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(54) Title: METHOD FOR DETERMINING MATURITY IN CONIFER SOMATIC EMBRYOS

(57) Abstract

The invention concerns measurement of sucrose series oligosaccharides, particularly sucrose, raffinose, and stachyose, and the dehydrin group proteins, in conifer somatic embryos as a measure of their biochemical maturity and readiness to germinate. The information gained is useful for evaluation of the effectiveness of the culture media, particularly that used for the development of the cotyledonary embryo stage. Somatic embryos having elevated levels of the oligosaccharides have been shown to have improved germination, especially those in the genus *Pinus*. The invention is also directed to conifer somatic embryos having elevated levels of sucrose series oligosaccharides and dehydrin group proteins.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/08314

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/68 G01N33/50 A01H4/00 C12N5/04

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 6 G01N A01C A01H C12N

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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 183 757 A (ROBERTS DANE R) 2 February 1993 cited in the application see abstract see column 7, line 1 - line 54; figure 2 ---	8-12
A	US 5 563 061 A (GUPTA PRAMOD K) 8 October 1996 cited in the application see the whole document ---	1-12
A	US 5 036 007 A (GUPTA PRAMOD K ET AL) 30 July 1991 cited in the application see the whole document --- -/--	1-12

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 August 1998	20. 11. 98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Routledge, B

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International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 034 326 A (PULLMAN GERALD S ET AL) 23 July 1991 cited in the application see the whole document ---	1-12
A	US 4 957 866 A (GUPTA PRAMOD K ET AL) 18 September 1990 cited in the application see the whole document -----	1-12

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INTERNATIONAL SEARCH REPORT

In' ·national application No.
PCT/US 98/08314

Box I Observations where certain claims were found unsearchable (Continuation of item 1 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 II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

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

see additional 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 an additional fee, this Authority did not invite payment of any additional fee.

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.:

1-12

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 98/08314

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-12

Methods for detecting biological readiness e.g. biochemical maturity (claims 1-7) or germination readiness (claims 8-12) of conifer somatic embryos by measuring a biochemical feature e.g. sucrose series oligosaccharide (claims 1-7) or dehydrin group protein content (claims 8-12) and making a comparison with results achieved from zygotic embryos at a similar developmental stage. Conifer somatic embryos of defined sugar content (claims 2-7).

2. Claim : 13

Method of inducing dehydrin group proteins in conifer somatic embryos by treating cotyledonary stage embryos at a minimum of 95% relative humidity for at least four days or until the dehydrin group proteins develop

INTERNATIONAL SEARCH REPORT

›rmation on patent family members

International Application No
PCT/US 98/08314

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