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- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
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(54) Title: THERMOSTABLE RUBISCO ACTIVASE AND USES THEREOF

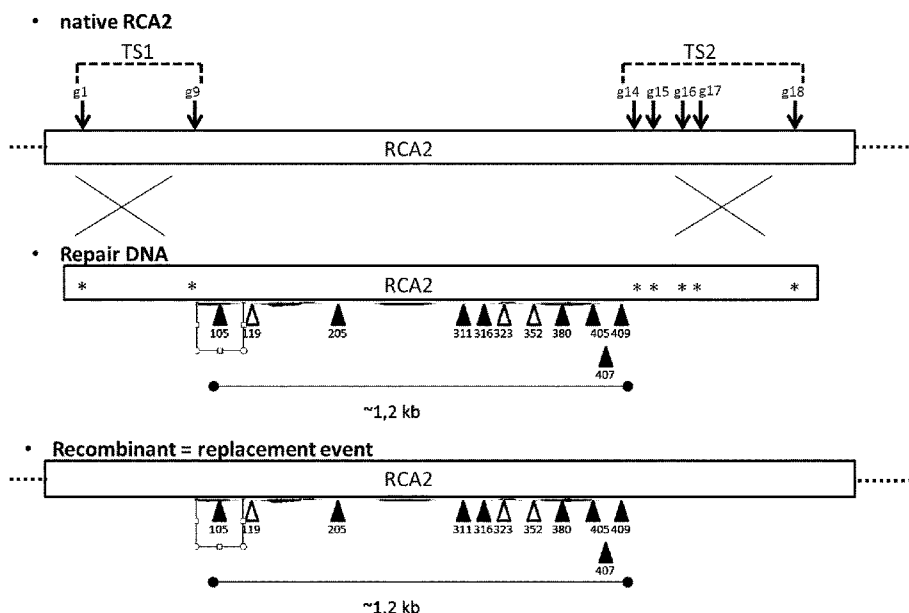


Figure 5

(57) Abstract: The present invention relates to the field of agriculture. In particular, the invention provides a thermostable Rca proteins, a recombinant gene, plants comprising the recombinant genes and a method to improve thermotolerance of a cereal plant under stress conditions.



**Declarations under Rule 4.17:**

- *as to the identity of the inventor (Rule 4.17(i))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*
- *of inventorship (Rule 4.17(iv))*

**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))*
- *with sequence listing part of description (Rule 5.2(a))*

**(88) Date of publication of the international search report:**

13 February 2020 (13.02.2020)

INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2019/066480

A. CLASSIFICATION OF SUBJECT MATTER  
INV. C12N9/88 C12N15/82  
ADD.

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)  
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 practicable, search terms used)

EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/074891 A2 (BASF PLANT SCIENCE GMBH [DE]; REZEAU CHRISTOPHE [FR] ET AL.) 26 June 2008 (2008-06-26)	14-18, 22-25, 30-32, 47-49, 53,54
Y	claims 95-123; sequence 293 ----- -/--	1-3

Further 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 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  29 October 2019	Date of mailing of the international search report  15/01/2020
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Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer  Marchesini, Patrizia
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## INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2019/066480

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ANSHUMAN KUMAR ET AL: "Arabidopsis thaliana expressing a thermostable chimeric Rubisco activase exhibits enhanced growth and higher rates of photosynthesis at moderately high temperatures", PHOTOSYNTHESIS RESEARCH ; OFFICIAL JOURNAL OF THE INTERNATIONAL SOCIETY OF PHOTOSYNTHESIS RESEARCH, SPRINGER, BERLIN, DE, vol. 100, no. 3, 9 June 2009 (2009-06-09), pages 143-153, XP019686936, ISSN: 1573-5079	1
Y	"material and methods"; figures 5-7, 9 the whole document	1-3, 14-18, 22-25, 30-32, 47-50, 53,54
Y	----- I. KUREK ET AL: "Enhanced Thermostability of Arabidopsis Rubisco Activase Improves Photosynthesis and Growth Rates under Moderate Heat Stress", THE PLANT CELL ONLINE, vol. 19, no. 10, 26 October 2007 (2007-10-26), pages 3230-3241, XP055081575, ISSN: 1040-4651, DOI: 10.1105/tpc.107.054171 the whole document	1-3, 14-18, 22-25, 30-32, 47-50, 53,54
Y	----- A. ELIZABETE CARMO-SILVA ET AL: "The Regulatory Properties of Rubisco Activase Differ among Species and Affect Photosynthetic Induction during Light Transitions", PLANT PHYSIOLOGY, vol. 161, no. 4, 15 February 2013 (2013-02-15), pages 1645-1655, XP055627612, Rockville, Md, USA ISSN: 0032-0889, DOI: 10.1104/pp.112.213348 the whole document	1-3, 14-18, 22-25, 30-32, 47-50, 53,54
Y	----- WO 2018/009632 A1 (US AGRICULTURE [US]; UNIV IOWA STATE RES FOUND INC [US]) 11 January 2018 (2018-01-11)  claims 1-19  ----- -/--	1-3, 14-18, 22-25, 30-32, 47-50, 53,54

INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2019/066480

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>US 2018/094328 A1 (POKKULURI PHANI R [US] ET AL) 5 April 2018 (2018-04-05)</p> <p>claims 1-24</p> <p style="text-align: center;">-----</p>	<p>1-3, 14-18, 22-25, 30-32, 47-50, 53,54</p>

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/EP2019/066480

## 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

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 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.:

2, 3, 24, 25, 50(completely); 1, 14-18, 22, 23, 30-32, 47-49, 53  
54(partially)

### 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: 2, 3, 24, 25, 50(completely); 1, 14-18, 22, 23, 30-32, 47-49, 53, 54(partially)

Relates to a method for increasing the ratio of a thermostable Rca protein in cereals by i) providing to cells of a cereal plant a recombinant gene comprising a promoter and a nucleic acid encoding an Rcalb protein and variants thereof depicted by SEQ ID No.8 or having at least 90% identity to SEQ ID No.8; and ii) reducing the expression of endogenous Rca2. Also relates to a recombinant gene comprising a nucleic acid depicted by SEQ ID No.: 7 encoding a Rca 1beta protein depicted by SEQ ID No.: 8. Also relates to vectors, host cells, plants and methods comprising or using said recombinant gene.

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2. claims: 4, 26(completely); 1, 7-12, 14-23, 29-32, 41, 43-49, 51-54, 57, 58(partially)

Relates to a method for increasing the ratio of a thermostable Rca protein in cereals by providing to cells of a cereal plant a recombinant gene comprising a promoter and a nucleic acid encoding a thermostable Rca2 protein variant; and ii) reducing the expression of endogenous Rca2. Also relates to a recombinant gene comprising a nucleic acid encoding a thermostable Rca2 protein depicted by SEQ ID No.: 30 or 33. Also relates to vectors, host cells, plants and methods comprising or using said recombinant gene.

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3. claims: 5, 6, 13, 27, 28, 42(completely); 1, 7-12, 14-23, 29-32, 41, 43-49, 51-54, 57, 58(partially)

Relates to a method for increasing the ratio of a thermostable Rca protein in cereals by providing to cells of a cereal plant a recombinant gene comprising a promoter and a nucleic acid encoding a thermostable Rca2 protein variant; and ii) reducing the expression of endogenous Rca2. Also relates to a recombinant gene comprising a nucleic acid encoding a thermostable Rca2 protein depicted by SEQ ID No.: 32 or 35. Also relates to vectors, host cells, plants and methods comprising or using said recombinant gene.

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4. claim: 1(partially)

Relates to a method for increasing the ratio of a thermostable Rca protein in cereals comprising introducing into cells at least one thermostable Rca2 allele encoding SEQ ID No.: 32 or 35. Also relates to recombinant gene comprising said SEQ ID No 32 or 35, as well as plants comprising said recombinant gene.

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

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5. claims: 33, 34

Relates to a knock out allele of an Rca2 gene

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6. claims: 35-40

Relates to a recombinant gene capable of suppressing specifically the expression of the endogenous Rca2 gene.

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7. claims: 55, 56

Relates to a method of increasing thermostability of a Rca2 protein comprising introducing amino acid substitutions to SEQ ID No.: 4.

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2019/066480

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2008074891 A2	26-06-2008	AR 064512 A1	08-04-2009
		BR PI0721100 A2	11-02-2014
		CA 2671401 A1	26-06-2008
		CN 101583720 A	18-11-2009
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		EP 2069505 A2	17-06-2009
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		EP 2395092 A2	14-12-2011
		EP 2395093 A2	14-12-2011
		EP 2405011 A2	11-01-2012
		US 2010077502 A1	25-03-2010
		US 2014182015 A1	26-06-2014
		WO 2008074891 A2	26-06-2008
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WO 2018009632 A1	11-01-2018	NONE	
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US 2018094328 A1	05-04-2018	NONE	
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