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- with international search report (Art. 21(3))
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- with sequence listing part of description (Rule 5.2(a))

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20 August 2009

(54) Title: SOYBEANS WITH REDUCED ISOFLAVONES

### Graphic representation of the TILLING® process.

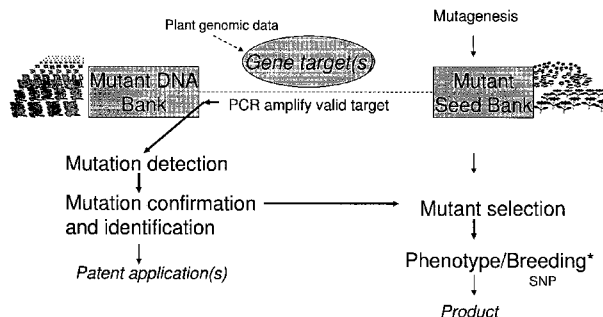


Figure 1

(57) Abstract: Non-naturally occurring soybean plants and seeds having reduced isoflavones are provided. A series of independent non-transgenic human-induced mutations found in one or more isoflavone synthase genes of soybean; soybean plants having these mutations in one or more isoflavone synthase genes; and a method of creating and finding similar and/or additional mutations of the isoflavone synthase gene by screening pooled and/or individual DNA of soybean plants. The results are soybean plants and seeds having reduced isoflavones.



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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 08/69258

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A01H 5/00 (2009.01)

USPC - 800/312

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 (2009.01)

USPC - 800/312

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

USPC - 800/278, 800/281, 800/295, 800/313 - see keyword below

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

PubWEST(USPT,PGPB,EPAB,JPAB); Medline, Google: non-naturally, soybean plant, isoflavone, reduced, IFS, isoflavone synthase, mutation, expression, stop, missense, transgene, IFS1, progeny, seed, food, plant, pollen, cell, tissue, ethyl methanesulfonate, 0.5%, genomic, amplifying, primer, mutagen. GenCore 6.3: SEQ ID NO:3

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---	US 2006/0005276 A1 (FALCO et al.) 05 Jan 2006 (05.01.2006), Abstract, para [0010], [0018], [0134], [0169], [0173], [0181], [0184], [0189], [0293], [0297], and [0405]	1-4, 8, 21-23
Y		5-7, 9-11, 24-29
Y	US 2005/0289669 A1 (WAN et al.) 29 Dec 2005 (29.12.2005), para [0003], [0013], and [0170]	5-7, 9-11
Y	US 2007/0118920 A1 (LEON et al.) 24 May 2007 (24.05.2007), para [0024], [0056], [0076], [0079]-[0080], [0084], [0115], [0141], [0155], and 0227]	24-29
Y	US 2006/0242735 A1 (FADER et al.) 26 Oct 2006 (26.10.2006), para [0053]-[0154]	10-11, 29

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

Date of the actual completion of the international search

22 June 2009 (22.06.2009)

Date of mailing of the international search report

**29 JUN 2009**

Name and mailing address of the ISA/US

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 08/69258

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:

Group I: claims 1-11, 21-29, drawn to a non-naturally occurring soybean plant that produces seeds having an isoflavone level reduced as compared to a wild type plant, restricted to IFS1 gene of SEQ ID NO:3, and a method of producing said plant.

Group II: claims 1-9, 12, 21-28, drawn to a non-naturally occurring soybean plant that produces seeds having an isoflavone level reduced as compared to a wild type plant, restricted to IFS1 gene of SEQ ID NO:1, and a method of producing said plant.

Group III, claims 1-7, 13-15, 21-29, drawn to a non-naturally occurring soybean plant that produces seeds having an isoflavone level reduced as compared to a wild type plant, restricted to IFS2 gene of SEQ ID NO:4, and a method of producing said plant.

\*\*\*\*\*Continued in extra sheet\*\*\*\*\*

- 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. [X] 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.:
1-11, 21-29, restricted to SEQ ID NO: 3.

- 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.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 08/69258

Continuation of:

Box No III (unity of invention is lacking)

Group IV, claims 1-7, 16, 21-28, drawn to a non-naturally occurring soybean plant that produces seeds having an isoflavone level reduced as compared to a wild type plant, restricted to IFS2 gene of SEQ ID NO:2, and a method of producing said plant.

Group V+, claims 1-7, 17-20, 21-29, drawn to a non-naturally occurring soybean plant, wherein said isoflavone level is reduced by a non-transgenic mutation of an IFS1 gene and a non-transgenic mutation of an IFS2 gene, and a method of producing said plant. Should additional fee be paid, Applicant is invited to elect specific mutations in a specific combination of IFS1 and IFS2 genes to be searched. The exact claims searched will depend on Applicant's election.

The inventions listed as Groups I-V+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Although Groups I-V+ share the technical feature of a non-naturally occurring soybean plant that produces seeds having an isoflavone level reduced as compared to a wild type plant, this shared technical feature is obvious over the paper entitled "Genome-wide analyses of the structural gene families involved in the legume-specific 5-deoxyisoflavonoid biosynthesis of *Lotus japonicus*" by Shimada et al. (DNA Res. Feb 2007, 14(1):25-36) that teaches targeted approaches to the legume genomics using as an example the genes encoding enzymes involved in the biosynthesis of the legume-specific 5-deoxyisoflavonoid of *L. japonicus* (abstract). Shimada et al. further teaches that isoflavonoids have significant ecological and physiological functions and that reverse genetic approaches such as TILLING is useful for functional genomic studies of leguminous plants (pg 35, col 2). As the above plant and a method for producing it is obvious in view of Shimada et al., the plant and the method cannot be considered a special technical feature that would otherwise unify the groups.

Furthermore, the claimed SEQ ID NOs are taught by prior art and do not have significant structural similarities with one another. Specifically, Direct Genbank Submission AF195798 (16-FEB-2000; [Retrieved from the Internet 21 Apr 2009: <<http://www.ncbi.nlm.nih.gov/nuccore/6979519>>) discloses the claimed isoflavone synthase gene of SEQ ID NO: 1. Without significant structural similarities, the compounds lack a shared special technical feature. In the absence of a shared special technical feature, the inventions lack unity with one another.

Groups I-V+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.