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(54) Title: DUAL PURPOSE POLLENIZER WATERMELONS

(57) Abstract: The application relates to the field of plant breeding, in particular watermelon breeding. Provided are diploid watermelon plants (and seeds from which these plants can be grown) which produce small, diploid, red watermelon fruits. Also provided are small, diploid watermelon fruits having an average weight of less than 1.8 kg.

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Dual Purpose Pollenizer Watermelons

FIELD OF THE INVENTION

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The present invention relates to the field of watermelon breeding and watermelon improvement. Provided are new diploid watermelon plants (2n = 2x = 22) and seeds from which such plants can be grown, which produce very small diploid fruits. The diploid watermelon plants are preferably suitable as pollenizers in triploid watermelon (2n = 3x = 33) production, whereby the pollenizers therefore have a dual purpose: providing sufficient viable pollen to pollinate female flowers of triploid plants (which after pollination produce triploid, seedless watermelon fruits) and/or to provide small ("mini"- or personal size), edible diploid fruits on the pollinizer plants themselves.

BACKGROUND OF THE INVENTION

Seedless watermelon (*Citrullus lanatus* (Thunb.) Matsum. And Nak.) production involves using pollen from diploid male parent plants to fertilize flowers of tetraploid (2n = 4x = 44) maternal parent plants. Pollination of the tetraploid flowers with diploid pollen leads to hybrid F1 seeds which are triploid (Kihara, 1951, Proceedings of American Society for Horticultural Science 58: 217-230; Eigsti 1971, Hort Science 6: 1-2). The triploid hybrid plants, grown from these F1 seeds, are self-infertile as they produce sterile pollen due to chromosome imbalance (Fehr, 1987). The triploid hybrids, therefore, need to be pollinated by a diploid pollenizer to produce watermelon fruit. Triploid plants are, therefore, interplanted with pollenizer plants for fruit production. The "seedless" fruit produced after pollination on the triploid hybrid plant are often not truly seedless, but may contain some undeveloped, small, pale seeds, which are edible.

For optimal seedless watermelon fruit set, sufficient viable pollen is required. Plants are generally planted at a ratio of 1 pollenizer per every 2-4 triploid plants. Triploid plants and pollenizers are either planted in separate rows (e.g. 1 row of pollenizer and 2-4 rows of triploids), or interplanted within rows (e.g. planting 1 pollenizer plant in between 2 to 3 triploid plants in the same row), or interplanted in narrow rows between rows of triploids (see US 2006/0168701 Table 2). The fruit produced on the pollenizer plants preferably has a different rind pattern from the fruit on the triploid hybrids, so that these can be easily distinguished. Until now generally the fruits produced on dedicated pollenizer plants are not harvested or discarded and only the seedless triploid fruits are sold.

In the last years, several dedicated pollenizer plants have been developed, which provide sufficient staminate flowers and sufficient viable pollen throughout the season to increase triploid fruit yield. These dedicated pollenizers include for example varieties Polimax and Jenny (Nunhems), Sidekick (Harris Morin), Companion (Seminis) and the Super-Pollenizers SP-1 and SP-4 (Syngenta). These dedicated pollenizers can be divided into two categories based on their vegetative growth type, which is

either of the standard vine length e.g. Jenny and SP-1 and SP-4, or the 'compact' vine length, e.g. Companion or Sidekick.

Some pollenizers produce diploid fruits which could be marketable, while others produce fruits that are unsuitable for consumption and marketing. Dittmar (2006, MSc Thesis North Carolina State University, Horticultural Science, Characterization of diploid watermelon pollenizers and utilization for optimal triploid watermelon production and effects of halosulfuron post and post-dir on watermelon) evaluated different pollenizers for the potential marketability of their fruits and concluded that Mickeylee, SF800, MiniPool, Jenny and Pinnacle have a fruit quality that could potentially be marketed. Average fruit weight of these was 5.1 kg, 10.7 kg, 3.9 kg, 3.3 kg and 2.9 kg respectively (Dittmar 2006, *supra*). The smallest diploid fruits were produced by Sidekick (1.0 kg, with dimensions of 12.3 x 11.9 cm length: width Dittmar 2006, supra) and SP-1 (2.0 kg, with dimensions of 17.5 x 15.4 cm length: width (Dittmar 2006, supra), but neither of these produce marketable fruits. The fruits of Sidekick are very poor quality pink-fleshed and those of SP-1 are white-fleshed and have a low brix value. Due to the non-marketable fruits, these pollenizers are referred to as being "non-harvestable pollenizers".

US2009/0288183 (Gold Seed Co. LLC) describe a pollinizer called "Escort-4" which produces small fruits having reduced sugar for type 2 diabetics, referred to as a "dual purpose reduced sugar watermelon". The fruits are said to have an average weight of 4.0 lbs (1.8 kg) and a size of 5-7 inches long (12.7 - 17.7 cm) x 4-5 inches wide (10.1 - 12.7 cm). The fruits of Escort-4 are said to have approximately 1/3 less sugar content than commercial diploid varieties, such as Sangria (Syngenta Inc.).

It is an object of the invention to provide dual purpose watermelon pollenizers producing small, edible (i.e. marketable) diploid fruit with an average fruit weight of less than 2.0 kg, preferably less than 1.8 kg or 1.7 kg, more preferably equal to or less than 1.6, 1.5, 1.4, 1.3, 1.0, 0.9, 0.8, 0.7 kg, and even more preferably equal to or less than about 0.65 kg, such as equal to or less than 0.6 kg, 0.5 kg, 0.4 kg or 0.3 kg. In one embodiment the fruits are preferably red fleshed, more preferably dark red fleshed, with a RHS rating of 39 or higher (not pink red or coral red or yellow-red). In another embodiment the average fruit brix is at least about 7.5% or higher.

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It is a further object of the invention to provide dual purpose watermelon pollenizers producing small, edible (i.e. marketable) diploid fruit with an average fruit weight of less than 0.9 kg, such as equal to or less than 0.8 kg, 0.7 kg, 0.65 kg, 0.6 kg, 0.5 kg, 0.4 kg or 0.3 kg, but above 0.25 kg. In one embodiment the fruits are preferably red fleshed, more preferably dark red fleshed with a RHS rating of 39 or higher (not pink red or coral red or yellow-red). In another embodiment the average fruit brix is at least about 7.5% or higher.

GENERAL DEFINITION

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The verb "to comprise" and its conjugations is used in its non-limiting sense to mean that items following the word are included, but items not specifically mentioned are not excluded. In addition, reference to an element by the indefinite article "a" or "an" does not exclude the possibility that more than one of the element is present, unless the context clearly requires that there be one and only one of the elements. The indefinite article "a" or "an" thus usually means "at least one", e.g. "a plant" refers also to several cells plants, etc. Similarly, "a fruit" or "a plant" also refers to a plurality of fruits and plants.

As used herein, the term "plant" includes the whole plant or any parts or derivatives thereof, preferably having the same genetic makeup as the plant from which it is obtained, such as plant organs (e.g. harvested or non-harvested fruits, leaves, etc.), plant cells, plant protoplasts, plant cell- or tissue-cultures from which whole plants can be regenerated, plant calli, plant cell clumps, plant transplants, seeds from which the plant can be grown and seeds produced by the plant, seedlings, plant cells that are intact in plants, plant clones or micropropagations, or parts of plants, such as plant cuttings, embryos, pollen, ovules, fruits (e.g. harvested tissues or organs), flowers, leaves, clonally propagated plants, roots, stems, root tips, grafts (scions and/or root stocks) and the like. Also any developmental stage is included, such as seedlings, cuttings prior or after rooting, etc.

It is, thus, understood that herein a watermelon plant, such as a triploid plant or pollenizer plant, encompasses not only an ungrafted plant, but also a plant with a rootstock of a different plant, such as a gourd or squash rootstock, another watermelon rootstock, a transgenic rootstock, etc.

As used herein, the term "variety" or "cultivar" means a plant grouping within a single botanical taxon of the lowest known rank, which can be defined by the expression of the characteristics resulting from a given genotype or combination of genotypes.

The term "allele(s)" means any of one or more alternative forms of a gene at a particular locus, all of which alleles relate to one trait or characteristic at a specific locus. In a diploid cell of an organism, alleles of a given gene are located at a specific location, or locus (loci plural) on a chromosome. One allele is present on each chromosome of the pair of homologous chromosomes. A diploid plant species may comprise a large number of different alleles at a particular locus. These may be identical alleles of the gene (homozygous) or two different alleles (heterozygous).

The term "locus" (loci plural) means a specific place or places or a site on a chromosome where for example a gene or genetic marker is found.

"Diploid plant" refers to a plant, vegetative plant part(s), or seed from which a diploid plant can be grown, having two sets of chromosome, designated herein as 2n.

"Triploid plant" refers to a plant, vegetative plant part(s), or seed from which a triploid plant can be grown, having three sets of chromosomes, designated herein as 3n.

"Tetraploid plant" refers to a plant, vegetative plant part(s), or seed from which a tetraploid plant can be grown, having four sets of chromosomes, designated herein as 4n.

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"Pollenizer plant" or "pollenizer" refers to the (inbred or hybrid) diploid plant, or parts thereof (e.g. its pollen or scion), suitable as pollenizer for inducing fruit set on triploid plants. A pollenizer plant is, thus, able to lead to good fruit set (and good triploid fruit yield) of triploid plants, by producing an appropriate amount of pollen at the appropriate day-time and for an appropriate period of time, e.g. at least during peak flowering time of the triploid female plants. A good triploid fruit yield is, for example, a yield comparable to the yield obtainable when using Polimax (produced by Nunhems) as pollenizer (see e.g. Example 3).

"Dual purpose pollenizer" refers to a pollenizer plant which also produces edible diploid fruits on the pollenizer plant itself (through self-pollination) and also is suitable to be used as a pollenizer in triploid (seedless) watermelon production. This definition is independent of whether or not the plant is actually being used as a pollenizer in triploid fruit production, i.e. it can also be used for diploid fruit production on its own.

The term "edible" is used herein to refer to fruits marketable for human consumption, especially fresh consumption of the fruit flesh. The fruits have at harvest at least good, preferably very good flavor properties (i.e. taste and odor). To have good flavor properties the fruits preferably have an average level of Total Soluble Solids of at least about 7.5% or more. Good fruit flesh color is also an important criterion for marketability for human consumption. For red-fleshed fruits it is an embodiment that the flesh color has an average RHS rating of at least 39 or above. If red-fleshed fruits are measured on a scale of 1 (white) to 10 (dark red), the fruits have an average rating of at least 7 or more.

"Hybrid triploid plant" is a triploid plant grown from hybrid, triploid seed obtained from cross fertilizing a male diploid parent with a female tetraploid parent.

"Seedless fruit" are triploid fruit which contain no or few mature seeds. The fruit may contain one or more small, edible, white ovules. Plants which produce seedless fruit may herein be referred to as "seedless".

"Interplanting" refers to the combination of two or more types of seeds and/or transplants sown or transplanted on the same field, especially the sowing and/or transplanting of pollenizers in the same field as triploid hybrid plants (for seedless fruit production on the triploid plants and diploid fruit production on the pollenizer plants). For example, the pollenizer may either be planted in separate rows or interplanted with the triploid plants in the same row (e.g. in hills within each row). Pollenizers may also

be planted in between rows of triploids. Also seeds of pollenizers and triploid hybrids may be mixed prior to seeding, resulting in random seeding. The transplants of the triploid hybrid plants and/or pollenizer plants may also comprise a rootstock of a different plant. Suitable rootstocks are known in the art. Also encompassed are methods where the triploid hybrid plant and the pollenizer plant are grafted together onto one rootstock.

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"Planting" or "planted" refers to seeding (direct sowing) or transplanting seedlings (plantlets) into a field by machine or hand.

"Vegetative propagation" refers to propagation of plants from vegetative tissue, e.g. by *in vitro* propagation or grafting methods (using scions).

- "Vegetative type" or "growth type" or "vine type" refers to the combination of growth characteristics of the vegetative parts of a plant line or variety, such as (average) internode length, (average) length of the main vine, (average) length of the shortest and longest branch, average number of primary branches, etc.

 Three vegetative types can be distinguished: The "normal/standard vine type", the "compact vine type" and an "intermediate vine type" between these two.
- "Compact vine type" refers to the vegetative type of a plant or plant line or variety having an average internode length of about 4.0 6.5 cm, especially an average internode length of equal to or below 6.5 or 6.0 cm, e.g. equal to or below 5.0, 4.5 or 4.0 cm and/or an average longest branch of equal to or less than about 170 cm, preferably 160 cm, preferably equal to or less than 150 cm or 145 cm, preferably equal to or less than 140 or 130 cm. Examples of compact vine types are Companion and Sidekick (US 7,314,79).

A "standard vine type" refers to the vegetative type of a plant or plant line or variety having an average internode length of more than 6.5 cm, preferably equal to or more than about 7, 8, 9, 10, 11 or 12 cm and/or an average longest branch of equal to or more than 225 cm, e.g. equal to or more than 230 cm, 250 cm, 300 cm, 350 cm or more. Examples are varieties Ace, Jenny, SP-1, SP-4.

An "intermediate vine type" refers to the vegetative type of a plant or plant line or variety which falls between the standard and compact vine type as defined above. It has an average internode length of less than 10.5 cm, e.g. equal to or less than 10, 9, 8, 7, 6 or 5 cm, preferably about 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 or 9.0 cm and/or an average longest branch of equal to or less than about 220 cm. The longest branch is on average preferably about 175 – 220 cm, e.g. preferably about 175, 180, 185, 190, 195, 200, 205, 210, 215 or 220 cm.

Throughout this document "average" and "mean" are used interchangeably and refer to the arithmetic mean.

DETAILED DESCRIPTION

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The invention provides plants (and seeds from which such plants are grown) of the species *Citrullus lanatus*, wherein said plant is suitable as a pollenizer in triploid watermelon production, is diploid and produces marketable diploid fruits having an average weight of less than 2.0 kg, preferably less than 1.8 kg, more preferably equal to or less than 1.7, 1.6, 1.5, 1.4, 1.3, 1.2, 1.0 kg, more preferably equal to or less than 0.9, 0.8, 0.7, 0.65 kg, such as equal to or less than 0.6, 0.5, 0.4, 0.35, 0.3 or 0.25 kg at harvest (i.e. at maturity). In a further embodiment average fruit size is equal to or less than about 0.9 kg (such as 0.8, 0.7, 0.65, 0.6, 0.5, 0.4 kg) but above 0.25 kg. To determine average fruit characteristics, such as average fruit weight, of a plant line or hybrid according to the invention several plants of a line or hybrid are grown in one location and 3, 4, 5 or more fruits are harvested from 2, 3, or more plants of the same line and e.g. weighed. Reference plant lines (such as known pollenizers) can be grown at the same location (e.g. in the same trial or under the same environmental conditions) as a comparison. The average fruit weight according to the invention is in one embodiment preferably less than 0.65 kg, more preferably equal to or less than about 0.6, 0.5, 0.4, 0.3 or 0.25 kg. In another embodiment the average fruit size is less than 0.65 kg but above 0.25 kg.

Fruit diameters can be variable, but preferably average fruit length is less than 12 cm, more preferably equal to or less than about 11.5 or 11 cm, such as equal to or less than 10.5, 10.0, 9.5, 9.0, 8.5 or 8.0 cm, while the average fruit width is preferably less than 11, 10.5 or 10 cm, such as equal to or less than 9.5, 9.0, 8.5, 8.0 cm. Thus, average fruit dimensions range have ranges of 8.0 – 12.0 cm average length x 8.0 – 11 cm average width. Preferably at least fruit length is on average below 12 cm, preferably below 11.5 cm, more preferably about 11 cm or less. Preferably, both length and width of the fruits is on average about 11 cm or less. In one embodiment the average length by width is 10 x 11 cm, or less. In one embodiment average fruit dimensions for both lengths and width are equal to or below 10.5 cm. These dimensions are particularly encompassed for the diploid fruit weights of less than 0.65 kg on average. For the heavier diploid fruits (e.g. less than 1.8 kg but above 0.65 kg) also larger dimensions are encompassed herein, such as an average length of equal to or less than 17 cm (such as 16, 15, 14, 13, 12 cm) and an average width of equal to or less than 15 cm, such as 14, 13, 12 cm).

The fruits, i.e. the fruit flesh, produced on the pollenizer plants are edible at maturity. The flesh color of the mature fruits of the diploid plants according to the invention is in one embodiment red, having an RHS mini color chart value (Royal Horticultural Society mini color chart) of 39 or higher, especially between 39 and 41. Also, the average percent Total Soluble Solids (% TTS; herein also referred to as degrees Brix, or °Brix) of the fruits is at maturity at least about 7.5%, preferably at least about 8 % or 8.5%, more preferably at least about 9 % or 9.5%, and even more preferably at least about 10 %, 10.5%, 11%, 11.5%, 12%, 12.5%, 13%, or more. Average TSS can, for example, be determined using a

refractometer as described in the Examples. The average percentage TSS of the diploid fruits produced by the pollenizer plants according to the invention can be increased by traditional breeding techniques, e.g. by crossing the plants provided herein with watermelon plants comprising high TSS and selection of progeny (e.g. obtainable by one or more selfings and/or backcross populations) producing fruits with higher TSS values while maintaining small fruit size.

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As mentioned, the diploid fruits according to the invention are edible, i.e. they have fruit quality characteristics which make them marketable for human consumption. This means that the fruits have good flavor properties (no off-flavors etc.). For fruits to have at least good flavor properties a minimum average brix of at least about 7.5 degrees is desired. Flavor properties can be determined and scored (e.g. as bad, good, very good) by trained test-panels using known methods for evaluating sensory properties of fruits (Karen L. Bett, Ch 13, Fresh-Cut Fruits and Vegetables, Science, Technology, and Market; Edited by Olusola Lamikanra, CRC Press 2002, Print ISBN: 978-1-58716-030-1). Selection for good flavor includes test panels to select against bitterness and other unpleasant flavors, such as caramel flavor. Watermelon checks of varieties which have good flavor properties (e.g. Allsweet, Crimson Sweet) are preferably included in the test.

The fruits should preferably also not be susceptible to what is known as "fruit cracking" and/or should preferably not contain the brittle gene as present in SP-1. The Super Pollenizers such as SP-1 (as described in WO03/075641) bear brittle fruits, which makes the fruits (in particular the fruit flesh) unmarketable as fresh produce (although the seeds contained within the fruits can be harvested and marketed). Crack-resistance is generally selected for during breeding (e.g. in field observations and/or using for example pressure tests or other tests), as cracking is an undesired fruit quality characteristic. See also Haikun *et al.* 2010, Acta Hort. (ISHS) 871:223-230).

Other fruit characteristics can be introduced by traditional breeding methods (see further below) and thereby combined with pollenizers producing small, edible diploid fruits according to the invention. For example plants can be selected which produce small, edible fruits, as described above, with increased or reduced rind thickness, increased or reduced rind brittleness, various skin/rind colors (e.g. light green; dark green; green-striped with narrow, medium or wide stripes; grey types; with or without spotting; Golden yellow) and rind surfaces (e.g. furrowed or smooth surface), flesh structure / flesh firmness, different fruit shapes (elongate, oval, blocky, spherical or round), higher brix content, higher lycopene and/or vitamin content, different sugar: acid ratios, very good fruit flavour, etc. Also the combination of small edible fruits with another flesh color than red is possible, for example genetic determinants for yellow flesh or orange flesh or white flesh may be introduced, e.g. by backcross breeding with another color-type. See Guner and Wehner 2004, Hort Science 39(6): 1175-1182, in particular pages 1180-1181 describing genes for fruit characteristics. Generally important breeding objectives are early maturity, high fruit yield, high internal fruit quality (good uniform color, high sugar, proper sugar: acid ratio,

good flavor, high vitamin and lycopene content, firm flesh texture, non-fibrous flesh texture, freedom from defects such as hollow heart, rind necrosis, blossom-end rot or cross stitch and good rind characteristics and cracking-resistance).

Rind thickness is a characteristic which influences damage during handling and transporting (too thin or too brittle rind), but thin rinds may also be desirable for consumers. In one embodiment the fruits have a thin rind, such as an average rind thickness (measured on the side) of at least about 0.2 cm, 0.3 cm, 0.4 cm, but less than 0.5 cm, more preferably less than or equal to 0.4 cm. Thus in one embodiment the rind is thicker than the rind of SP-4 fruit, but thinner than the rind of Polimax, and optionally thinner than the rind of Sidekick fruit. For certain embodiments thicker rinds may be desired and small fruits having a rind of 0.5 or more cm, such as 0.6 and 0.7 cm are also encompassed herein. The rind of the fruits preferably does not crack easily (i.e. is cracking-resistant), both in fruits with thin rinds and thick rinds.

In one embodiment of the invention, the fruits preferably also do not have a brittle rind and/or an explosive rind as described in WO03/075641 on page 13 and 14, i.e. the fruits do not break under pressure in the range of 90 to 140 g/mm².

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Flesh firmness of the diploid fruits is preferably at least about 0.8 (average firmness in kg as in Example 1), more preferably at least about 0.9, 1.0, or more. Flesh firmness can be increased by e.g. crossing with watermelons having firmer fruit flesh and selection for firmer flesh without increasing fruit size. Plants producing fruits with ultra-firm flesh are, for example, described in US2006/0005284.

The diploid plants provided herein are suitable as pollenizers, which means that they produce sufficient pollen at the right time of the day and for an appropriate period of time to induce fruit set in triploid hybrids, leading to a (average) triploid fruit yield at least comparable to that obtained when using e.g. Polimax as pollinator. However, the plants according to the invention need not be sold or marketed as pollenizer for triploid fruit production and need not be used as pollenizer in triploid fruit production. They may also be marketed and/or used solely for diploid fruit production on their own.

In one embodiment the pollenizer plants preferably produce a large number of male flowers at the appropriate time during flowering of the triploid hybrids, preferably at least about 35 open male flowers at day 15 and/or at least about 30 open male flowers at day 22 from the first day of flowering, although the number of male flowers is not critical in determining triploid fruit set, as long as sufficient pollen is produced by the available male flowers to lead to good triploid fruit set. As the pollenizer plants are also used for diploid fruit production, also sufficient female flowers must be produced by the pollenizers to ensure their dual purpose.

As can be seen in the Examples Table 3, many open male and female flowers were present over the 3 week period counted. At the flowering date (the date when more than 50% of the plant in the plot has male/female flowers) the 11 pollenizer lines had on average 12.6 open male and 3.9 open female flowers. Each line generally had significantly more open male flowers than SP-4 and Polimax. At day 15 and 22 from 1st day of flowering all lines produce significantly more open male flowers than Sidekick, SP-4 and Polimax, all having at least 35 open male flowers at day 15 and at least 30 open male flowers at day 22. However, Polimax is a very good pollenizer for triploid hybrids, despite the fact that the number of male flowers is significantly lower than in the hybrids provided in the Examples. Polimax had only about 18 open male flowers at day 15 and day 20. The pollenizers according to the invention, therefore, may have a lower number of male flowers than in the Examples in Table 3, e.g. plants having about the same number of open male flowers as Polimax are encompassed herein, i.e. pollenizers having at least about 15, 18, 20, 25, 30, or more open male flowers at day 15 and/or at day 22 from the 1st day of flowering are also an embodiment of the invention.

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Also the number of open female flowers was good, indicating that the pollinizers are indeed suitable for dual-purposes, i.e. pollination and fruit production of triploid hybrids and/or pollination and fruit production on the diploid plants themselves (fruits produced by self-pollination of the pollenizer plant).

In one embodiment of the invention the pollenizer plants are dual purpose pollenizer plants. In particular, the average number of open male flowers at day 22 from flowering is at least 15, 18, 20, 25 or 30 or more.

In one embodiment the pollenizer plants according to the invention are hybrid diploids (F1 diploids) and not open pollinated (OP).

In yet a further embodiment of the invention, the dual purpose pollenizer plants are not of the "edible seed watermelon" or "confectionary" type, as for example produced in variety San Juan (Native Seeds). These types of watermelon are produced to harvest the seeds for consumption or seed oil production and the fruits produce large, black or red edible seeds with soft seed coats, see e.g. Zhang 1996, Cucurbit Genetics Cooperative Report 19:66-67 (article 24) and Zhang and Wang, 1990, Cucurbit Genetics Cooperative Report 13:43-44 (article 16). In contrast, the seeds produced in the diploid fruits according to the invention are small (preferably tomato seed size to medium seed size, but not large) and are not suitable for seed harvest and seed consumption. In one embodiment of the invention the seeds of the diploid fruits are on average shorter than or equal to 5 mm in length, for example shorter than or equal to 4 mm, 3.5 mm, 3 mm, 2.5 mm, 2 mm, 1.5 mm or 1 mm in length.

The dual purpose pollenizer plants not only produce very small, edible fruits, but also high numbers of marketable fruits. In one embodiment a plant according to the invention produces at maturity on average

at least about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more fruits per plant, more preferably at least 25 fruits, more preferably at least about 27, 28, 29, 30, 35 or more fruits.

The pollenizer plants according to the invention can be combined with different vine types, such as compact vine type, standard vine type or, most preferably an "intermediate" vine type using normal breeding techniques. For example the pollenizers according to the invention may be crossed with a standard vine type and progeny may be selected which have an intermediate vine type.

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It is understood that it is also an object of the invention to provide seeds from which the pollenizer plants described herein can be grown. Also seedlings, scions and rootstocks, as well as cells and tissues of the pollenizer plants are encompassed herein. Such plant parts comprise the genetic determinants for producing dual purpose pollenizer plants according to the invention. Thus whole plants obtained from seedlings, scions and rootstocks, as well as cells and tissues of the pollenizer plants retain all the physiological and morphological characteristics of the pollenizer plants according to the invention when grown under the same environmental conditions.

It is a further object of the invention to provide a plurality of diploid watermelon fruits obtainable on a pollenizer plant according to the invention as described above, and/or seeds present in those fruits. Thus, in one embodiment, harvested diploid fruits are provided, such as packaged whole fruits or fruit parts and/or processed fruits or fruit parts.

Also progeny of the plants according to the invention are provided herein, such as seeds obtainable by crossing a pollenizer plant described herein with another watermelon plant and/or by selfing a plant according to the invention to produce F1 seeds (and F1 plants grown from these seeds, as well as fruit produced by self-pollinating the F1 plants).

Further provided are plant cells, cell cultures or tissue cultures of plants according to the invention, as well as root stocks, scions, transplants and vegetative propagations of plants according to the invention or of progeny thereof.

In one aspect, a representative sample of seeds of the plants according to the invention are deposited under accession number (WH 9306), or accession number NCIMB 41773 (WH9307), or accession number (WH9308), or accession number (WH9309), or accession number (WH9311), or accession number (WH9313), or accession number (WH9317), or accession number (WH9318), or accession number (WH9319), or accession number (WH9320), or accession number (WH9321).

Plants having the genetic determinants for producing small, edible fruits are, therefore, obtainable from the deposited seeds. The genetic determinants (i.e. the combination of genes) for producing small, edible fruits can be transferred to other watermelon plants, for example to create other diploid pollenizers with this phenotype or other diploids (open pollinated or inbred lines, or hybrid diploids). This can be done by using the pollenizers according to the invention as a parental line in breeding methods, i.e. as male or female parent in a cross with another watermelon plant. Known breeding methods can be used alone or in combination, such as (but not limited to) recurrent selection, pedigree breeding, backcross breeding, inbred development, hybrid testing, marker assisted breeding, etc. Diploids may also be used for tetraploid development, using e.g. colchicine treatment. Progeny are then selected which retain the small fruit dimensions and fruit weight, a brix of at least 7.5% and dual purpose pollenizer characteristics, all as described herein.

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Other watermelon plants may be used as a starting point to develop dual purpose pollenizer plants producing small, edible fruits according to the invention. For example, small fruited cultivars or lines may be used as starting material, such as for example Sidekick (US 7,314,979) and/or SP-1 (WO 03/075641) and/or watermelon plants carrying the "tomato seed" mutant (gene ts) described in Guner and Wehner (2004, HortScience 39(6):1175-1182) and obtainable from gene curator of the Cucurbit Genetics Cooperative), and selecting for fruit quality characteristics (e.g. high brix, good flavor), small fruit size (e.g. small fruit dimensions and e.g. less than 0.9 kg weight) and small seed size in the diploid fruits, as well as pollenizer characteristics (e.g. many male flowers) as described herein.

Selection for small seed size encompasses selecting for an average seed length of equal to or less than 8 mm, preferably 7mm, 6 mm or more preferably equal to or less than 5.0 mm, preferably equal to or less than 4.5 mm, e.g. equal to or less than about 4.0 mm, 3.5 mm, 3.0 mm, 2.5 mm or 1.5 mm or 1.0 mm seed length.

In one embodiment a breeding method for producing diploid dual pupose pollenizers according to the invention is provided, comprising:

- a) providing a breeding population of diploid watermelon plants, and
- b) selecting progeny for small fruit size, small seed size, high brix, good flesh color and pollenizer characteristics (all as described throughout this application).

Plants obtainable by this method are encompassed herein. As mentioned, the breeding population can be provided by using at least one, preferably two small-fruited parents and crossing these, to generate an F1 and further progeny generations (F2, etc.). For example, one of the plants provided herein (e.g. WH9307) is used in step a) and is crossed to another watermelon plant. The progeny generations are then selected for at least the characteristics described in b).

In one embodiment one or more hybrid plants is provided, obtained from crossing a pollenizer plant according to the invention with another watermelon plant and harvesting the F1 seeds of said cross. The F1 seeds may then be grown into F1 plants and self pollinated or sib-pollinated to produce F2 seeds. If the parents used in the initial cross differ by one or more characteristics (e.g. disease resistance and fruit size), the F2 population will segregate for these trait(s) and the breeder can select plants in this and/or further progeny generations (F3, F4, etc.) which combine the desired traits (e.g. small fruit size and disease resistance). Alternatively, the F1 may be backcrossed to the recurrent parent (e.g. the pollenizer according to the invention into which a trait is to be introduced) or the F1 may be selfed to produce an F2 population segregating for the trait of interest and selected F2 plants having the trait of interest may be backcrossed to the recurrent parent.

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Thus, one or more traits not present in the pollenizer plants according to the invention can be introduced into a pollenizer plant according to the invention, while maintaining the genetic determinants for small diploid fruits. For example other fruit characteristics as described above can be introduced (e.g. darker red flesh color, higher brix, firmer flesh, a different flesh color, etc.), or any other traits can be introduced, such as one or more QTLs for high yield, disease resistance genes, stress tolerance genes (e.g. water stress tolerance), etc. resistance to fungal-, bacterial-, viral- diseases, root-knot nematodes and/or insect pests may be introduced. For example, resistance to Fusarium wilt (*Fusarium oxysporum fsp. niveum* race 0, race 1 and/or race 2, and/or race 3, and/or other new races which may develop), Anthracnose (*Colletotrichum lagenarium* races 1-7, or other new races), Gummy stem blight, powdery mildew, *Verticillium* wilt, bacterial fruit blotch, papaya ringspot virus (PRSV), watermelon mosaic virus (WMV) or zucchini yellow mosaic virus (ZYMV).

Resistance to Fusarium wilt races 0 and 1 is present in many commercial varieties, and also resistance to race 2 has been identified in PI296341 and PI271769 and is also present in SP-4 (US 7,550,652). Anthracnose resistance to race 1 is for examples present in SP-4 and resistance to race 2 in AU-Sweet Scarlet (AW-82-50CS) (Breeder: Alabama Agric. Expt. Station, Auburn University). Crimson Sweet has the Ar-1 gene, which provides resistance to anthracnose races 1 and 3. Gummy stem blight resistance is also found in Plant Introduction lines.

- Also provided is a method for producing diploid and triploid watermelon fruits in one field, said method comprising:
 - (a) interplanting diploid pollenizer plants according to the invention and triploid hybrid plants in one field,
 - (b) allowing pollination of flowers of the triploid hybrid plants with pollen of the diploid pollenizer plants and allowing pollination of flowers of the diploid pollenizer plants with pollen of the diploid pollenizer plants,
 - (c) harvesting fruits produced on the triploid hybrid plants and, optionally, harvesting fruits produced on the diploid pollenizer plants.

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In one embodiment of the invention the diploid fruits and/or the triploid fruits are edible and red-fleshed, white-fleshed, orange-fleshed or vellow-fleshed.

5 In a further embodiment the rind of the diploid fruits is not yellow or Golden (as controlled by the recessive gene go) (Barham 1965, Proc Ameri Soc Hort Sci 67: 487-489).

Interplanting in one field may be either done by seeding or transplants of the pollenizer and triploids. Various interplanting methods can be used, as known in the art and various ratios of pollenizer: triploid hybrid may be used. One row of pollenizer plants may for example be present at at least every 2, at least every 3 or at least every 4 rows of triploids, but other methods of interplanting may also be used.

Any triploid hybrid may be used, such as known triploid hybrid varieties.

15 Pollination is usually done by bees, and bee hives can be provided to the fields unless sufficient wild bees are naturally present. Pollination can also be performed by manual or mechanical means. Harvest at maturity may be done by hand or mechanized.

The diploid fruit may be distinguished from the triploid fruit based on the smaller fruit size of the diploid fruit, and/or alternatively by a different rind pattern. In one embodiment the rind of the diploid fruits according to the invention is not yellow or golden. Preferably harvested diploid and triploid fruit are placed into different containers. Thus, in one embodiment a container comprising solely small diploid fruits according to the invention is provided. Any type of container may be used, e.g. cartons, boxes, etc.

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Also a method for producing small diploid edible watermelon fruits having an average weight of less than 1.8 kg, 1.7 kg, 1.0 kg, 0.9 kg, 0.8 kg, 0.7 kg, preferably equal to or less than 0.65 kg (but in one embodiment larger than 0.25 kg), is provided comprising:

- (a) growing a plant according to the invention, i.e. a pollenizer as described herein,
- (b) pollinating the female flowers of said plant with pollen of said plant,
- (c) harvesting the fruits produced on said plant.

Thus, by self-pollination of a diploid plant according to the invention, small edible diploid fruits are produced. The pollenizers according to the invention may be grown in a field without other watermelon plants being present, and the small diploid fruits may be harvested and placed in containers for transport. Step (b) may be performed by allowing insect pollination or any other means of pollinating.

In one embodiment a diploid pollenizer plant (or a seed from which the plant can be grown) capable of producing small edible diploid fruits having an average weight of less than 1.8 kg, 1.7 kg, 1.0 kg, 0.9 kg, 0.8 kg, 0.7 kg or equal to or less than 0.65 kg (but in one embodiment larger than 0.25 kg) is provided, wherein a representative sample of seed containing the genetic elements for producing said small fruits has been deposited under accession number (WH 9306), or accession number NCIMB 41773 (WH9307), or accession number (WH9308), or accession number (WH9309), or accession number (WH9311), or accession number (WH9313), or accession number (WH9317), or accession number (WH9318), or accession number (WH9319), or accession number (WH9320), or accession number (WH9321).

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Especially, pollenizer hybrid WH 9307 comprises the genetic elements for producing said small, edible fruits according to the invention and a representative sample of seeds have been deposited under accession number NCIMB 41773. Thus, when referring herein to seed deposits of pollenizers according to the invention, this pollenizer is referred to as a representative plant according to the invention, but seeds of the other hybrids or lines are also suitable and when reference to WH9307 or progeny thereof (or parts of any of these) is made, the other hybrids or lines mentioned herein are equally implied.

In one embodiment a hybrid watermelon seed is provided, having as a male or female parent (preferably as a male parent) a diploid pollenizer plant capable of producing small edible diploid fruits having an average weight of less than 1.8 kg, 1.7 kg, 1.0 kg, 0.9 kg, 0.8 kg, 0.7 kg or equal to or less than 0.65 kg (but in one embodiment larger than 0.25 kg) is provided, wherein a representative sample of seed containing the genetic elements for producing said small diploid fruits has been deposited under accession number (WH 9306), or accession number NCIMB 41773 (WH9307), or accession number (WH9308), or accession number (WH9319), or accession number (WH9317), or accession number (WH9318), or accession number (WH9319), or accession number (WH9320), or accession number (WH9321).

In one embodiment the fruit of a cross-pollination between a diploid pollenizer plant as pollen donor capable of producing small diploid fruits having an average weight of less than 1.8 kg, 1.7 kg, 1.0 kg, 0.9 kg, 0.8 kg, 0.7 kg or equal to or less than 0.65 kg (but in one embodiment larger than 0.25 kg) is provided, wherein a representative sample of seed containing the genetic elements for producing said small fruits has been deposited under accession number (WH 9306), or accession number NCIMB 41773 (WH9307), or accession number (WH9308), or accession number (WH9319), or accession number (WH9317), or accession number (WH9318), or accession number (WH9319), or accession number (WH9320), or accession number (WH9321), and the pistillate flowers of another

watermelon plant is provided. The other watermelon plant is preferably a triploid hybrid and the fruit is triploid and preferably seedless.

- In one embodiment a diploid fruit and/or an inbred diploid seed is provided produced by self pollinating
 a diploid pollenizer plant capable of producing small edible diploid fruits having an average weight of
 less than 1.8 kg, 1.7 kg, 1.0 kg, 0.9 kg, 0.8 kg, 0.7 kg or equal to or less than 0.65 kg (but in one
 embodiment larger than 0.25 kg) is provided, wherein a representative sample of seed containing the
 genetic elements for producing said small diploid fruits has been deposited under accession number
 (WH 9306), or accession number NCIMB 41773 (WH9307), or accession number
 (WH9308), or accession number (WH9309), or accession number (WH9311), or accession
 number (WH9313), or accession number (WH9317), or accession number (WH9318),
 or accession number (WH9319), or accession number (WH9320), or accession number
 (WH9321).
- In one aspect of the invention plants according to the invention and fruits according to the invention are obtainable from WH 9306, WH9307, WH9308, WH9309, WH9322, WH9313, WH9317, WH9318, WH9319, WH9320, WH9321, or of progeny of any of these.
- Plants obtained (derived), or obtainable (derivable), from plants according to the invention (e.g. from deposited seeds) include, therefore, plants obtained by breeding methods, such as selfing, crossing, backcrossing, recurrent selection, double haploid production, marker assisted selection, clonal propagations, transformants, etc., whereby the derived plants produce small, edible fruits according to the invention.
- In another aspect of the invention plants having essentially all the morphological and/or physiological characteristics of WH 9306, WH9307, WH9308, WH9309, WH9322, WH9313, WH9317, WH9318, WH9319, WH9320, WH9321, or of progeny of any of these, are provided. Representative examples of physiological and morphological characteristics are provided in Tables 1-5.
- Also provided is a progeny plant of WH 9306, WH9307, WH9308, WH9309, WH9322, WH9313, WH9317, WH9318, WH9319, WH9320 or WH9321, obtained by further breeding with said plant, wherein said progeny plant has essentially all physiological and morphological characteristics of said plant.
- Essentially all physiological and morphological characteristics include herein at least small, edible fruits and dual purpose pollenizer characteristics as described throughout the description and examples.

Also a plant part (e.g. a fruit, tissue, cell, cell culture, vegetative propagation, pollen, etc.) is provided which, when regenerated or grown into a whole plant, has essentially all the morphological and/or physiological characteristics of WH 9306, WH9307, WH9308, WH9309, WH9322, WH9313, WH9317, WH9318, WH9319, WH9320 or WH9321.

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Also provided is a plant derived from any of WH 9306, WH9307, WH9308, WH9309, WH9322, WH9313, WH9317, WH9318, WH9319, WH9320 or WH9321, having one or two physiological and/or morphological characteristics which are different from the WH plant listed and which otherwise has essentially all physiological and morphological characteristics of a plant designated WH 9306, WH9307, WH9308, WH9309, WH9322, WH9313, WH9317, WH9318, WH9319, WH9320 or WH932, obtainable by further breeding with the WH plant and/or by selecting a natural or induced mutant, or a somaclonal variant from a population of plants designated WH 9306, WH9307, WH9308, WH9309, WH9322, WH9313, WH9317, WH9318, WH9319, WH9320 or WH9321.

15 <u>DEPOSIT INFORMATION</u>

Applicant(s) maintain a deposit of at least 2500 seeds of hybrid pollenizers mentioned in the Examples, and parent inbred lines, at Nunhems B.V. Applicant has deposited hybrid WH 9307 at the NCIMB on 12 November 2010 under Accession number NCIMB 41773. Access to this deposit will be available during the pendency of this application to persons determined by the Commissioner of Patent and Trademarks to be entitled thereto upon request.

Subject to 37 C.F.R. § 1.808(b), all restrictions imposed by the depositor on the availability to the public of one or more deposits will be irrevocably removed upon the granting of the patent by affording access to a deposit of at least 2500 seeds with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Va. 20110 or National Collections of Industrial, Food and Marine Bacteria (NCIMB), 23 St. Machar Drive, Aberdeen, Scotland, AB24 3RY, United Kingdom. The deposit will be maintained for a period of 30 years, or 5 years after the most recent request, or for the enforceable life of the patent whichever is longer, and will be replaced if it ever becomes nonviable during that period. Applicant does not waive any rights granted under this patent on this application or under the Plant Variety Protection Act (7 USC 2321 et seq.).

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The following non-limiting Examples describe the production of diploid pollenizers and small, edible diploid fruits according to the invention. Unless stated otherwise in the Examples, methods for conventional watermelon breeding are used, such as e.g. described in Maynard 2001, Watermelons – Characteristics, Production and Marketing, ASHS Press; Mohr H.C. Watermelon Breeding in Mark J. Bassett (editor) 1986 Breeding Vegetable Crops, AVI Publishing Company.

WO 2012/069539 PCT/EP2011/070817

Example 1 - Breeding history

EXAMPLES

Breeding for the hybrids goes back to 1985, to in-house breeding lines and crosses of these with the variety Allsweet. Selected self pollinations were backcrossed with Tomato Seed OP (Sugar Baby mutant; ts gene) and selections were made for red flesh and small seed. Further self pollinations were selected for red flesh, small seeds and agronomic traits in general. Selections were crossed with a line with many male flowers, followed by several selfings and selection for the production of many male flowers and small red fruits.

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Example 2 – Pollenizer characteristics

2.1 – Materials and Methods

A field trial was conducted in Italy (Sant'Agata Bolognese-BO). Seeds were sown on 7 April 2010 and transplanted into the field on 21 May 2010 (100 cm within the row, 250cm between the rows). The plot contained 10 plants per line. Fruits were harvested on 26 / 27 July 2010 for evaluation.

2.1.1 Flowering

The number of open male and female flowers were counted for three plants per line on the flowering date (day 1 = 24 June 2010), and 8, 15 and 22 days after the flowering date (day 8 = 2 July 2010; day 15 = 9 July 2010; day 22 = 16 July 2010). The mean number of three plants per line was calculated.

2.1.2 Fruits

- Average fruit number: at maturity (26/27 July) the total number of fruits harvested from two plants was counted and the average fruit number determined.
- 25 2) Average fruit weight: mean of the weight of three fruits per line randomly harvested from two plants at maturity
 - 3) Brix: Value is the mean of three reading for three fruit, collected between the centre and the rind of the fruit; expressed in Degrees Brix (°) using the K71901 portable refractometer Mod. RLC ATC 0-18% (OPTECH).
- 30 Flesh structure (flesh firmness): Value is the mean of the reading of three fruits; expressed in kg 4) using the fruit pressure tester FT 011 (Cientec Instrumentos)
 - Flesh colour: evaluated using the Royal Horticultural Society mini colour chart 5) (http://www.rhs.org.uk/Plants/RHS-Publications/RHS-colour-charts)

35 2.1.3 Other fruit characteristics

Measurements were done for three fruits:

- 1) Fruit length (cm), indicated as FRT-cm L
- 2) Fruit diameter at midsection (cm), indicated as FRT-cm D

- 3) Fruit rind thickness (cm), blossom end, indicated as RND-cm BE
- 4) Fruit rind thickness (cm), side of fruit, indicated as RND-cm S.

Rind thickness is measured from the outer edge of the fruit to the boundary between white mesocarp and colored endocarp.

2.2 - Results

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2.2.1 – Fruit characteristics

Table 1 shows that the pollenizers according to the invention produced very small, red, edible fruits.

Table 1 – Fruit characteristics of fruits from 11 diploid pollenizers according to the invention and commercial diploid pollenizers (Sidekick, SP-4 and Polimax)

Dual Purpose	Average	Average	° Brix	Fruit	Flesh color
Pollenizers	fruit no.	fruit		flesh	(RHS)
(diploid		weight		structur	
hybrids)		(kg)		e (kg)	
WH 9306x	38.5	0.52	7.0	0.6	Red (41A)
WH 9307x	41.0	0.55	8.5	1.4	Red (41A)
WH 9308x	51.0	0.44	7.7	0.6	Red (41B)
WH 9309x	38.5	0.45	8.0	0.6	Red (41A)
WH 9311x	56.5	0.55	7.3	0.6	Red (41B)
WH 9313x	23.5	0.59	6.3	0.5	Red (39B)
WH 9317x	51.5	0.45	5.7	0.6	Red (41B)
WH 9318x	34.5	0.51	7.7	0.6	Red (39B)
WH 9319x	52.5	0.57	7.3	0.6	Red (39B)
WH 9320x	35.5	0.49	7.7	0.5	Red (41B)
WH 9321x	49.5	0.33	6.2	0.5	Red (39B)
Min – Max	23.5-56.5	0.33-0.59	5.7-8.5	0.5-1.4	Red (RHS
(average)	(43.0)	(0.49)	(7.2)	(0.6)	39B – 41A)
Commercial poli	lenizers:			•	,
Sidekick	27.5	0.75	7.7	0.6	Pink (31D)
SP-4	8.5	1.4	5.2	1.1	white
Polimax	5.0	2.3	10.3	1.0	red

As can be seen from Table 1, the diploid pollenizers according to the invention produced very small, red-fleshed edible fruits. Fruit weight in Table 1 is significantly smaller than that of commercial pollenizers such as Sidekick, SP-4 and Polimax.

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Table 2 – Fruit dimensions

Pollenizers (diploid hybrids)	Average length (cm)	Average width (cm)
	(FRT-cm_L)	(FRT-cm_D)
WH 9306x	11.33	10.17
WH 9307x	10.50	9.83
WH 9308x	10.50	9.67
WH 9309x	11.00	10.00
WH 9311x	10.38	9.88
WH 9313x	11.33	10.00
WH 9317x	10.83	9.17
WH 9318x	11.17	9.67
WH 9319x	10.67	9.33
WH 9320x	11.33	9.17
WH 9321x	9.67	8.67
Commercial pollenizers:	1	
Sidekick	12.00	11.33
SP-4	15.33	13.17
Polimax	16.50	16.00

Table 2 shows that the pollenizers according to the invention have on average smaller fruit dimensions than the commercial pollenizers. The fruit length is on average 11.33 cm or smaller, while in Sidekick the average fruit length is 12 cm. The diameter is also smaller, only 10.17 cm or less, compared to 11.33 cm in Sidekick. Thus fruit dimensions of equal to or below 11.33×10.17 cm (e.g. even as small as 9.67×8.67 cm) are significantly smaller than 12.00×11.33 cm in Sidekick.

Table 3 shows mean rind thicknesses per line, measured at two points of the fruit. Thin rinds are an advantage for consumption.

Table 3 – rind thickness at the blossom end and on the side of the fruit

Pollenizers (diploid hybrids)	Average rind	Average rind
	thickness – blossom	thickness – side (cm)
	end (cm)	(RND-cm_S)
	(RND-cm_BE;)	
WH 9306x	0.17	0.37
WH 9307x	0.27	0.43
WH 9308x	0.20	0.37
WH 9309x	0.20	0.40
WH 9311x	0.25	0.40
WH 9313x	0.23	0.47
WH 9317x	0.15	0.33
WH 9318x	0.20	0.30
WH 9319x	0.23	0.40
WH 9320x	0.20	0.40
WH 9321x	0.13	0.30
Commercial pollenizers:		
Sidekick	0.30	0.43
SP-4	0.10	0.10
Polimax	0.40	0.70

- The pollenizers according to the invention have good, although thin, rind thickness of between 0.30 and 0.47 cm. The rind is also not susceptible to cracking, giving the fruits good handling properties. Fruits do also not have a brittle or explosive rind.
- The rind pattern of the fruits of all pollenizers according to the invention is a Crimson Sweet type rind pattern (medium-striped or netted), but the small fruit size can also be combined with other rind colors using standard breeding methods.

2.2.2 – Flowering characteristics

Table 4 – Flowering characteristics of 11 diploid pollenizers according to the invention and commercial diploid pollenizers (Sidekick, SP-4 and Polimax)

Pollenizer	No. of o	pen male	flowers		No. of o	pen femal	le flowers	
	Day 1	Day 8	Day 15	Day 22	Day 1	Day 8	Day 15	Day 22
WH 9306x	18.3	48.3	49.0	35.7	4.7	2.0	0.0	1.3
WH 9307x	15.0	51.0	53.7	35.0	4.7	3.0	0.3	1.0
WH 9308x	12.3	45.0	36.0	41.7	4.3	4.7	0.0	2.0
WH 9309x	10.0	42.3	49.7	46.7	2.0	1.0	0.3	2.3
WH 9311x	11.7	54.7	57.0	61.7	1.3	6.3	0.7	3.0
WH 9313x	9.0	57.3	47.7	40.3	5.0	2.7	0.3	1.3
WH 9317x	12.0	56.7	68.3	82.3	5.0	4.7	0.7	5.3
WH 9318x	7.0	38.7	65.3	68.7	4.7	5.7	0.0	5.3
WH 9319x	18.3	71.7	54.3	56.7	3.7	3.3	0.3	1.3
WH 9320x	12.0	43.7	47.7	33.3	3.3	2.0	0.0	0.7
WH 9321x	13.3	42.7	65.0	56.7	4.3	5.3	0.0	3.3
Min –	7.0 -	38.7 -	36.0 -	33.3 –	1.3 –	1.0 –	0.0 –	0.7 –
Max	13.8	71.7	68.3	82.3	5.0	6.3	0.7	5.3
(average)	(12.6)	(50.2)	(54.0)	(50.8)	(3.9)	(3.7)	(0.2)	(2.5)
Commercial	•							
Sidekick	14.3	46.3	33.0	24.3	2,0	4.7	0.0	0.0
SP-4	13.3	11.7	29.7	28.7	2,3	1.3	2.3	1.0
Polimax	14.3	10.0	18.3	18.7	1.3	1.7	1.3	1.3

2.2.3 – Vegetative types

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The hybrids have a relatively compact growth type, with the (average/ pollenizer line of the) longest branch falling between 113.5 and 180.0 cm long and the shortest branch being between 57.0 and 80.0 cm long. The average number of primary branches per pollenizer line was between 3 and 3.5. The average number of secondary branches per pollenizer line at 30 cm and at 90 cm was between 56.0 and 88.5 (at 30 cm) and between 77.5 and 144.0 cm. Internode length ranged between 4.3 and 5.3 on average, depending on the line. Leaf length and width is also relatively compact, with leaf widths between 7.1 and 9.4 cm and lengths between 7.8 and 9.1 cm.

Example 3 – Use of pollenizers according to the invention

3.1 –Trial set-up

5 Three trials were carried out in Spain using pollenizers WH9317x, WH9318x, WH9320x and WH9321x for triploid fruit production on the triploid hybrid variety 'Fashion'.

<u>Trial 1:</u>

Location: green house

10 Trial dimensions: 3600 m²

Transplanting date: 19-March-2010

Harvest date: 16-June-2010

Scheme: pollenizers and triploids were in separate rows, alternating one row of triploid and one row of

pollenizer. Distance between rows was 3 meter, distance between plants in a row was 1 meter.

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Trial 2:

Location: open field

Trial dimensions: 2500 m²

Transplanting date: 24-March-2010

Harvest date: 6-July-2010

Scheme: pollenizers and triploids were interplanted in the same rows, with pollenizers making up 25% of the total plants. Distance between rows was 3 meters and distance between plants in a row was 1 meter.

25 <u>Trial 3:</u>

Location: open field

Trial dimensions: 1500 m²

Transplanting date: 20-March-2010

Harvest date: 2-July-2010

Scheme: pollenizers and triploids were interplanted in the same rows, with pollenizers making up 25% of the total plants. Distance between rows was 2 meters and distance between plants in a row was 1.8 meter.

3.2 - Trial Results

Table 5 - mean value* of the triploid fruit weight (kg) of Fashion (triploid hybrid) for three trials carried out in three locations in Spain 2010

	Trial 1	Trial 2	Trial 3
WH9317x	5,14	4,18	4,27
WH9318x	5,06	4,48	4,12
WH9320x	5,22	3,94	4,23
WH9321x	4,65	4,35	4,30
Average	5,02	4,24	4,23
(min-MAX)	(2,5-9,08)	(2,5-7,64)	(2,56-7,60)
Commericial			
Pollenizers			
Jenny	5,15	4,26	4,96
Polimax	5,30	3,90	4,54
SP-4	6,14	4,26	4,44
Sidekick	5,35		

^{*}Mean value: mean of the weights of the total marketable triploid fruits (>2,5 kg) harvested in an area of 60 m², 90 m² and 30 m² of the Trial 1, Trial 2 and Trial 3, respectively.

PCT

Print Out (Original in Electronic Form) (This sheet is not part of and does not count as a sheet of the international application)

0-1	Form PCT/RO/134 (SAFE) Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis)	
0-1-1	Prepared Using	PCT Online Filing
		Version 3.5.000.225 MT/FOP 20020701/0.20.5.20
0-2	International Application No.	PCT/EP2011/070817
0-3	Applicant's or agent's file reference	BCS102018-WO
1	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
1-1	page	16
1-2	line	16-20
1-3	Identification of deposit	
1-3-1	Name of depositary institution	NCIMB NCIMB Ltd.
1-3-2	Address of depositary institution	Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen AB21 9YA, United Kingdom
1-3-3	Date of deposit	12 November 2010 (12.11.2010)
1-3-4	Accession Number	NCIMB 41773
1-5	Designated States for Which Indications are Made	All designations

FOR RECEIVING OFFICE USE ONLY

	This form was received with the international application: (yes or no)	YES
0-4-1	Authorized officer	Koestel, Gilbert

FOR INTERNATIONAL BUREAU USE ONLY

	This form was received by the international Bureau on:	
0-5-1	Authorized officer	

CLAIMS:

- 1. A plant of the species *Citrullus lanatus*, wherein said plant is diploid and produces edible, diploid fruits having an average weight of less than 0.9 kg at maturity of said fruit.
- 2. The plant according to claim 1, having an average fruit weight of equal to or less than 0.65 kg at maturity of said fruit.
 - 3. The plant according to claim 1 or 2, wherein the average percent Total Soluble Solids (TSS) of said fruits is at least about 8 %, preferably at least about 9 %, more preferably at least about 10 %.
- 4. The plant according to any one of the preceding claims, wherein the fruit flesh has a red color, having a RHS color chart rating of 39 or higher.
 - 5. The plant according to any one of the preceding claims, wherein the plant produces at least 30 male flowers at day 22 from flowering.
 - 6. The plant according to any one of the preceding claims, wherein said plant produces an average number of fruits of at least 10 per plant.
- The plant according to any one of the preceding claims, wherein said fruits have an average fruit length x width of 10 cm x 11 cm, or less at maturity of said fruit.
 - 8. A diploid watermelon fruit obtainable from a plant according to any one of the preceding claims, having a fruit weight of less than 0.9 kg at maturity of said fruit.
- 9. The diploid watermelon fruit according to claim 8, wherein said fruit has an average fruit weight of equal to or less than 0.65 kg at maturity of said fruit.
 - 10. A container comprising a plurality of fruits according to claim 8.
 - 11. A watermelon plant or seed having the plant according to claim 1 as male or female parent.
 - 12. A transplant or vegetative propagation of a plant according to claim 1.
 - 13. Seeds or transplants from which a plant according to claim 1 can be grown.
- 25 14. The plant according to claim 1 obtainable from seeds deposited under accession number NCIMB 41773.
 - 15. A method for producing diploid and triploid watermelon fruits in one field, said method comprising:

- (a) interplanting diploid pollenizer plants according to claim 1 and triploid hybrid watermelon plants in one field,
- (b) allowing pollination of flowers of the triploid hybrid plants with pollen of the diploid pollenizer plants and allowing pollination of flowers of the diploid pollenizer plants with pollen of the diploid pollenizer plants,
- (c) harvesting fruits produced on the triploid hybrid plants and, optionally, harvesting fruits produced on the diploid pollenizer plants.
- 16. A method for producing edible, diploid watermelon fruits having an average weight of less than 0.9 kg, said method comprising:
- 10 (a) growing a plant according to claim 1,

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- (b) pollinating the female flowers of said plant with pollen of said plant,
- (c) harvesting the fruits produced on said plant.
- 17. The method according to claim 16, wherein the genetic elements for producing small fruits having an average weight of less than 0.9 kg are obtainable from seed deposited under accession number NCIMB 41773.
 - 18. A diploid watermelon pollenizer plant producing small edible fruits having an average weight of less than 0.9 kg, wherein a representative sample of seed containing the genetic elements for producing said small fruits has been deposited under accession number NCIMB 41773.
- Seed of a diploid watermelon line capable of producing edible fruit of less than 0.9 kg upon
 self-pollination, wherein a representative sample of seed has been deposited under accession number NCIMB 41773.
 - 20. A plant cell, an ovule, pollen, a root-stock or a scion of a plant according to claim 1.
 - 21. The plant according to any one of claims 1 to 7, wherein the plant is a hybrid.

INTERNATIONAL SEARCH REPORT

International application No PCT/EP2011/070817

A. CLASSIFICATION OF SUBJECT MATTER INV. A01H5/08 A01H5/00

ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols) A01H

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

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

EPO-Internal, BIOSIS, MEDLINE, WPI Data

C. DOCUM	ENTS CONSIDERED TO BE RELEVANT	
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DITTMAR PETER J ET AL: "Maximum Potential Vegetative and Floral Production and Fruit Characteristics of Watermelon Pollenizers", HORTSCIENCE, AMERICAN SOCIETY OF HORTICULTURAL SCIENCE, ALEXANDRIA, VA, US, vol. 44, no. 1, 1 February 2009 (2009-02-01), pages 59-63, XP009145720, ISSN: 0018-5345	1,20
Α	table 5	2-19,21
A	WO 00/70933 A1 (SUNSEEDS INC [US]; ELMSTROM GARY W [US]) 30 November 2000 (2000-11-30) page 7, lines 6-11	1-21
	-/	

Further documents are listed in the continuation of Box C.	See patent family annex.
"A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family
Date of the actual completion of the international search 23 December 2011	Date of mailing of the international search report $30/12/2011$
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Bilang, Jürg

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/070817

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 7 820 884 B2 (ZUCKERBRAUN ELIEZER [US] ZUCKERBRAUN ELIEZER [IL]) 26 October 2010 (2010-10-26) cited in the application column 5, lines 34-48	1-21
A	column 5, lines 34-48 WO 03/075641 A2 (SYNGENTA PARTICIPATIONS AG [CH]; ZHANG XINGPING [US]; WILLIAMS TOM VAR) 18 September 2003 (2003-09-18) cited in the application abstract	1-21

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2011/070817

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US 7820884	В2	26-10-2010	NONE		
WO 03075641	A2	18-09-2003	AU BR EP JP MX US US WO	2003216413 A1 0308228 A 1487256 A2 2005518814 A PA04008512 A 2005050597 A1 2006168701 A1 03075641 A2	22-09-2003 28-06-2005 22-12-2004 30-06-2005 06-12-2004 03-03-2005 27-07-2006 18-09-2003