PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IB2012/003045	19.12.2012	19.12.2011

International Patent Classification (IPC) or both national classification and IPC
INV. C12N15/82 A01H5/00 C07K14/415 C12N9/16 C12N9/78

Applicant
SZKOLA GLOWNA GOSPODARSTWA WEIJSKEIGO W WARSZAWIE

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Fax: +49 89 2399 - 4465	see form PCT/ISA/210	Mundel, Christophe Telephone No. +49 89 2399-7314

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-20
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-20
Industrial applicability (IA)	Yes:	Claims	1-20
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 The present application refers to isolated nucleic acid molecule comprising at least two of SEQ ID NO: 4 (Arabidopsis thaliana PAD4), SEQ ID NO: 5 (A. thaliana LSD1) and SEQ ID NO: 6 (A. thaliana EDS1) or SEQ ID NO: 7 (Populus trichocarpa PAD4), SEQ ID NO: 8 (P. trichocarpa LSD1) and SEQ ID NO: 9 (p. trichocarpa EDS1), a vector comprising such a nucleic acid, a host cell comprising the nucleic acid or the vector, a plant or plant cell comprising the nucleic acid or the vector. The application further refers to methods of regulating growth and increasing biomass production in plant using such a nucleic acid.

2 Reference is made to the following documents:

D1 WO 2009/114733 A2 (CERES INC [US]; ZHOU FASONG [US]; PICCOLO KERSTIN [US]; SOSA JULISSA [) 17 September 2009 (2009-09-17)

D2 HUANG XIAOZHEN ET AL: "The Arabidopsis LSD1 gene plays an important role in the regulation of low temperature-dependent cell death",
NEW PHYTOLOGIST,
vol. 187, no. 2, 2010, pages 301-312,
ISSN: 0028-646X

D3 P. MUHLENBOCK ET AL: "Lysigenous Aerenchyma Formation in Arabidopsis Is Controlled by LESION SIMULATING DISEASE1",
THE PLANT CELL ONLINE,
vol. 19, no. 11, 16 November 2007 (2007-11-16), pages 3819-3830, XP055069037,
ISSN: 1040-4651, DOI: 10.1105/tpc.106.048843

D4 MUHLENBOCK PER ET AL: "Chloroplast Signaling and LESION SIMULATING DISEASE1 Regulate Crosstalk between Light Acclimation and Immunity in Arabidopsis",
PLANT CELL,
vol. 20, no. 9, September 2008 (2008-09), pages 2339-2356,
ISSN: 1040-4651

3 **Novelty; Article 33(2) PCT.**

None of the prior art documents discloses an isolated nucleic acid comprising at least two amino acid sequences chosen from among amino acid sequences having at least 60% identity with SEQ ID NO:4, at least 60% identity with SEQ ID NO: 5 and at least 60% identity with SEQ ID NO: 6 or chosen from among amino acid sequences having at least 60% identity with SEQ ID NO: 7, at least 60% identity with SEQ ID NO: 8 and at least 60% identity with SEQ ID NO: 9.

Therefore, the claims have to be considered as novel in the sense of Article 33(2) PCT.

4 **Inventive step; Article 33(3) PCT.**

4.1 At the filing date of the present application, it was already well-known in the art that the LSD1 protein was implicated in light acclimation, cold stress, oxidative stress and hypoxia tolerance in plants.

The patent application D1 discloses transgenic plants overexpressing A. thaliana LSD1 and having enhanced tolerance to oxidative stress induced by L-arginine (example 8).

The document D2 discloses the implication of LSD1 in cold stress. The lsd1-3 mutant disclosed in D2 shows a chilling-sensitive phenotype (Abstract). It is clear from the teaching of D2 that PAD4 and EDS1 are required for the chilling-sensitive phenotype of the lsd1-3 mutant (Abstract).

The document D3 shows that the LSD+, PAD4 and DES1 proteins are implicated in lysigenous aerenchyma formation in response to hypoxia (Abstract). In this document, LSD1 has been shown to be a negative regulator of aerenchyma formation and EDS1 and PAD4 have been shown to be positive regulators of aerenchyma (p. 3827, left-hand column, lines 12-23).

The document D4 discloses the fact that LSD1 is implicated in light acclimation. The lsd1 mutant fails to limit programmed cell death under long photoperiods (>16h). This phenotype depends on EDS1 and PAD4 (Abstract).

The skilled person, knowing from D1 to D4 that LSD1 is implicated in various abiotic stresses and knowing from D2 to D4 that the action of LSD1 is dependent on PAD4 and EDS1 would have needed no inventive activity to generate a nucleic acid molecule comprising at least two amino acid sequences chosen from among LSD1, PAD4 and EDS1.

Therefore, claims 1-11 and 13-20 cannot be considered as inventive in the sense of Article 33(3) PCT.

4.2 All the examples of the present application deal with loss-of-function mutants. However, the claims of the present discloses, or at least do not exclude the (over)expression of the LSD1, PAD4 and EDS1 proteins in the plants. There is no evidence in the present application that overexpression of at least two of LSD1, PAD4 and EDS1 will result in increased biomass or growth. Therefore, it is not clear if the problem to be solved by the present application, i.e to increase the biomass or growth of plants, is indeed solved over the whole scope of the claims.

Thus, no inventive step can be recognized for claims 1-20.

4.3 In the examples of the present application, only some specific double loss-of-function mutations have been associated with specific phenotypes.

In Arabidopsis, the eds1/lsd1 results in increased siliques yield under both laboratory and field conditions, increased seed yield under both laboratory and field conditions and reduced water use under laboratory and field conditions.

Therefore, only a nucleic acid comprising a nucleic acid attenuating the expression of both LSD1 and EDS1 has been shown to solve the problem of increasing growth or biomass in a plant. Only claims directed to this specific embodiment could be considered as inventive.

Re Item VIII

Certain observations on the international application

Clarity; Article 6 PCT.

1 Claim 1 refers to sequences having at least 60% identity with SEQ ID NO: 4, etc... Lots of the sequences encompassed by claim 1 will encode non functional polypeptides. It is not clear what would be the problem to be solved by such non functional polypeptides.

This remark also applies to claim 14.

2 Claim 3 refers to allelic variants or homologues of the nucleotide sequences of claim 1 or 2. It is not clear what should be the technical characteristics of such allelic variants or homologues, what renders the scope of the claim unclear.

It is even doubtful that the terms "allelic variant" or "homologues" make any sense when referring to "artificial" nucleic acids as the ones in claims 1 and 2.

3 Claim 4 refers to the DNA fragment according to any one of claims 1-3. Claims 1-3 refer to nucleic acids, not to DNA fragments. Therefore, it is not clear what should be seen as a DNA fragment according to claim 4.

Claim 4 further refers to the fact that one or more functional characteristics of the protein are retained. It is not clear which functional characteristics of the plant should be retained. Moreover, it is not clear how this should be achieved in terms of technical fetaures of the plant.

4 Claim 6 refers to flanking exogenous sequences. It is not clear what should be the technical characteristics of such sequences what renders the scope of the claim unclear.

5 In claim 7, it is probably not the cell that should be operably linked to an expression control sequence.

6 Claim 11 refers to conditions that permit integration of the expression cassette into the genome of the plant. It is not clear which conditions are meant what renders the scope of the claim unclear.

This remark also applies to claim 12.

7 In the transgenic plant of claim 15, it is not clear which sequence should be overexpressed/attenuated to obtain the desired phenotype what renders the claim completely unclear.