Title: DNA EXTRACTION FROM SEEDS USING OSMOTICUM

Abstract: The invention broadly relates to methods of fragmenting seed by use of mechanical devices such as crushing pins or other crushing devices with a preconditioned hard seed. Methods of preconditioning the seeds to soften the seed for more effective fragmentation which are adapted to extract DNA yields and/or DNA quality are shown. Extracted DNA yield from alkali-soaked seeds can be significantly increased by adding an osmoticum to a seed-soften ing alkali soaking solution used for pretreatment of the seeds prior to crushing them. The osmoticum inhibits and reduces liquid uptake by the seeds, but the seeds are weakened enough to be crushed with steel beads, without requiring the use of crushing pins or other crushing devices.
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(88) Date of publication of the international search report: 8 August 2013
### DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>WO 2010/108082 A1 (COPE, JM et al.), September 23, 2010; abstract; page 24, lines 9-17; page 26, lines 1-6; page 27, lines 13-31</td>
<td>15, 16</td>
</tr>
<tr>
<td>Y</td>
<td>US 2009/0286301 A1 (TAO, F et al.), November 19, 2009; abstract; paragraphs [0202], [0207], [0266], [0270], [0348]</td>
<td>1-10, 20</td>
</tr>
<tr>
<td>Y</td>
<td>SHAO, S et al. The Outermost Cuticle Of Soybean Seeds: Chemical Composition And Function During Imbibition. Journal of Experimental Botany, 11 January 2007, Vol. 58, No. 5, pp. 1071 - 1082; abstract; page 1073, column 1, paragraph 2; page 1074, column 2, paragraph 2</td>
<td>1-10</td>
</tr>
<tr>
<td>Y</td>
<td>GAO, S et al. Development Of A Seed DNA-Based Genotyping System For Marker-Assisted Selection In Maize. May 22, 2008, Mol. Breeding, 22 May 2008; Vol. 22, pp 477-494; DOI: 10.1007/s11032-008-9192-4; page 482, column 1, paragraph 1 to column 2, paragraph 1; figure 1</td>
<td>11-14, 22</td>
</tr>
<tr>
<td>Y</td>
<td>US 2530272 A (THRASHER, WB), November 4, 1950; column 3, lines 20-37; column 4, lines 10-22</td>
<td>19-21</td>
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<tr>
<td>Y</td>
<td>US 7229304 B2 (FEAZEL, RJ et al.), June 12, 2007; abstract; column 1, lines 27-52; column 2, lines 20-35</td>
<td>22</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C.

- **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
- **I** 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**

16 March 2013 (16.03.13)

**Date of mailing of the international search report**

**17 JUN 2013**

**Name and mailing address of the ISA/US**

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

**Authorized officer:**
Shane Thomas
PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)
## DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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</thead>
<tbody>
<tr>
<td>Y</td>
<td>US 2007/0207485 A1 (DEPPERMAN, K et al.), September 6, 2007; abstract; paragraphs [0076], [0077]</td>
<td>12</td>
</tr>
</tbody>
</table>
INTERNATIONAL SEARCH REPORT

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:

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.

Group I: Claims 1-10, 15-18 and 22 are directed toward a method for extracting DNA from seeds, said method comprising: a. pretreating said seeds by soaking the seeds in a pretreatment solution comprising: i. an alkali in a concentration sufficient to soften said seed and optionally; ii. an osmoticum at a concentration sufficient to enhance the yield of said DNA compared to a process comprising pretreating said seeds only with an alkali; b. crushing said seeds; c. extracting said DNA from said crushed seeds.

Group II: Claims 11-14 and 19-21 are directed toward a method of fragmenting plant material comprising the steps of: pretreating plant material in a well with a solution; disrupting the pretreated seed with the reducer elements with vigorous shaking or collision forces resulting in fragmented seed material.

---Continued on Supplemental Page---

1. [X] 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.:

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.

Form PCT/ISA/210 (continuation of first sheet (2)) (July 2009)
The inventions listed as Groups I-II 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: the special technical features of Group I include: a method for extracting DNA from seeds, said method comprising: an alkali in a concentration sufficient to soften said seed and optionally; ii. an osmoticum at a concentration sufficient to enhance the yield of said DNA compared to a process comprising pretreating said seeds only with an alkali; b. crushing said seeds; c. extracting said DNA from said crushed seeds, which are not present in Group II; Group II having special technical features including: a method of fragmenting plant material comprising the steps of: pretreating plant material in a well with a solution; disrupting the pretreated seed with the reducer elements with vigorous shaking or collision forces resulting in fragmented seed material.

Groups I-II share the technical features including a method of fragmenting seeds, said method comprising: a. pretreating said seeds by soaking the seeds in a pretreatment solution; and b. crushing said seeds.

However, these shared technical features are previously disclosed by US 2008/0207932 A1 to Donaldson, et al. (hereinafter 'Donaldson'). Donaldson discloses a method of fragmenting seeds (abstract), said method comprising: a. pretreating said seeds by soaking the seeds in a pretreatment solution (Claims 1, 6); and b. crushing said seeds (abstract; Claims 1, 6, 8).

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