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D E C I S I O N
of 28 June 2005

Case Number: T 0539/04 - 3.3.8

Application Number: 00926773.3

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Language of the proceedings: EN

Title of invention:
Transgenic plant and methods

Applicant:
Syngenta Participations AG

Opponent:

-

Headword:
Transgenic plants/SYNGENTA

Relevant legal provisions:
EPC Art. 82, 123(2), 84, 54, 56, 83

Keyword:
"Main request - lack of unity (no)"
"Added subject-matter (no)"
"Clarity (yes)"
"Novelty (yes)"
"Inventive step (yes)"
"Sufficiency of disclosure (yes)"

Decisions cited:

-

Catchword:

-



Case Number: T 0539/04 - 3.3.8

D E C I S I O N
of the Technical Board of Appeal 3.3.8
of 28 June 2005

Appellant: Syngenta Participations AG
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Decision under appeal: Decision of the Examining Division of the
European Patent Office posted 23 September 2003
refusing European application No. 00926773.3
pursuant to Article 97(1) EPC.

Composition of the Board:

Chairman: L. Galligani
Members: F. L. Davison-Brunel
C. Rennie-Smith

Summary of Facts and Submissions

I. European patent application No. 00 926 773.3 was published as WO 00/60061 with the title "Transgenic plant and methods".

Originally filed claims 1, 2, 4, 5, 8, 9, 16 and 25 read as follows:

"1. A plant cell comprising a heterologous polynucleotide encoding a gene product that is expressed in the plant cell wherein the gene product has trichothecene resistance activity.

2. A plant comprising a plant cell of claim 1, wherein the plant is resistant to a trichothecene.

4. The plant of claim 1 [*sic*], wherein the plant is resistant to a fungus that produces a trichothecene, preferably a trichothecene comprising a C-3 hydroxyl group.

5. The plant according to claim 4, wherein the plant is resistant to *Fusarium*, preferably to *Fusarium graminearum*.

8. The plant according to any one of claims 1 to 7, wherein the heterologous polynucleotide comprises a sequence substantially similar to SEQ ID No: 1, 5 or 7.

9. The plant according to claim 8, wherein the heterologous polynucleotide comprises the nucleic acid sequence of SEQ ID No: 1, 5 or 7.

16. The plant according to any one of claims 2 to 15, wherein the plant is a wheat, maize, barley or rice plant.

25. A method of producing a fungal resistant plant comprising

- (a) transforming a plant cell with a heterologous gene encoding a gene product wherein the gene product increases resistance to a trichothecene;
- (b) expressing the gene product at a biologically significant level;
- (c) regenerating the plant cell into a plant; and
- (d) selecting a plant with increased resistance to a trichothecene; and
- (e) optionally, selfing or outcrossing [sic] the plant obtained in step (d)."

II. The application was refused by the examining division for lack of unity, lack of novelty and inventive step. Claims 1 and 2 of the amended claim request then on file read as follows:

"1. A plant cell comprising a heterologous polynucleotide encoding a gene product that is expressed in the plant cell wherein the gene product has trichothecene resistance activity, and wherein said heterologous polynucleotide comprises a nucleotide sequence having at least 65% sequence identity to SEQ ID NO:1.

2. The plant according to claim 1, wherein the plant is resistant to a fungus that produces a trichothecene, comprising a C-3 hydroxyl group."
- III. The appellant lodged an appeal against this decision enclosing an amended set of claims with the notice of appeal, paid the appeal fee and, then, duly filed a statement of grounds of appeal together with a new main request.
- IV. The examining division did not rectify the contested decision and referred the appeal to the board of appeal (Art. 109 EPC).
- V. On 7 July 2004, the appellant sent further submissions together with a new main request which comprised 9 claims.

Claims 1 and 4 thereof read as follows:

"1. A wheat plant, which is resistant to a fungus that produces a trichothecene that comprises a C-3 hydroxyl group, said wheat plant comprising a plant cell, wherein said plant cell comprises the nucleic acid sequence of SEQ ID NO:1 and wherein said plant is resistant to *Fusarium* infection.

4. A method for producing a wheat plant that is resistant to a fungus that produces a trichothecene that comprises a C-3 hydroxyl group comprising the steps of

- a) transforming a wheat plant cell with a heterologous gene comprising the nucleic acid sequence of SEQ ID NO:1;
- b) expressing the gene product of said gene at a biologically significant level;
- c) regenerating the plant cell into a plant; and
- d) selecting a plant with increased resistance to the fungus; and, optionally,
- e) selfing or outcrossing the plant obtained in step d)."

Claims 2 and 5 respectively related to further features of the wheat plant of claim 1 and of the method of claim 4. Claim 3 related to a seed of the plant according to claim 1 or claim 2. Claims 6 to 8 respectively related to a method of preventing mycotoxin contamination of a wheat plant and/or a wheat plant's seed, to a method for reducing and/or preventing the growth of a fungus of the genus *Fusarium* on a wheat plant and to a method for producing wheat seed wherein the plant grown from the seed is fungal resistant, all three methods comprising the use of a plant of claim 1 or claim 2 or produced by a method according to claim 4 or claim 5. Claim 9 related to a further feature of the method of claim 8.

VI. The following documents are mentioned in the present decision:

(1): Kimura, M. et al., FEBS Letters, Vol. 435, pages 163 to 168, 1998;

(2): Kimura, M. et al., J.Biol.Chem., Vol. 273, No. 3, pages 1654 to 1661, January 1998;

(3): WO 99/09173 published on 25 February 1999;

(6): English translation of the Japanese patent laid open No. 2000-32985 published on 2 February 2000.

VII. The appellant's arguments in writing insofar as relevant to the present proceedings may be summarised as follows:

Article 82 EPC; lack of unity

The examining division decided that two separate inventions were claimed differing by the specific 3-O-acetyltransferase gene which had been introduced in the plant cells/plants. The main request now for consideration by the board only relates to one of these inventions (SEQ ID NO 1) and, therefore, the objection of lack of unity no longer arises.

Articles 123(2) and 84 EPC; added subject-matter, clarity

Claim 1 was based on claims 1, 2, 5, 9 and 16 as originally filed as well as on the paragraph bridging pages 3 and 4 and paragraph 7 on page 4 of the application as filed. Claim 4 found a basis in claims 18, 19 and 25 as originally filed as well as in the paragraph bridging pages 3 and 4. Claims 2, 3, 5, 6 to 9 respectively corresponded to claims 5, 17, 20 and 26, 21, 23, 27 and 28 as originally filed. All claims were clearly worded. The requirements of Articles 123(2) and 84 EPC were, thus, fulfilled.

Article 54 EPC; novelty

Document (1) did not disclose a wheat plant or seed as now claimed. It was also not concerned with methods for producing fungal resistant wheat plants. Document (2) was an earlier document published by the same laboratory which also failed to disclose fungal resistant wheat plants and methods for producing them. While concerned with producing transgenic plants that were resistant to *Fusarium*, document (3) disclosed the use of a gene encoding a ribosomal protein and not that of a gene encoding 3-O-acetyltransferase such as SEQ ID NO:1. Thus, all claims of the present invention, being limited to uses of SEQ ID NO:1 and to plants comprising said sequence, were novel over the prior art.

Article 56 EPC; inventive step

The closest prior art with respect to claim 1 was document (2) which mentioned that transgenic plants expressing 3-O-acetyltransferase might be valuable for control of wheat head scab.

The objective technical problem could be defined as the provision of wheat plants that were resistant to fungi which produced a trichothecene that comprised a C-3 hydroxyl group.

The invention solved this problem by the provision of wheat plants comprising the nucleic acid of SEQ ID NO:1 encoding a 3-O-acetyltransferase.

The above mentioned statement found in document (2) provided the skilled person with no more than a mere

invitation to experiment. Neither document (2) alone nor a combination of documents (2) and (1) led to the invention as now claimed as they did not provide a disclosure of SEQ ID NO:1. The same was true of document (3) insofar as the transgenic plants which it described contained a gene which was not related to SEQ ID NO: 1.

Furthermore, there was no certainty that the cloned gene would show the requisite activity when transformed into a wheat plant for the following reasons:

- it may not be satisfactorily expressed because of a different codon usage;
- not all genes encoding 3-O-acetyl transferase were induced in vivo by the fungal toxin;
- it was uncertain whether in the plant enough of the toxin could be converted to its 3-acetylated form - innocuous for yeast - for the fungus not to cause damages;
- it was uncertain whether C-3 acetylated trichothecene was not toxic to plants;
- the fact that document (1) mentioned plants which had become herbicide resistant when transformed with a bacterial gene did not necessarily imply that fungal resistant plants could be produced by transformation of a fungal gene because of the complex interactions which had developed during the co-evolution of pathogens and their hosts.

For these reasons, the skilled person had no reasonable expectation of success when attempting to obtain the transgenic plants now claimed. The requirements of Article 56 EPC were, thus, fulfilled.

VIII. The appellant requested that the decision under appeal be set aside and that a patent be granted on the basis of the main request filed on 7 July 2004.

Reasons for the decision:

Article 82 EPC; lack of unity

1. The examining division came to a finding of lack of unity in relation to the main request then on file (see claims 1 and 2, section II, supra). It was observed that the claims covered plant cells and plants containing either one of two genes encoding 3-O-acetyl transferases conferring trichothecene resistance. The common concept linking the inventions was seen as the same mechanism of action of the enzymes. The examining division held that this concept was neither novel nor inventive as such genes were already known in the prior art, and, therefore, concluded that unity had to be denied.

2. The claims now on file are directed to plant cells and plants (methods of production and use thereof) transformed by a **specific** gene encoding a protein conferring trichothecene resistance, having the nucleotide sequence identified as SEQ ID NO: 1. Consequently, there is no basis any longer for an

objection of lack of unity. The requirements of Article 82 EPC are fulfilled.

Articles 123(2) and 84 EPC

3. The subject-matter of claim 1 (section V supra) is the result of the introduction in originally filed claim 4 of the features of the transgenic plants given in originally filed claims 5, 9 and 16 - they themselves being dependent on originally filed claim 4, see section I supra.

The subject-matter of claim 4 (section V, supra) is the result of the introduction in originally filed claim 25 of the features disclosed in the application as filed, in the passage bridging pages 3 and 4, on page 4, lines 21 to 23 and on page 5, lines 15 and 16. These features may be combined with the subject-matter of claim 25 because they are originally disclosed as being parts of a method "to provide a plant of the invention".

Claims 2 and 3 respectively correspond to originally filed claims 5 and 17, claim 5 corresponds to originally filed claim 26 and claims 6 to 9 respectively correspond to originally filed claims 21, 23, 27 and 28.

The requirements of Article 123(2) EPC are fulfilled.

4. The board does not see any lack of clarity in the wording of the claims. There is support in the description for the claimed subject-matter, in

particular in the examples relating to transgenic wheat.
The requirements of Article 84 EPC are fulfilled.

Article 54 EPC; novelty

5. The claims enjoy priority rights from 31 March 1999, ie from the filing date of the first priority document, US 09/282 995 as this document, pages 2 to 4, discloses the same subject-matter as is now claimed.

6. The three documents on file which mention/disclose transgenic plants having incorporated a gene encoding a protein susceptible of imparting trichothecene resistance to the plants are documents (6), (2) and (3). Document (6) was published on 2 February 2000 ie after the priority date of the now claimed subject-matter. It is not prior art and, therefore, cannot be taken into account for the assessment of novelty. Document (2) is concerned with the protective effect of expressing the gene *Tri101* encoding 3-O-acetyltransferase in fungal organisms producing trichothecene and in transformed yeast. The possibility of expressing it in plants is mentioned in one sentence on the page 1661, left-hand column which reads: "... a transgenic plant expressing *Tri101* might be valuable for control of wheat head scab and reduce the use of agricultural chemicals." This information per se cannot be considered as an enabling disclosure of wheat transgenic plants such as claimed.

7. Document (3) published on 25 February 1999 is state of the art pursuant to Article 54(2) EPC. It discloses transgenic maize tissue cultures which have become resistant to trichothecene (examples 6 and 7). The resistance results from the integration in high

molecular weight maize DNA of a mutant gene encoding a modified ribosomal protein. In the passage bridging pages 7 and 8, it is hypothesized that the trichothecene is not able to bind to the modified protein and that, for this reason, the plant becomes resistant to the toxin. The ribosomal protein and its encoding DNA have sequences which are different from, respectively, that of the acetyltransferase enzyme encoded by SEQ ID NO: 1 and that of SEQ ID NO: 1. If only for this reason, the plant cells comprising the DNA identified as SEQ ID NO: 1 (methods of production and use) are different from those described in document (3). All present claims directly or indirectly referring to SEQ ID:1, the claimed subject-matter as a whole is novel.

8. The requirements of Article 54 EPC are fulfilled.

Article 56 EPC; inventive step

9. The closest prior art is document (2). It discloses that the *Fusarium* species produces the mycotoxin trichothecene. *Fusarium graminearum* is said to protect itself against the toxin by transforming it into an inactive, 3-O-acetylated derivative. The reaction is carried out by the enzyme 3-O-acetyltransferase which is encoded by *Tri101*. The cloning of the *Fusarium graminearum Tri101* cDNA is reported and the cDNA sequence is disclosed as well as the deduced 451 amino acid sequence of the enzyme (cf. Fig. 4). Furthermore, it is shown that when the cDNA is introduced and expressed into yeast, the yeast cells become resistant to trichothecene. On page 1661, it is suggested that "a *transgenic plant expressing Tri 101 might be valuable*

for control of wheat head scab and reduce the use of agricultural chemicals."

10. Starting from the closest prior art, the problem to be solved can be defined as providing a method to produce a plant, more specifically a wheat plant, which is resistant to a fungus producing trichothecene.
11. The solution provided is to make the wheat transgenic for the gene encoding 3-O-acetyltransferase from *Fusarium sporotrichioides*, said gene comprising the nucleic acid of SEQ ID No:1.
12. Taking into account the disclosure in document (2) that trichothecene is produced by the members of the *Fusarium* species, and also the above mentioned suggestion (point 9, supra, last sentence), this approach to fungal resistance in plants is considered to have been obvious to try. The question which remains to be answered is whether or not there was a reasonable expectation of success when carrying out the experiment.
13. In this respect, the appellant argued that the expression of the *Fusarium graminearum* 3-O-acetyltransferase gene in yeasts - which resulted in resistance to trichothecene - was not indicative that the expression of the equivalent *Fusarium sporotrichioides* gene in plants would have the same effect. To back up this argument, document (1) (page 167, para. 4.2) was referred to, as it taught that the expression of 3-O-acetyltransferase was not always responsible for trichothecene resistance. In fact, the auto-resistance of *Fusarium sporotrichioides* itself was not due to the expression of the

- 3-O-acetyltransferase gene although this gene was functional. Furthermore, it was argued that the codon usage being quite different in fungi and in plants, one could not be sure that the level of fungal gene expression in wheat would be enough for the plant to become resistant to the fungus.
14. In the absence of any documents on file which could be regarded as raising doubts as to the soundness of these arguments, the board is prepared to accept them as indicative that there was no reasonable expectation of success when, having cloned from *Fusarium sporotrichioides* a gene equivalent to the one described in document (2), the skilled person tried to use it to make a transgenic plant resistant to *Fusarium* infection. Under these circumstances, the subject-matter of the claims on file is considered to involve an inventive step.
15. Two further points are worth mentioning. In its decision, the examining division expressed the view that, since the expression in plants of a bacterial gene encoding an antibiotic resistance (*S. hygroscopicus* Bar gene; document (1), discussion) was sufficient to make the plant resistant to this antibiotic (ie herbicide tolerant), it was obvious that the expression in plants of a fungal mycotoxin resistance gene would result in the plant becoming resistant to the mycotoxin/fungus producing it. The appellant answered that the complex interactions which develop between pathogens and their hosts could not be compared to the simple enzymatic reaction leading to the detoxification of a herbicide. Irrespective of the relative merits of the examining division's view or the

appellant's answer, the issue is not relevant as inventive step can already be acknowledged on the basis of the arguments mentioned in point 13, supra.

16. Document (3) describes a method for producing a mycotoxin resistant plant whereby the plant's translational machinery (ribosomal protein) is altered so that the mycotoxin can no longer interfere with translation. This earlier approach is so completely different from the one now claimed - which relies on the introduction into the plant of a foreign gene which encodes an enzyme capable of rendering the toxin inoperative - that it has no bearing on inventive step.

Article 83 EPC; sufficiency of disclosure

17. This issue does not seem to have been considered during examination. The board is satisfied that the patent specification provides enough technical information including the sequence of the 3-O-acetyltransferase encoding gene, the construction of the recombinant plasmids, methods for the transformation of immature embryos and bioassays for the skilled person to be able to reproduce the invention. The requirements of Article 83 EPC are fulfilled.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the first instance with the order to grant a patent on the basis of claims 1 to 9 filed on 7 July 2004 and a description to be adapted thereto.

The Registrar:

The Chairman:

A. Wolinski

L. Galligani