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(54) Title: METHODS AND COMPOSITIONS FOR PRODUCING PLANTS WITH ELEVATED BRIX

(57) Abstract: The invention provides compositions and methods relating to the production of *Cucumis melo* plants with elevated Brix and/or enhanced fruit color. The invention further provides plants, plant parts, and seeds comprising such elevated Brix. The invention allows introgression of the identified high Brix QTL in an desired genetic background of a sexually compatible plant species.



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A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A01H 5/08; C12N 5/04; C12Q 1/68 (2013.01)

USPC - 800/263, 309; 435/6.11

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8): A01H 5/00, 5/06, 5/04, 1/02, 5/08, 5/02, 5/10, 5/12; C12N 5/04; C12Q 1/68 (2013.01)

USPC: 800/263, 309, 317.4; 435/411, 6.11, 6.12

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

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

MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google; Google Scholar; Science Direct; ProQuest; 'Brix,' 'cucumis melo,' introgressed, sugar, 'linkage group,' 'genetic markers,' melon, 'QTL'

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2011/0283402 A1 (BUNN, TB et al.) November 17, 2011; paragraphs [0022], [0024], [0025]	1-22, 26-29, 31-36
A	US 2003/0135886 A1 (ZAMIR, D et al.) July 17, 2003; paragraphs [0018], [0034], [0053]	1-22, 26-29, 31-36
A	US 8053631 B2 (BROGLIE, KE et al.) November 8, 2011; column 3, lines 29-31, 38; column 6, line 28	1-22, 26-29, 31-36
A	US 2007/0039076 A1 (BOUKHAROV, AA et al.) February 15, 2007; paragraph [0020]; Claim 1	1-22, 26-29, 31-36

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

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Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

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

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

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

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

Groups I+: Claims 1-22, 26-29, 31-36, SEQ ID NOs: 1, 74

Remark on Protest

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

---Continuation of Box No. III - Observations where unity of invention is lacking:

Groups I+: Claims 1-36, a high Brix content contributing QTL defined by flanking markers NU0219671 (i.e., SEQ ID NO: 1) and NU0218915 (i.e., SEQ ID NO: 74) on Cucumis melo linkage group 4; Exemplary Election: a high Brix content contributing QTL defined by flanking markers NU0220114 (i.e., SEQ ID NO: 75) and NU0219142 (i.e., SEQ ID NO: 109) on Cucumis melo linkage group 10

-Continued from 1. (ii):

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+: Claims 1-36 and a high Brix content contributing QTL defined by flanking markers NU0219671 (i.e., SEQ ID NO: 1) and NU0218915 (i.e., SEQ ID NO: 74) on Cucumis melo linkage group 4 are directed toward a Cucumis melo plant comprising at least a first introgressed chromosomal region conferring increased Brix content relative to a plant lacking the region, wherein the region is selected from the group consisting of a high Brix content contributing QTL defined by flanking markers NU0219671 (i.e., SEQ ID NO: 1) and NU0218915 (i.e., SEQ ID NO: 74) on Cucumis melo linkage group 4, a high Brix content contributing QTL defined by flanking markers NU0220114 (i.e., SEQ ID NO: 75) and NU0219142 (i.e., SEQ ID NO: 109) on Cucumis melo linkage group 10, and a high Brix content contributing QTL defined by flanking markers NU0220114 (i.e., SEQ ID NO: 75) and NU0220323 (i.e., SEQ ID NO: 83) on Cucumis melo linkage group 10; a method of obtaining Cucumis melo germplasm comprising the steps of: a) assaying Cucumis melo plants or a seed thereof for the presence of at least a first genetic marker genetically linked to a chromosomal region conferring increased Brix content relative to a plant lacking the region, wherein the region is selected from the group consisting of a high Brix content contributing QTL defined by flanking markers NU0219671 (i.e., SEQ ID NO: 1) and NU0218915 (i.e., SEQ ID NO: 74) on Cucumis melo linkage group 4, a high Brix content contributing QTL defined by flanking markers NU0220114 (i.e., SEQ ID NO: 75) and NU0219142 (i.e., SEQ ID NO: 109) on Cucumis melo linkage group 10, and a high Brix content contributing QTL defined by flanking markers NU0220114 (i.e., SEQ ID NO: 75) and NU0220323 (i.e., SEQ ID NO: 83) on Cucumis melo linkage group 10; and b) selecting at least a first Cucumis melo plant or seed thereof comprising the genetic marker and the QTL that confers increased Brix content; and a method of Cucumis melo plant breeding comprising: a) assaying Cucumis melo plants or seeds that produce the plants for the presence of at least a first genetic marker genetically linked to a chromosomal region conferring increased Brix content relative to a plant lacking the region, wherein the region is a high Brix content contributing QTL defined by flanking markers NU0219671 (i.e., SEQ ID NO: 1) and NU0218915 (i.e., SEQ ID NO: 74) on Cucumis melo linkage group 4, a high Brix content contributing QTL defined by flanking markers NU0220114 (i.e., SEQ ID NO: 75) and NU0219142 (i.e., SEQ ID NO: 109) on Cucumis melo linkage group 10, or a high Brix content contributing QTL defined by flanking markers NU0220114 (i.e., SEQ ID NO: 75) and NU0220323 (i.e., SEQ ID NO: 83) on Cucumis melo linkage group 10; and b) selecting at least a first Cucumis melo plant or seed that produces the plant comprising the genetic marker and the QTL that confers increased Brix; and c) crossing the first Cucumis melo plant with itself or a second Cucumis melo plant to produce progeny Cucumis melo plants comprising the QTL that confers increased Brix.

A high Brix content contributing QTL defined by flanking markers NU0219671 (i.e., SEQ ID NO: 1) and NU0218915 (i.e., SEQ ID NO: 74) on Cucumis melo linkage group 4 will be searched without the payment of any additional fees. Additional members of the "+" group can be searched upon the payment of additional fees. An Exemplary Election would be: a high Brix content contributing QTL defined by flanking markers NU0220114 (i.e., SEQ ID NO: 75) and NU0219142 (i.e., SEQ ID NO: 109) on Cucumis melo linkage group 10. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

Groups I+ share the technical features including a Cucumis melo plant comprising at least a first introgressed chromosomal region conferring increased Brix content relative to a plant lacking the region, wherein the region is a high Brix content contributing QTL defined by flanking markers; a method of obtaining Cucumis melo germplasm comprising the steps of: a) assaying Cucumis melo plants or a seed thereof for the presence of at least a first genetic marker genetically linked to a chromosomal region conferring increased Brix content relative to a plant lacking the region, wherein the region is a high Brix content contributing QTL defined by flanking markers; and b) selecting at least a first Cucumis melo plant or seed thereof comprising the genetic marker and the QTL that confers increased Brix content; and a method of Cucumis melo plant breeding comprising: a) assaying Cucumis melo plants or seeds that produce the plants for the presence of at least a first genetic marker genetically linked to a chromosomal region conferring increased Brix content relative to a plant lacking the region, wherein the region is a high Brix content contributing QTL defined by flanking markers; and b) selecting at least a first Cucumis melo plant or seed that produces the plant comprising the genetic marker and the QTL that confers increased Brix; and c) crossing the first Cucumis melo plant with itself or a second Cucumis melo plant to produce progeny Cucumis melo plants comprising the QTL that confers increased Brix.

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However, these shared features are previously disclosed by US 2011/0055947 A1 to Chung, et al. (hereinafter 'Chung'). Chung discloses a Cucumis melo plant (abstract) comprising at least a first introgressed chromosomal region (paragraphs [0087]-[0089]) conferring increased Brix content relative to a plant lacking the region (paragraphs [0083], [0089], [0121], [0122]; Tables 4, 5; examples of desirable characteristics include degrees of Brix), wherein the region is a high Brix content contributing (paragraphs [0083], [0121], [0122]) QTL (paragraph [0065]) defined by flanking markers (paragraphs [0105], [0108]); a method of obtaining Cucumis melo germplasm (paragraph [0087]) comprising the steps of: a) assaying Cucumis melo plants or a seed thereof for the presence of at least a first genetic marker (paragraph [0099]) genetically linked to a chromosomal region conferring increased Brix content relative to a plant lacking the region (paragraphs [0089], [0121], [0122]; Tables 4, 5; examples of desirable characteristics include degrees of Brix), wherein the region is a high Brix content contributing (paragraphs [0083], [0121], [0122]) QTL (paragraph [0065]) defined by flanking markers (paragraphs [0105], [0108]); and b) selecting at least a first Cucumis melo plant or seed thereof comprising the genetic marker (paragraph [0099]) and the QTL (paragraph [0065]) that confers increased Brix content (paragraphs [0083], [0089], [0121], [0122]; Tables 4, 5; a means of determining melon maturity at an appropriate harvest time on a per melon basis, providing the means for consistent harvest of mature melons with high sugar levels and firm flesh); and a method of Cucumis melo plant breeding (paragraph [0002]) comprising: a) assaying Cucumis melo plants or seeds that produce the plants for the presence of at least a first genetic marker (paragraph [0099]) genetically linked to a chromosomal region conferring increased Brix content relative to a plant lacking the region (paragraphs [0083], [0089], [0121], [0122]; Tables 4, 5; examples of desirable characteristics include degrees of Brix), wherein the region is a high Brix content contributing (paragraphs [0083], [0121], [0122]) QTL (paragraph [0065]) defined by flanking markers (paragraphs [0105], [0108]); and b) selecting at least a first Cucumis melo plant or seed that produces the plant comprising the genetic marker (paragraph [0099]) and the QTL (paragraph [0065]) that confers increased Brix (paragraphs [0083], [0089], [0121], [0122]; Tables 4, 5; a means of determining melons with high sugar levels and firm flesh); and c) crossing the first Cucumis melo plant with itself or a second Cucumis melo plant to produce progeny Cucumis melo plants (abstract; Claim 58) comprising the QTL (paragraph [0065]) that confers increased Brix (paragraphs [0083], [0089], [0121], [0122]; Tables 4, 5; a means of determining melon maturity at an appropriate harvest time on a per melon basis, providing the means for consistent harvest of mature melons with high sugar levels and firm flesh).

Since none of the special technical features of the Groups I+ invention is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Chung reference, unity of invention is lacking.