSICUM SPECIES

The present invention relates to a method for transferring one or more genetic traits from a plant of the purple-flowered Capsicum species comprising said genetic trait, to a plant of the white-flowered Capsicum species. The invention further relates to the plants per se, as well as to the fruits, seeds and other plant parts derived from said plants.

The genus Capsicum belongs to the large family of the Solanaceae. Several species of the Capsicum group are valuable crops that are grown in open fields or under protected conditions in many countries all over the world. Capsicum species with pungent fruits are generally used as a spice, either fresh, dry or extracted. Species with non-pungent fruits in a big variety of shapes and colours, commonly known as sweet peppers, are widely used as vegetable.

The genus Capsicum can be divided in two distinct groups based on the flower colour: the white-flowered group, comprising e.g. the closely related species C. annuum, C. frutescens, C. baccatum, C. chacoense, C. galapagoense, C. praetermissum and C. chinense, and the purple-flowered group, comprising e.g. C. tovarii, C. pubescens, C. eximium and C. cardenasii. Of the white-flowered group Capsicum annuum, and its closely related species C. frutescens are the best known domesticated species, which are mainly used for the production of sweet and hot peppers for processing and consumption.

Capsicum, like other plants, is attacked by many

pests and diseases, including insects, nematodes, fungi, bacteria and viruses. One of such bacterial diseases is bacterial leafspot, caused by the bacterium Xanthomonas campestris pv. vesicatoria. Bacterial leafspot of Capsicum can be recognized by numerous angular spot on the leaves. Initially, the spots are water-soaked. Leaves infected at an early stage become deformed. Often the margins of affected leaves are rimmed with a narrow band of necrotic tissue. Infected Capsicum leaves drop prematuraly, by which the fruit is exposed to sun which may result in sun scald, secondary fruit rots and reduced yields. Capsicum fruits rarely show symptoms but may drop if infected early.

X. vesicatoria is widespread and damaging to Capsicum in particular in field-grown crops in warm-temperate and tropical countries, and thus has a large economic impact. Control of the disease relies principally on the production of plants from healthy (treated) seeds and on preventive steps taken during the entire season.

Useful resistances to such diseases, as well as other traits such as fruit quality characteristics etc., may exist in wild or other domesticated species of Capsicum, such as for example in C. pubescens which carries unique and specific traits, such as the resistance against certain races of the bacterium Xanthomonas vesicatoria, that are not present in the white flowering species. It thus may be advantageous to transfer such favourable genetic traits, such as any resistance gene, from the distinctly related purple-flowered C. pubescens to the commonly used white-flowered species such as C. annuum and/or C. frutescens.

It is well known, however, that strong crossing barriers of varying degree exist between the different species of the Capsicum genus. Some crosses have even been proven impossible, and other crosses are only possible with

the help of in vitro culture techniques, such as embryo rescue. In addition, in most cases the products derived from said crosses are completely or partially sterile which makes them not accessible for any further breeding purposes.

A need therefore exists for a method by which desirable genetic traits can be transferred between the different purple and white flowered Capsicum species.

The object of the present invention is to provide a method for transferring one or more genetic traits, such as, but not limited to, resistance genes, from plants of the purple-flowered Capsicum species to plants of the white-flowered Capsicum species.

This is achieved by providing a method, comprising the steps of:

(a) preparing a first hybrid plant comprising said genetic trait by crossing a first plant of the purple-flowered Capsicum species comprising said genetic trait with a second plant of another purple-flowered Capsicum species, and selecting from the progeny thereof plants comprising said genetic trait;

(b) preparing a second hybrid plant by crossing said first hybrid plant comprising said genetic trait, with a first plant of the white-flowered Capsicum species;

(c) crossing said second hybrid plant with said first plant of the purple-flowered Capsicum species comprising said genetic trait, and selecting from the progeny thereof plants comprising the genetic trait;

(d) preparing a third hybrid plant by crossing the plants obtained step c) with a second plant of the white-flowered Capsicum species, and selecting from the progeney thereof plants comprising the genetic trait.

According to the present invention, for the first time a genetic trait has been sucessfully transferred from a

purple-flowered Capsicum species to a white-flowered Capsicum species. The method of the present invention is based on a suitable combination of several interspecific crosses, as well as crosses that serve as a kind of bridge to the next species (Figure 1). The desired genetic traits can for example be followed by molecular markers or bio-assay according to well-known molecular biological techniques known to the skilled person.

According to a preferred embodiment of the present invention, the method further comprises:

(e) selecting plants obtained in step (d) not comprising the genetic trait and crossing these plants with said first plant of the purple-flowered Capsicum species comprising said genetic trait, and selecting from the progeny thereof plants comprising the genetic trait; and

(f) preparing a fourth hybrid plant by crossing the plants obtained step (e) with a second plant of the white-flowered Capsicum species, and selecting from the progeny thereof plants comprising the genetic trait. In this way, plants that are obtained in step (d) of the method of the invention that do not comprise the genetic trait, still can be used further in the method of the invention.

According to a further preferred embodiment, the method further comprises:

g) preparing a fifth hybrid plant by crossing the fourth hybrid plant comprising the genetic trait with a second plant of the white-flowered Capsicum species, and selecting from the progeny thereof plants comprising the genetic trait. Thus, a stable white-flowered plant is obtained comprising the genetic trait of interest derived from the purple-flowered species but with all the quality aspects of the white-flowered species.

Preferably, after step (b) the second hybrid plant is

self-pollinated and the progeny thereof is used in step (c) in order to fix the resistance, to improve fertility aspects and to obtain homozygous, genetically stable plants carrying the desired trait(s).

According to another preferred embodiment, the plants obtained in step (c) are self-pollinated and the progeny thereof is used in step (d), again in order to fix the resistance, to improve fertility aspects and to obtain homozygous, genetically stable plants carrying the desired trait(s).

In addition, according to other preferred embodiments, the plants obtained in step (d) are self-pollinated and the progeny thereof is used in step (e), and/or the plants obtained in step (e) are self-pollinated and the progeny thereof is used in step (f).

According to the present invention embryo rescue techniques are applied whenever it is beneficial to the process in terms of obtaining hybrid plants or speeding up the process.

Preferably, the first plant of the purple-flowered Capsicum species is selected from the group consisting of C. pubescens, C. eximium, and C. cardenasii, more preferably the first plant of the purple-flowered Capsicum species is C. pubescens. The greatest genetic distance in the genus Capsicum is between the species C. pubescens and C. annuum/frutescens. Direct crosses between these species have been proven impossible until now, even with the help of embryo rescue. By using the method of the present invention, however, a totally new gene pool becomes accessible to the species of C. annuum and/or C. frutescens.

According to the invention, the second plant of the purple-flowered Capsicum species is selected from the group consisting of C. pubescens, C. eximium, and C. cardenasii,

preferably the second plant of the purple-flowered Capsicum species is C. eximium or C. cardenasii, preferably C. eximium. Preferably, the first plant is selected from another species than the second plant of the purple-flowered Capsicum species.

In a preferred embodiment of the invention, the first plant of the white-flowered Capsicum species is selected from the group consisting of C. baccatum, C. frutescens, C. chinense, C. annuum, and hybrids thereof. Preferably, the first plant of the white-flowered Capsicum species is C. frutescens.

The second plant of the white-flowered Capsicum species is preferably selected from the group consisting of C. baccatum, C. frutescens, C. chinense and C. annuum, and hybrids thereof, more preferably the second plant of the white-flowered Capsicum species is C. chinense, C. annuum and/or a hybrid thereof.

According to the present invention a hybrid is a plant obtained from a cross between two different species of the Capsicum genus, e.g. a cross between a plant of the species C. chinense and a plant of the species C. annuum (interspecific cross), or a plant obtained from a cross between populations or cultivars of a single species (intraspecific cross).

According to a further preferred embodiment of the the invention, the genetic trait is a resistance gene against a specific Capsicum disease attacking all cultivated species of Capsicum. By transferring one or more resistance genes from distinctly related species of the Capsicum genus to commonly used species not naturally having such resistance genes, new resistant plants are provided. As a consequence, the use of environmental undesirable chemical treatments may for example be reduced or even abandoned. However, also other

genetic traits, such as other resistance genes, fruit quality characteristics such as shape, taste and colour, improved yield, fruit set, and resistance against abiotic stress (e.g. cold/salt) may be transferred using the method of the present invention.

In a particular preferred embodiment, the resistance gene is the Bs4 gene, conveying resistance to the bacterial disease Xanthomanas campestris pv. vesicatoria race I, II, IV and VI.

The present invention further relates to plants, obtainable by the method as described above.

In particular, the invention relates to plants of the white-flowered Capsicum species, comprising a genetic trait derived from a plant of the purple-flowered Capsicum species, said plants comprising a genetic trait which normally is not present in the white-flowered species.

In a preferred embodiment, the plant is selected from the group consisting of C. baccatum, C. frutescens, C. chinense C. annuum, and hybrids thereof, preferably, the plant is C. chinense, C. annuum and/or hybrids thereof.

According to a preferred embodiment of the invention, the genetic trait is a resistance gene against a Capsicum disease, in particular the resistance gene is the Bs4 gene, conveying resistance to the bacterial disease Xanthomanas campestris pv. vesicatoria race I, II, IV and VI.

Furthermore, the present invention relates to fruits, seeds and other plant parts derived from the plants described above, wherein the other plant parts may be selected from the group consisting of seeds, cuttings, runners, and meristem.

The present invention is illustrated by the following Example and Figures, which are not intended to limit the scope of the invention in any way.

Figure 1 schematically shows the steps of the method

of the present invention;

Figure 2 is a photograph of a C. eximium plant (A) and a C. pubescens PI235047 (B) plant with the desired resistance gene used in a first step of a preferred embodiment of the method of the invention;

Figure 3A shows an example of a first hybrid plant, i.e. the product obtained in the first step of the method of the invention; figure 3B is a photograph of C. frutescens PI238059;

Figure 4 shows an example of a second hybrid plant (A), and photographs of C. annuum (B) and C. chinense (C);

Figure 5 is a photograph of the product of the self-pollinated ((C. pubescens X C. eximium) X C. frutecens) X (C. chinense X C. annuum); and

Figure 6 shows C. annuum plants carrying the resistance genes derived from C. pubescens.

EXAMPLE

Methods:

Seeds were sown on vermiculate, potted into rockwool, blocks, and fertilised and propagated until flowering. Flowerbuds were emasculated and pollinated with the chosen fatherlines. Between 24-28 days after pollination fruits were harvested, surface sterilised and opened under sterile conditions. Embryos from all seeds were isolated aseptically and transferred to embryo rescue (ER) medium as described by Sibi et al., (Ann. Amelior. Plantes, 29: 583-606, 1979). After 1-2 weeks well developed plants were transferred to MS medium (Murashige T. and Skoog F., Physiol. Plant 15: 473-497, 1962), supplemented with 0.8 % agar and 20 g/l sucrose. Plants with a well developed root system were subsequently

transferred to the greenhouse and adapted carefully to its conditions.

Leaves of 4-6 weeks old plants were detached and inoculated with Xanthomonas campestris vesicatoria race VI to select the resistant plants. The bacterial clone is stored in the freezer and grown on agar medium one week before inoculation at 22° C. The suspension for inoculation is prepared by resuspending the bacteria in demineralised water. The bacteria suspension was applied to the leaves with a syringe. Inoculated leaves are kept in boxes covered with glass in a climate room at 22°C for two days. Leaves of resistant plants show a light brown and dry spot at the site of inoculation caused by a hypersensitivity reaction (HR). Leaves of susceptible plants show no reaction or a water soaked lesion at the site of inoculation depending on the time of incubation. Absence of a hypersensitivity reaction thus is indicative of the absence of the Bs4 gene. Resistant control is C. pubescens PI235047, susceptible control is a susceptible C. pubescens accession.

Crosses:

A first hybrid plant was prepared by crossing Capsicum pubescens PI235047 (male, Figure 2B) (United States Department of Agriculture), comprising the Bs4 resistance gene, with Capsicum eximium (female, Figure 2A). 37 hybrid plants (Figure 3A), comprising the resistance gene, were obtained.

A second hybrid plant was subsequently prepared by crossing C. frutescens PI 238059 (male, Figure 3B) with the first hybrid plants comprising the resistance gene (female). None of the second hyrid plants plants derived from this cross expressed the resistance.

Subsequently, the second hybrid plant (female, Figure

4A) was crossed with Capsicum pubescens PI235047 (male). Two resistant plants were obtained. These plants were fertile and accordinlgy F2 seeds were obtained.

In addition, hybrids of C. annuum (Figure 4B) and C. chinense (Figure 4C) were made by crossing individuals of C. chinense PBC306 (Asian Vegetable Research and Development Center) with individuals of a C. annuum plant. The product of this cross was again crossed with C. annuum. Subsequently F2 seeds were obtained.

Subsequently, a third hybrid plant (Figure 5) was prepared by crossing the resistant plants derived from the cross of the second hybrid plant with C. capsicum or plants derived from the F2 seeds thereof, with plants derived from the F2 seeds of the C. annuum/C. chinense hybrid. The resistant products from this cross were further crossed with pure C. annuum lines.

In addition, plants derived from said cross that were susceptible for Xcv6, were crossed again with C. pubescens PI235047. The resistant plants derived from that cross were then selfed and F2 seeds were obtained. Resistant plants derived from that cross were subsequently again crossed with a line derived from the hybrid Abdera AMA12. This hybrid is a cross between C. chinense and C. annuum. The resistant plants resulting from said last cross were selfed and F2 seeds were obtained. In addition, the plants derived from the selfing of the last mentioned cross with PI235047 were further crossed with several C. annuum parental lines.

As a result, C. annuum plants were obtained carrying the Bs4 resistance gene derived from C. pubescens (Figure 6). These result clearly shows that it is possible to introduce traits from the purple-flowered Capsicum species into the white-flowered Capsicum species, using the method of the present invention.

2005337085 11 Nov 2011

CLAIMS

1. Method for transferring one or more genetic traits from a plant of the purple-flowered Capsicum species C. pubescens comprising said genetic trait, to a plant of the white-flowered Capsicum species C. chinense, C. annuum and/or a hybrid thereof, said method comprising the steps:

(a) preparing a first hybrid plant comprising said genetic trait by crossing a first plant of the purple-flowered Capsicum species C. pubescens comprising said genetic trait with a second plant of the purple-flowered Capsicum species C. eximium, and selecting plants comprising said genetic trait;

(b) preparing a second hybrid plant by crossing said first hybrid plant comprising said genetic trait, with a first plant of the white-flowered Capsicum species C. frutescens;

(c) crossing said second hybrid plant with said first plant of the purple-flowered Capsicum species C. pubescens comprising said genetic trait, and selecting from the progeny thereof plants comprising the genetic trait;

(d) preparing a third hybrid plant by crossing the plants obtained step c) with a white-flowered hybrid of C. chinense and C. annuum, C. chinense, or C. annuum, and selecting from the progeny thereof plants comprising the genetic trait.

2. Method as claimed in claim 1, further comprising

(e) selecting plants obtained in step (d) not comprising the genetic trait and crossing these plants with said first plant of the purple-flowered Capsicum species C. pubescens comprising said genetic trait, and

2005337085 11 Nov 2011

selecting from the progeny thereof plants comprising the genetic trait; and

(f) preparing a fourth hybrid plant by crossing the plants obtained step (e) with a white-flowered hybrid of C. chinense and C. annuum, C. chinense, or C. annuum, and selecting from the progeny thereof plants comprising the genetic trait.

3. Method as claimed in claim 2, further comprising:

(g) preparing a fifth hybrid plant by crossing the fourth hybrid plant comprising the genetic trait with a white-flowered hybrid of C. chinense and C. annuum, C. chinense, or C. annuum, and selecting from the progeny thereof plants comprising the genetic trait.

4. Method as claimed in claim 1, wherein after step (b) the second hybrid plant is self-pollinated, and the progeny thereof is used in step (c).

5. Method as claimed in any of the previous claims, wherein the plants obtained in step (c) are self-pollinated and the progeny thereof is used in step (d).

6. Method as claimed in claim 2, wherein the plants obtained in step (d) are self-pollinated and the progeny thereof is used in step (e).

7. Method as claimed in any of the claims 2 to 6, wherein the plants obtained in step (e) are self-pollinated and the progeny thereof is used in step (f).

8. Method as claimed in any of the claims 1 to 7, wherein the genetic trait is a resistance gene against a Capsicum disease.

2005337085 11 Nov 2011

9. Method as claimed in claim 8, wherein the resistance gene is the Bs4 gene, conveying resistance to the bacterial disease Xanthomanas campestris pv. vesicatoria race I, III and IV.

10. Plant of the white-flowered Capsicum species C. chinense, C. annuum and/or a hybrid thereof comprising a genetic trait derived from a plant of the purple-flowered Capsicum species C. pubescens.

11. Plant as claimed in claim 10, wherein the genetic trait is a resistance gene against a Capsicum disease.

12. Plant as claimed in claim 11, wherein the resistance gene is the Bs4 gene, conveying resistance to the bacterial disease Xanthomanas campestris pv. vesicatoria race I, III and IV.

13. Fruits, seeds and plant parts derived from a plant as claimed in any of the claims 10 to 12.

14. Method for transferring one or more genetic traits from a plant of the purple-flowered Capsicum species C. pubescens comprising said genetic trait, to a plant of the white-flowered Capsicum species C. chinense, C. annuum and/or a hybrid thereof substantially as herein described.

15. Plant of the white-flowered Capsicum species C. chinense, C. annuum and/or a hybrid thereof substantially as herein described.

16. Method for transferring one or more genetic traits from a plant of the purple-flowered Capsicum species

2005337085 11 Nov 2011

C. pubescens comprising said genetic trait, to a plant of the white-flowered Capsicum species C. chinense, C. annuum and/or a hybrid thereof substantially as herein described with reference to the examples.

17. Plant of the white-flowered Capsicum species C. chinense, C. annuum and/or a hybrid thereof substantially as herein described with reference to the examples.

18. Method for transferring one or more genetic traits from a plant of the purple-flowered Capsicum species C. pubescens comprising said genetic trait, to a plant of the white-flowered Capsicum species C. chinense, C. annuum and/or a hybrid thereof substantially as herein described with reference to the figures.

19. Plant of the white-flowered Capsicum species C. chinense, C. annuum and/or a hybrid thereof substantially as herein described with reference to the figures.

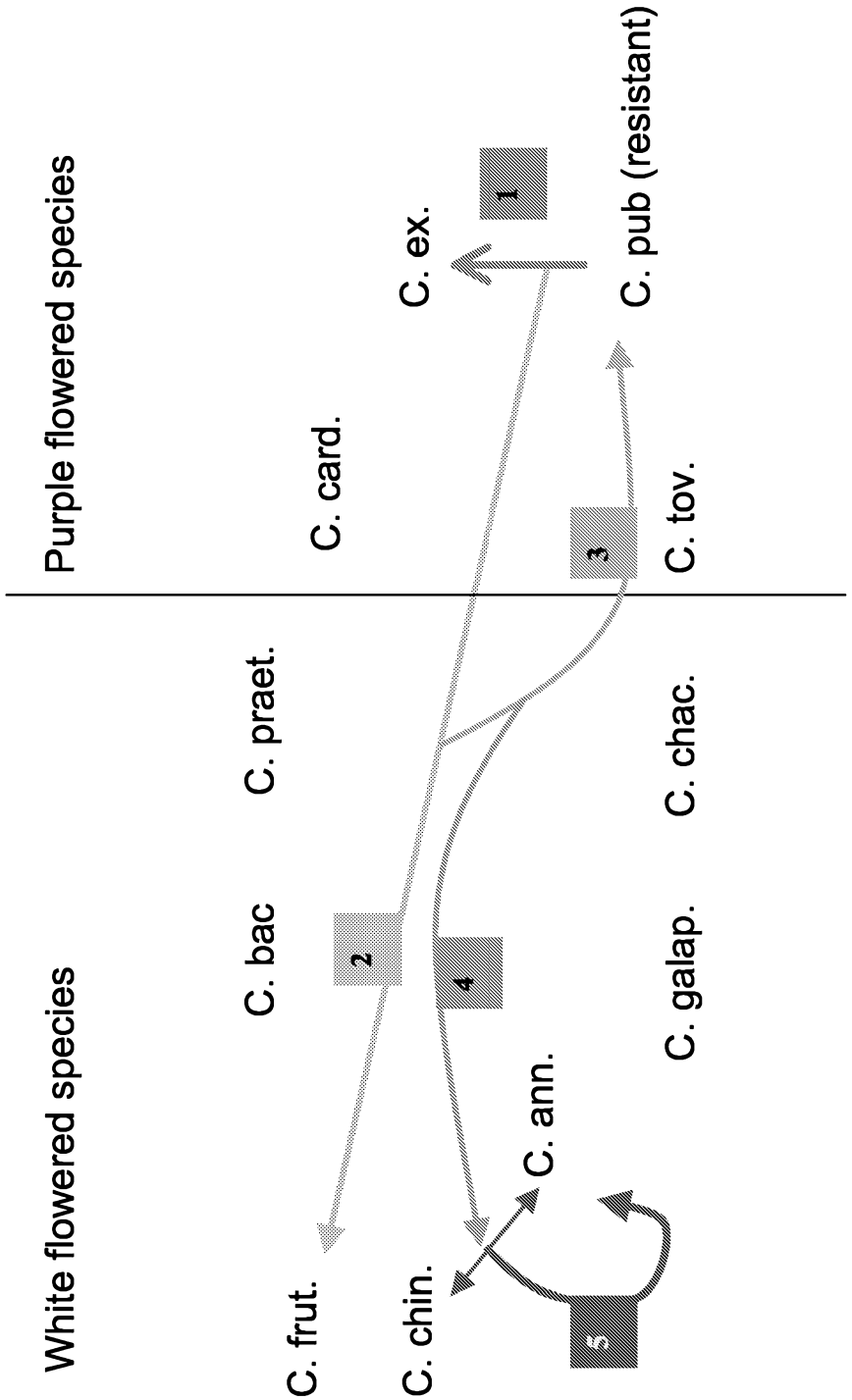
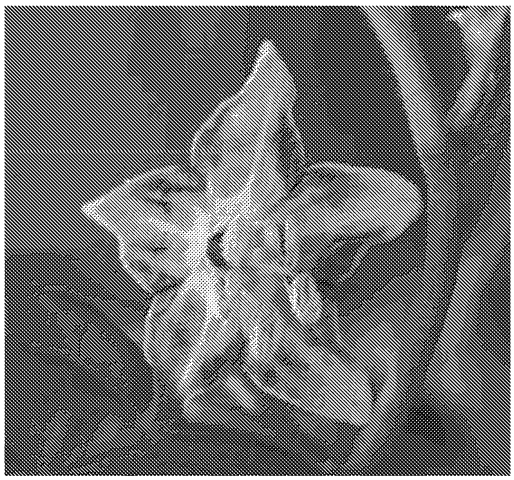

Purple flowered species
White flowered species
C. ex.
C. pub (resistant)
C. card.
C. tov.
C. praet.
C. bac
C. chac.
C. galap.
C. ann.
C. frut.
C. chin.
1
2
3
4
5

FIG. 1



C. eximium

FIG. 2A

C. pubescens plants with desired resistance

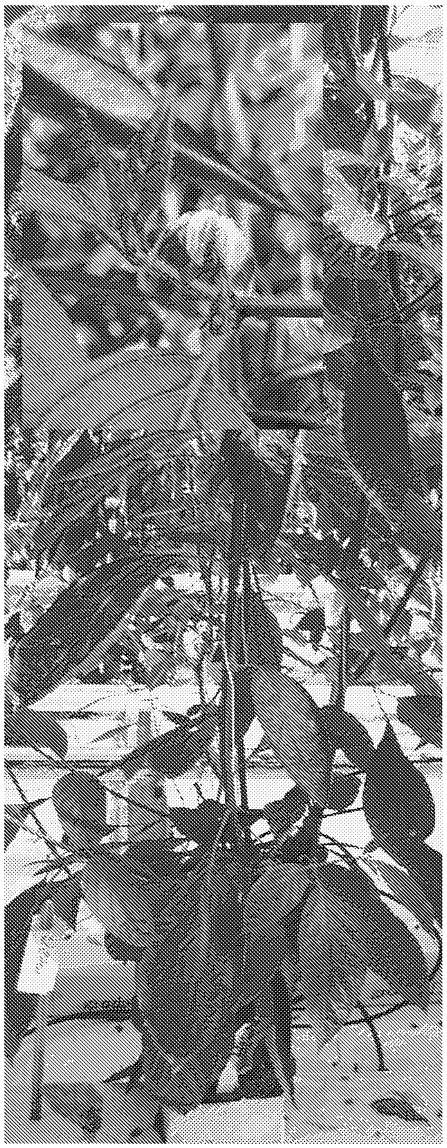
FIG. 2B

Product of step I

FIG. 3A

C. frutescens

FIG. 3B



Product of C.pub, C. exi and C. frut.

FIG. 4A

C. annuum

FIG. 4B

C. chinense

FIG. 4C

Product of self pollinated C.pub, C. ex, C. frut plant combined with C. chin and C. ann. hybrid

FIG. 5

FIG. 6A

FIG. 6B

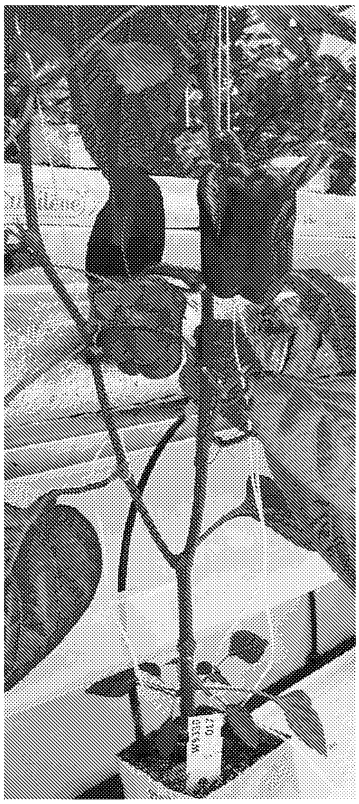


FIG. 6C

FIG. 6D