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**Datasheet for the decision  
of 1 June 2017**

**Case Number:** T 2241/13 - 3.3.08

**Application Number:** 07867133.6

**Publication Number:** 2027295

**IPC:** C12Q1/68

**Language of the proceedings:** EN

**Title of invention:**

A METHOD TO IDENTIFY DISEASE RESISTANT QUANTITATIVE TRAIT LOCI  
IN SOYBEAN AND COMPOSITIONS THEREOF

**Applicant:**

Monsanto Technology LLC

**Headword:**

Soybean resistance pathogens/MONSANTO

**Relevant legal provisions:**

EPC Art. 54, 56, 83, 84, 114(2), 123(2)  
RPBA Art. 13

**Keyword:**

Main request and seventh auxiliary request - admitted into the  
appeal proceedings (yes)  
Main request - added subject-matter (yes)  
Seventh auxiliary request - inventive step (no)

**Decisions cited:**

**Catchword:**



**Beschwerdekammern**  
**Boards of Appeal**  
**Chambres de recours**

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Case Number: T 2241/13 - 3.3.08

**D E C I S I O N**  
**of Technical Board of Appeal 3.3.08**  
**of 1 June 2017**

**Appellant:**  
(Applicant)

Monsanto Technology LLC  
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**Representative:**

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**Decision under appeal:**

**Decision of the Examining Division of the  
European Patent Office posted on 14 June 2013  
refusing European patent application No.  
07867133.6 pursuant to Article 97(2) EPC.**

**Composition of the Board:**

**Chairman** P. Julià  
**Members:** B. Stolz  
D. Rogers

## **Summary of Facts and Submissions**

- I. The applicant/appellant filed an appeal against the decision of an examining division whereby European patent application No. 07 867 133.6 (published as WO 2008/054546, hereinafter "the patent application") was refused. The examining division decided that claims 1 to 4, 8, 12 and 13, filed under cover of a letter dated 22 March 2013, lacked novelty and inventive step.
- II. With the statement of its grounds of appeal, the appellant filed a new main request.
- III. The appellant was summoned to oral proceedings. In a communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA) annexed to the summons, the appellant was informed of the board's provisional, non-binding opinion on some of the issues of the appeal proceedings.
- IV. Under cover of a letter dated 2 May 2017, the appellant replied to the board's communication and filed a new main request and first to sixth auxiliary requests.
- V. Oral proceedings were held on 1 June 2017. In the course of these proceedings, the appellant submitted a seventh auxiliary request and withdrew the first to sixth auxiliary requests.
- VI. Claim 1 of the main request reads as follows:  
  
"1. A method for assaying a soybean plant for disease resistance, comprising the steps of:  
  
(a) obtaining a part of a leaf of said soybean plant;

(b) cultivating said part of a leaf in a media consisting essentially of water, wherein said media is capable of maintaining said part for up to 2 months;

(c) exposing said part of a leaf to a plant pathogen; and

(d) assessing said disease resistance in said part of a leaf upon exposure to said plant pathogen, wherein said plant pathogen is selected from *Phakopsora pachyrhizi*, *Phakopsora meibomia* (Asian Soybean Rust), *Colletotrichum truncatum*, *Colletotrichum dematium* var. *truncatum*, *Sclerotinia sclerotiorum* (Sclerotinia stem rot), *Septoria glycines* (Brown Spot), *Phomopsis longicola* (Stem blight), *Phomopsis* spp. (Phomopsis seed decay), *Peronospora manshurica* (Downy Mildew), *Rhizoctonia solani* (Rhizoctonia root and stem rot, Rhizoctonia aerial blight), *Cercospora kikuchii* (Purple Seed Stain), *Alternaria* sp. (Target Spot), *Cercospora sojina* (Frogeye Leafspot), *Corynespora cassiicola* (Target spot), *Pseudomonas savastanoi* pv. *glycinea* (Bacterial blight), *Xanthomonas axonopodis* pv. *glycines* (Bacterial pustule), *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (Bacterial tan spot), *Fusarium solani* f. sp. *glycines* (sudden death syndrome), *Phialophora gregata* (brown stem rot) and *Sclerotinia* (stemrot)."

Dependent claims 2 to 10 refer to specific embodiments of the method of claim 1.

VII. Claim 1 of the seventh auxiliary request reads as follows:

"1. A method for assaying a soybean plant for disease resistance upon exposure to a plant pathogen, said method comprising the steps of:

- (a) obtaining a piece of a leaf of said soybean plant;
- (b) cultivating said piece of a leaf in a media consisting of water and optionally containing ingredients necessary to sustain the plant pathogen or the piece of the leaf, wherein said media is capable of maintaining said piece of the leaf viable for up to 2 months;
- (c) exposing said piece of the leaf to a plant pathogen; and
- (d) assessing said disease resistance in said piece of a leaf upon exposure to said plant pathogen,

wherein said plant pathogen is *Phakopsora pachyrhizi* or *Phakopsora meibomiaae*."

Dependent claims 2-8 refer to specific embodiments of the method of claim 1.

VIII. The following documents are cited in this decision:

D1: L.D. Owens and D.E. Cress, 1985, *Plant Physiology*, Vol. 77, 87-94;

D8: H. Ding *et al.*, 1994, *Physiological and Molecular Plant Pathology*, Vol. 44, 363-378;

D9: E.W. Orlandi *et al.*, 1992, *Physiological and Molecular Plant Pathology*, Vol. 40, 173-180;

D10: J.J. Burdon and D.R. Marshall, 1981, *Plant Disease*, Vol. 65, No. 1, 44-45;

D11: B. Mieslerová et al., 2000, Journal of Phytopathology, Vol. 148, 303-311;

D12: R.G. Pratt, 1996, Phytopathology, Vol. 86, No. 9, 923-928;

D14: A. Lebeda, 1984, Scientia Horticulturae, Vol. 24, 241-249;

D15: T.-F. Hsieh and J.-W. Huang, 2001, Plant Pathology Bulletin, Vol. 10, 37-44.

IX. The arguments of the appellant, as far as relevant for the present decision, can be summarised as follows:

*Main request*

*Article 123(2) EPC*

Basis for the feature "consisting essentially of water" in step (b) of claim 1 could be found in the paragraph bridging pages 8 and 9 of the patent application. There it was stated that the medium comprising water could additionally contain any ingredients necessary to sustain the pathogen or plant tissue so long as the additional ingredients did not interfere with the expression of resistance. In Example 3 of the patent application, sterile deionized water was put into each well of the 6-well tissue culture plate containing the part of the soybean leaf and, after application of the aqueous suspension with the plant pathogen, the media contained water and less than 0.00025% Tween 20 (page 50, lines 20 to 28). It was therefore implicit that the medium consisted essentially of water. A person skilled in the art, when reading Example 3 of the patent application, would have implicitly and unambiguously understood that the culture media consisted essentially

of water. The feature "wherein said media is capable of maintaining said part for up to 2 months" was disclosed on page 51, line 6 of the patent application.

*Seventh auxiliary request*

*Article 114(2) EPC and Article 13 RPBA*

The seventh auxiliary request addressed objections raised by the board for the first time at the oral proceedings and therefore, it should be admitted into the appeal proceedings.

*Article 123(2) EPC*

Basis for the feature "a piece of a leaf" could be found, *inter alia*, on page 5, lines 14 and 15 and, in particular, in Example 3, page 50, lines 18-20 of the patent application. The feature "media consisting of water and optionally containing ingredients necessary to sustain the plant pathogen or the piece of leaf" was based on the paragraph bridging pages 8 and 9. The feature "wherein said media is capable of maintaining said piece of leaf viable for up to 2 months" was disclosed on page 51, line 6. The skilled person immediately understood this disclosure to refer not only to a medium consisting of water as in Example 3 but also to a medium comprising additional ingredients necessary to sustain a piece of a leaf or the plant pathogen. If a medium consisting of water was capable of maintaining a piece of a leaf for up to two months, this had to be all the more true for a medium comprising water and additional ingredients necessary to sustain a piece of a leaf or the plant pathogen.



*Article 84 EPC*

The term viable in the feature "wherein said media is capable of maintaining said piece of the leaf viable for up to 2 months" referred to the susceptibility of the piece of leaf to infection by the pathogen. In this context, the term was not open to any other interpretation and it was thus not ambiguous.

*Article 56 EPC*

The closest state of the art, document D10, described a method for assaying soybean plants for resistance to soybean rust caused by *Phakopsora pachyrhizi*. An intact, detached leaf was placed on the surface of a water agar support medium containing gibberellic acid and the leaf was sprayed with spores of *P. pachyrhizi*. Subsequently, the leaf was sprayed with distilled water. This method differed from the method of claim 1 by several features. The method of claim 1 used a piece of a leaf instead of a whole detached leaf, and this piece of a leaf was cultivated in a medium which allowed for a simplified application of the pathogen spores via the culture medium, and for the piece of a leaf to be viable for up to two months. The technical problem thus consisted in providing an improved method for assaying a soybean plant for resistance to *P. pachyrhizi* or *P. meibomia*e. The solution to this problem as defined in claim 1 was not obvious. The leaf discs described in the prior art were less stable and they could not be maintained in culture for up to two months.

- X. The appellant requested that the decision under appeal be set aside and a patent be granted on the basis of

the main request filed under cover of a letter dated 2 May 2017 or, alternatively, upon the basis of the seventh auxiliary request filed at the oral proceedings before the board on 1 June 2017.

## **Reasons for the Decision**

### Main Request

1. The main request, filed with appellant's reply to a communication pursuant to Article 15(1) RPBA, comprises amendments that intend to overcome the objections raised by the board in this communication (cf. points III and IV *supra*). These objections had not been raised during the procedure before the examining division. The amendments are a straightforward reply to the board's provisional, non-binding opinion expressed in the communication. Therefore, the board, in the exercise of its discretion, decides to admit the main request into the appeal proceedings (Article 114(2) EPC, Article 13 RPBA).

### *Article 123(2) EPC*

2. Step (b) of claim 1 comprises the cultivation of a part of a leaf "in a media consisting essentially of water" (cf. point VI *supra*).
3. It was not disputed that the patent application does not explicitly disclose this feature. The appellant submitted however that the paragraph bridging pages 8 and 9 of the patent application disclosed it implicitly (cf. point IX *supra*).
4. The two sentences of this paragraph read:

*"The present invention further provides that the media used in the method for selection **is comprised of** water that is untreated, distilled or deionized. The media **can contain any ingredients necessary to sustain** the pathogen or plant tissue, so long as the ingredients do not interfere with the expression of resistance as conferred by the QTL"* (emphases added by the board).

5. The appellant submitted that the term "consisting essentially of" did not exclude the presence of additional components in minor amounts. The major, essential constituent was however water.
  
6. While it is true that the major constituent of any culture medium is water, the two sentences recited in point 4 above do not provide a basis for defining a culture medium as "consisting essentially of water". According to the second sentence, any ingredient necessary to sustain the pathogen or the plant can be added to the culture or selection medium, the addition being limited only by the proviso that the ingredient does not interfere with the expression of resistance but without any limitation to the concentration or amount of this ingredient in said medium. An ingredient necessary to sustain the pathogen or the plant is however an essential ingredient of a culture medium. When read in context, the two sentences of this paragraph therefore do not directly and unambiguously disclose a culture medium "consisting essentially of water".
  
7. The sole other section of the patent application describing a culture or selection medium is Example 3. It describes a method for assaying disease resistance comprising the incubation of pieces of leaves with

sterile deionized water. In a first step, leaves are cut into pieces and placed on top of Whatman filter papers sitting in the wells of a 6-well tissue culture plate. Two milliliters of water are added into each well, i.e. a medium consisting only of water (page 50, lines 18 to 22). Spores of Asian Soybean Rust are suspended in sterile deionized water containing 0.01% Tween 20 at a concentration of  $1 \times 10^5$  spores per milliliter (cf. page 50, lines 22 to 24) and, in a next step, 50 microliters of spore suspension is applied to each piece of a leaf (cf. page 50, lines 24 to 27). These first and second steps correspond to steps (b) and (c), respectively, of the method of claim 1. The exposure medium in which the spores are suspended is not a culture or selection medium, nor is the culture or selection medium defined by the ingredients present in said exposure medium. Indeed, during monitoring of the disease progression, the wells have not to dry out and thus, when needed, further culture or selection medium is added, namely deionized water (cf. page 50, lines 27 to 32). Thus, Example 3 describes the use of a culture or selection medium consisting of water, it does however not directly and unambiguously disclose the use of a medium consisting essentially of water. There is no formal basis for this feature in Example 3 of the patent application nor in the patent application as a whole.

8. Claim 1 therefore contains subject-matter which extends beyond the content of the application as filed and contravenes Article 123(2) EPC.

Seventh auxiliary request

*Article 114(2) EPC and Rule 13 RPBA*

9. The seventh auxiliary request was filed at the oral proceedings before the board (cf. point V *supra*). This auxiliary request differs from the main request by amendments to claim 1, deletion of claims 2 and 4, and replacement of the term "a part of a leaf" by "a piece of a leaf" in all claims. These amendments are a direct response to issues mentioned in the board's communication pursuant to Article 15(1) RPBA and/or at the oral proceedings before the board. These issues had not been addressed during examination before the examining division. The amendments neither raise new issues nor create a fresh case. The board, in the exercise of its discretion, decides to admit the seventh auxiliary request into the appeal proceedings (Article 114(2) EPC, Article 13 RPBA).

*Article 123(2) EPC*

10. The subject matter of claim 1 is disclosed on page 5, lines 1 to 7, describing a method for assaying soybean plants for disease resistance comprising the steps of (a) detaching a plant tissue from a soybean plant, (b) cultivating said tissue in a media, (c) exposing said tissue to a plant pathogen, and (d) assessing said tissue for, *inter alia*, resistance to disease caused by the pathogen, in combination with references to the use of a portion of a leaf (e.g. page 5, lines 14 and 15), the paragraph bridging pages 8 and 9 describing the media used in this method, and Example 3 disclosing the use of a cut piece of a leaf. Example 3 (page 51, line 6) furthermore discloses that the leaf section used in this assay remains viable for up to 2 months. If a

piece of leaf remains viable for up to two months in a medium consisting of water, as in Example 3, it is directly and unambiguously derivable that this is also the case in a medium containing ingredients necessary to sustain the plant pathogen or the piece of leaf.

11. Claim 1 of the seventh auxiliary request does not contravene Article 123(2) EPC.

*Article 84 EPC*

12. In the context of claim 1, the person skilled in the art understands the feature "wherein said media is capable of maintaining said piece of the leaf viable for up to 2 months" as referring to the capability of the medium to maintain the piece of the leaf in a state in which it is susceptible to infection by the pathogen and the development of disease symptoms. The subject matter of claim 1 is thus clearly defined (Article 84 EPC).

*Article 83 EPC*

13. In the decision under appeal, the examining division did not raise any objection under Article 83 EPC nor does the board see any reason of its own to do so. The patent application discloses the method of claim 1 sufficiently clear and complete for it to be carried out by a person skilled in the art (Article 83 EPC).

*Article 54 EPC*

14. Claim 1 is limited to a method for assaying a soybean plant for disease resistance upon exposure to *Phakopsora pachyrhizi* or *Phakopsora meibomia*. Document D8, cited in the decision under appeal as anticipating

the subject-matter of claim 1 of the request before the examining division, describes the infection of soybean leaf discs with *Phytophthora megasperma*. Document D1, cited in the board's communication pursuant to Article 15(1) RPBA, describes the infection of soybean leaves by several *Agrobacterium* strains. None of the prior art documents on file discloses a method for assaying a soybean plant for resistance to *Phakopsora pachyrhizi*, or *Phakopsora meibomia*, comprising all the features of claim 1 (Article 54 EPC).

*Article 56 EPC*

15. Document D10 describes the screening of soybean plants for resistance to *P. pachyrhizi* and represents the closest state of the art. Newly expanded leaves were detached from soybean plants and placed, abaxial surface up, on a 0.4% water agar support medium containing 5 ppm gibberellic acid (cf. page 44, middle column), i.e. they were placed on a support agar comprising water and an ingredient necessary to sustain the leaf. Leaves were then exposed to pathogen spores, sprayed with distilled water, incubated and assessed for disease resistance (cf. page 44, middle and right columns).
  
16. The appellant argued that the method specified in claim 1 differed significantly from the method disclosed in document D10 in at least two aspects. First, a piece of a leaf is used instead of a detached leaf. Second, the piece of leaf is cultivated in a medium, i.e. submerged in a culture medium, instead of putting it on a water agar support medium. The submersion of the piece of the leaf in a culture medium improved its stability and, via the application of spores to the culture medium, simplified the way of infection of the piece of leaf.

The technical problem underlying the claimed invention therefore consisted in the provision of an improved method for assaying a soybean plant for resistance to *P. pachyrhizi* (cf. point IX *supra*).

17. The board notes however that the application of pathogen spores to the culture medium is a feature of dependent claim 4 but not of claim 1.

Claim 4 specifies three different ways of applying the spores to the piece of leaf, the first one consisting of a "direct application of the pathogen to said piece of a leaf". Claim 1 thus comprises methods where at least one surface of the piece of leaf is exposed such that the spores can be applied directly to it. Indeed, in Example 3 of the patent application, the spores are applied to the piece of leaf, which rests on three layers of Whatman filter paper, using an airbrush (page 50, lines 24-27). The second method mentioned in dependent claim 4 is the "inclusion of the pathogen in said culture media". Any advantage resulting from the application of the pathogen spores directly to the culture medium is thus the result or consequence of a specific embodiment of the more generally defined method of claim 1, and therefore not relevant for the definition of the technical problem underlying claim 1.

Furthermore, since one surface of the piece of leaf can be exposed in the method of claim 1, the feature "cultivating said piece of a leaf in a media" does not limit the claim to methods where said piece of a leaf is submerged in a culture medium but encompasses all possible ways of bringing a piece of leaf in contact with said culture medium. This is in line with Example 3 of the patent application in which, as stated above, three layers of Whatman No. 1 filter paper are used to



physically support the piece of leaf. Any alleged increase in stability as a result of cultivating the piece of leaf submerged in a medium is therefore not relevant for defining the technical problem underlying claim 1.

18. According to claim 1, the media used for cultivating the piece of a leaf must be capable of maintaining said piece of a leaf viable for up to 2 months. The medium described in document D10 was capable of maintaining leaves of *Glycine tabacina* Accession #1257 for at least 28 days (Table 1: latent period (number of days from inoculation until the first pustule erupted) 23 days, plus 5 days (number of active pustules 5 days after the first pustule erupted)). There is no evidence that the medium described in document D10 is not capable of maintaining a detached leaf viable for up to two months.
19. It follows from the above considerations that the sole technical difference between the method of claim 1 and the method described in document D10 relevant for the assessment of inventive step is the use of a piece of a leaf instead of a detached, intact leaf. This difference does not however result in any unexpected technical effect.
20. Starting from document D10, the objective technical problem to be solved is therefore defined as the provision of an alternative method for assaying a soybean plant for resistance to *P. pachyrhizi*.
21. As a solution to this problem, the patent application proposes the method of claim 1. The board has no doubts that the claimed method solves this technical problem.

However, the claimed solution does not involve an inventive step.

22. There is ample prior art on file showing that leaf disc assays were widely used in the field of assaying plants for resistance to pathogens, such as, for instance document D8, published in 1994, stating: "*In commonly used leaf disc tests, infection droplets are applied to the surface of the floating disc ...*" (cf. the last paragraph on page 365).

This is corroborated by documents D11 (cf. page 304, left-hand column, last paragraph), D12 (page 924, right-hand column, 4th paragraph), D14 (page 242, left-hand column, last paragraph), and D15 (paragraph bridging pages 38/39).

Moreover, the use of leaf discs of soybean plants, i.e. the use of a piece of a leaf, in pathogen infection assays was also well known in the art (cf. document D8, page 365, last paragraph; see also document D9, page 175, describing "*Leaf disc K+/H+ assays*").

23. The person skilled in the art was therefore well aware of the use of leaf disc based assays for testing the resistance to plant pathogens. The use of a leaf disc, i.e. a piece of a leaf, instead of a detached, intact leaf was thus an obvious alternative to a person skilled in the art trying to solve the above mentioned technical problem.
24. Claim 1 of the seventh auxiliary request lacks an inventive step in view of document D10 in combination with the skilled person's common general knowledge of the use of leaf discs for assaying plant resistance to

plant pathogens and, in particular, of soybean plants such as in document D8.

**Order**

**For these reasons it is decided that:**

The appeal is dismissed.

The Registrar:

The Chairman:



A. Wolinski

P. Julià

Decision electronically authenticated