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[Continued on next page]

(54) Title: MATERIALS AND METHODS FOR PUFA PRODUCTION, AND PUFA-CONTAINING COMPOSITIONS

Fig. 1 aerobic ω**6**ω3pathway pathway 18:2 49.12 18:3 49.12,15 ⊿6-desaturase 49-elongase 49-elongase 18:4 46,9.12,15 20:3 411,14,17 18:3 46,9,12 ∆8-desaturase ⊿6-elongase ∆8-desaturase 20:4 48,11,14,17 20:3 48,11,14 45-desaturase PKS (anaerobic) Sprecher pathway 20:5 45.8 11:14.17 e.g. bacteria e.g. mammals Acetyl-CoA 24:5 49, 12, 15, 18,21 22.4 47.10.13.16 22:5 47:10:13:16:19 Δ6-desaturase .14-desaturase 20:5 45,8,11,14,17 24.6 46,9,12,15,18,21 22:5 44,7,10,13,16 22-6 A4.7,10,13,16,19 **β-oxidation** DHA

(57) Abstract: T-DNA for expression of a target gene in a plant, wherein the T-DNA comprises a left and a right border element and at least one expression cassette comprising a promoter, operatively linked thereto a target gene, and downstream thereof a terminator, wherein the length of the T-DNA, measured from left to right border element and comprising the target gene, has a length of at least 30000 bp.



Published:

- with international search report (Art. 21(3))
- 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))
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INTERNATIONAL SEARCH REPORT

International application No PCT/EP2015/076631

| A. CLASSIFICATION OF SUBJECT MATTER INV. C12N15/82 ADD. | | | | | |
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| According to be been able to Detect Classification (IDC) and a ball making all along if a time and IDC | | | | | |
| 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) | | | | | |
| Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched | | | | | |
| Electronic da | ata base consulted during the international search (name of data bas | e and, where practicable, search terms use | d) | | |
| EPO-Internal, Sequence Search, WPI Data, BIOSIS, EMBASE | | | | | |
| C. DOCUME | ENTS CONSIDERED TO BE RELEVANT | | | | |
| Category* | Citation of document, with indication, where appropriate, of the rele | vant passages | Relevant to claim No. | | |
| X | WU G ET AL: "STEPWISE ENGINEERIN PRODUCE HIGH YIELDS OF VERY LONG-POLYUNSATURATED FATTY ACIDS IN PLINATURE BIOTECHNOLOGY, NATURE PUBLICATION OF THE PRODUCE | -CHAIN LANTS", LISHING | 1-8,11, 12,18,19 | | |
| X Furth | ner documents are listed in the continuation of Box C. | See patent family annex. | | | |
| * 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 | | 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 | | | |
| means being obvious to a person skilled in the art "P" document published prior to the international filing date but later than the priority date claimed "&" document member of the same patent family | | | | | |
| Date of the | actual completion of the international search | Date of mailing of the international search report | | | |
| 22 February 2016 | | 13/05/2016 | | | |
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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/076631

| | tion). DOCUMENTS CONSIDERED TO BE RELEVANT | I |
|-----------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| X | RUIFENG HE ET AL: "Agrobacterium-Mediated Transformation of Large DNA Fragments Using a BIBAC Vector System in Rice", PLANT MOLECULAR BIOLOGY REPORTER., vol. 28, no. 4, 2 March 2010 (2010-03-02), pages 613-619, XP055251246, NL ISSN: 0735-9640, DOI: 10.1007/s11105-010-0195-z the whole document page 617; figure 3 page 618, column 1 - paragraph 4 | 1 |
| A | CERTIK M ET AL: "Desaturase-defective fungal mutants: useful tools for the regulation and overproduction of polyunsaturated fatty acids", TRENDS IN BIOTECHNOLOGY, ELSEVIER PUBLICATIONS, CAMBRIDGE, GB, vol. 16, no. 12, 1 December 1998 (1998-12-01), pages 500-505, XP004143810, ISSN: 0167-7799, DOI: 10.1016/S0167-7799(98)01244-X the whole document figure 2 | 18,19 |

International application No. PCT/EP2015/076631

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: | | | |
| see additional sheet | | | |
| 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 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.: | | | |
| 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, 18, 19 | | | |
| 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. | | | |

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-12, 18, 19

T-DNA for expression of a target gene in a plant having at least 30000 bp; T-DNA thereof comprising any single gene of the tables given in the examples; plants comprising a T-DNA thereof; plants comprising a T-DNA comprising D5-desaturase, D6 elongase, D6-desaturase, omega-3-desaturase, D5-elongase and D4-desaturase; plant obtainable from progenies thereof; Method for creating a plant; method of plant oil production; plant oil thereof. Method for increasing the content of mead acid, comprising expressing in a plant D6-desaturase, D6 elongase and D5-desaturase. The method further expressing D12-desaturase, omega-3-desaturase, D5-elongase and/or D4-desaturase.

2. claims: 13-17

Method for analysing desaturase reaction specificity; method for analysing elongase reaction specificity; method for optimization of a metabolic pathway; and methods for determining CoA-dependence of a desaturase
