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D E C I S I O N
of 3 June 2004

Case Number: T 0078/01 - 3.3.8

Application Number: 84306494.0

Publication Number: 0142924

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Language of the proceedings: EN

Title of invention:
Insect resistant plants

Patentee:
Mycogen Plant Science, Inc.

Opponents:
Bayer BioScience N.V.
Monsanto Company
Syngenta Participations AG

Headword:
Insect resistant plants/MYCOGEN

Relevant legal provisions:
EPC Art. 83

Keyword:
"Main and auxiliary requests - sufficiency of disclosure (no)"

Decisions cited:
T 0612/92, T 0116/95

Catchword:
-

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Case Number: T 0078/01 - 3.3.8

D E C I S I O N
of the Technical Board of Appeal 3.3.8
of 3 June 2004

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Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted 2 November 2000 revoking European patent No. 0142924 pursuant to Article 102(1) EPC.**

Composition of the Board:

Chairman: C. Rennie-Smith
Members: P. Julia
M. R. Vega Laso

Summary of Facts and Submissions

I. The European patent no. 0 142 924 with the title "Insect resistant plants" was granted with seventy-nine claims. Four oppositions were filed on the grounds of Articles 100(a),(b) EPC. In its decision issued on 28 November 1993, the opposition division revoked the patent on the grounds that the amendments of the main request and those of the first and second auxiliary requests then on file offended against Articles 123(2)(3) EPC.

II. The patentees lodged an appeal and with the statement of grounds of appeal filed a new main request and auxiliary claim requests. In response to the comments of respondents I to III (opponents 01 to 03, the former opponents 03 and 04 having in the meantime merged and became one party), the appellants filed a new main request and four new auxiliary requests. During the oral proceedings before the board hearing that appeal, the appellants filed a new main request which was held to fulfil the requirements of Articles 123(2)(3) and 84 EPC (cf. T 116/95 of 26 April 1999). The board further decided to remit the case to the opposition division for further prosecution.

III. The opposition division in its interlocutory decision of 2 November 2000 decided that none of the requests then on file - the main request and auxiliary requests 1 to 4 filed in the previous appeal proceedings and auxiliary requests 5 and 6 filed in the further opposition proceedings - fulfilled the requirements of Article 83 EPC and the patent was revoked.

IV. The patentee lodged an appeal against this decision and filed a statement of grounds of appeal.

V. Respondents I and II (Opponents 01 and 02) filed replies to the grounds of appeal.

VI. The board summoned the parties to oral proceedings and, in a communication annexed to the summons, identified the main issues to be discussed at the oral proceedings.

VII. In reply to the board's communication, the appellant announced its intention not to attend the oral proceedings and respondents I and II submitted further observations.

VIII. Oral proceedings took place on 3 June 2004 in the absence of the appellant.

IX. Claim 1 of the **main request** read:

"A plant comprising plant cells which are genetically modified to contain an insecticide structural gene which is a bacterial gene or a modified bacterial gene, under control of a plant expressible promoter, whereby expression of said gene renders said plant insect resistant, provided that said cells are not tobacco cells containing the vector pA-ocs-B-proI-ESI as disclosed in EP-A-0140556."

Claims 2 to 6 concerned further embodiments of the plant of claim 1. Claims 7 to 31 were directed to a plant tissue comprising plant cells defined as in claim 1. Claims 32 to 44 and claim 77 were directed to

a vector comprising an insecticide structural gene and a plant expressible promoter as defined in claim 1 to render plant tissue comprising plant cells insect resistant. Claims 45 to 49 and claim 78 referred to a bacterial strain transformed with a vector defined as in claim 32. Claim 50 related to specific plasmids, whereas claims 51 and 52 concerned strains comprising these plasmids. Claim 53 to 76 and claim 79 related to a method of genetically modifying a plant cell to render plant tissue comprising such modified cells insect resistant, by transforming the cell with a vector as defined in claim 32.

X. Claim 1 of the **first auxiliary request** read:

"A plant comprising plant cells which are genetically modified to contain and express an insecticide structural gene which is a bacterial gene or a modified bacterial gene, under control of a plant expressible promoter, provided that said cells are not tobacco cells containing the vector pA-ocs-B-proI-ESI as disclosed in EP-A-0140556."

XI. Claim 1 of the **second auxiliary request** read:

"A plant comprising plant cells which are genetically modified to contain an insecticide structural gene which is a bacterial gene or a modified bacterial gene, under control of a plant expressible promoter, provided that said cells are not tobacco cells containing the vector pA-ocs-B-proI-ESI as disclosed in EP-A-0140556."

XII. Claim 1 of the **third auxiliary request** read:

"A plant comprising plant cells which are transformable by *Agrobacterium* and which are genetically modified to contain an insecticide structural gene which is a bacterial gene or a modified bacterial gene, under control of a plant expressible promoter, whereby expression of said gene renders said plant insect resistant, provided that said cells are not tobacco cells containing the vector pA-ocs-B-proI-ESI as disclosed in EP-A-0140556."

XIII. Claim 1 of the **fourth auxiliary request** read:

"A dicotyledonous plant comprising plant cells which are genetically modified to contain an insecticide structural gene which is a bacterial gene or a modified bacterial gene, under control of a plant expressible promoter, whereby expression of said gene renders said plant insect resistant, provided that said cells are not tobacco cells containing the vector pA-ocs-B-proI-ESI as disclosed in EP-A-0140556."

XIV. Claim 1 of the **fifth auxiliary request** read:

"A plant comprising plant cells which are genetically modified to contain an insecticide structural gene which is a *Bacillus thuringiensis* crystal protein gene or a modified *Bacillus thuringiensis* crystal protein gene, under control of a plant expressible promoter, said gene being expressible in said plant cells so as to render said plant insect resistant, provided that said cells are not tobacco cells containing the vector pA-ocs-B-proI-ESI as disclosed in EP-A-0140556."

XV. Claim 1 of the **sixth auxiliary request** read:

"A dicotyledonous plant comprising plant cells which are genetically modified to contain an insecticide structural gene which is a *Bacillus thuringiensis* crystal protein gene or a modified *Bacillus thuringiensis* crystal protein gene, under control of a plant expressible promoter, whereby expression of said gene renders said plant insect resistant, provided that said cells are not tobacco cells containing the vector pA-ocs-B-proI-ESI as disclosed in EP-A-0140556."

Claims 2 to 79 of each of the auxiliary requests were as claims 2 to 79 of the main request but relating to and defined as the subject of claim 1 of each of the corresponding auxiliary requests.

XVI. The following documents are referred to in the present decision:

D1: J. Schell and M.V. Montagu, Bio/Technology, April 1983, pages 175 to 180;

D3: K.A. Barton and W.J. Brill, Science, February 1983, Vol. 219, pages 671 to 676;

D15: E.E. Murray et al., Plant Molecular Biol., 1991, Vol. 16, pages 1035 to 1050;

D17: M.J. Adang et al., in "Molecular Strategies for Crop Protection", Ed. C.J. Arntzen and C. Ryan, Alan R. Liss, Inc. N.Y., 1987, pages 345 to 353;

D22: M. Vaeck et al., Nature, July 1987, Vol. 327, pages 33 to 37;

D37: A. Ledeboer and V. Malik, Bio/Technology, April 1983, pages 169 to 171;

D46: C.H. Shaw, Chemistry and Industry, December 1984, pages 817 to 824;

D49: L.K. Miller et al., Science, February 1983, Vol. 219, pages 715 to 721;

D76: R.F. Barker et al., Plant Mol. Biol., 1983, Vol. 2, pages 335 to 350;

P11: EP-A-0 193 259 (publication date: 03.09.86)

E5: Declaration of Perlak, dated 02.09.94 (Perlak I);

E11: Declaration of Keith A. Walker, dated 01.02.95 (Walker I);

E14: Declaration of Keith A. Walker, dated 30.06.97 (Walker II);

E17: Declaration of J. Leemans, dated 11.07.95 (Leemans II).

XVII. The appellant's arguments in writing, insofar as they are relevant to the present decision, may be summarised as follows:

The patent in suit disclosed three genes (tmr, tms and tml) involved in the induction of tumour growth and

referred to the advantageous deletion of the tumour-inducing genes tmr and tms for regenerating transformed plants. The deletion of the tml gene, though desirable, was not essential for regenerating normal, healthy plants. The patent was exemplified with disarmed vectors (lacking tmr and tms genes) that regenerated normal plants.

Post-published documents only showed that the level of insect resistance for plants transformed with the full-length insecticide structural gene from *Bacillus thuringiensis* (Bt) was relatively low. Declaration E14 demonstrated a significant insect resistance using the full-length Bt gene. Insect resistance did not mean insect killing since resistance could be obtained by sub-lethal levels of Bt toxin too. Insect bioassays for detecting expression of the Bt gene showed to be more sensitive than methods for detecting the Bt protein (ELISA). There was no need to demonstrate a correlation between expression of Bt protein and insecticide properties of the transformed plants. Such a correlation only represented an arbitrary requirement for a higher level of insect resistance. In this respect, the relevance of several parameters in the insect bioassay had not been recognized in the technical evidence relied on by the respondents: in particular, the leaf damage rating system was subjective and arbitrary, type of leaves (young or old, top, middle or bottom leaves) and age of the plants were not appropriate.

Document D17 showed insect resistant plants transformed with the full-length Bt gene. The fact that some transformed plants showed no resistance was irrelevant

since the levels of Bt expression were very variable. Declaration E11 demonstrated the presence of the full-length Bt gene in the R1 progeny of the transformed plants of document D17 and the effects on insect growth due to the expression of this gene. The problems associated with the detection of mRNA and Bt protein were irrelevant in as much as there were unequivocal data showing transformed plants resistant to insects.

Document D22 also showed plants transformed with the full-length Bt gene and having an insecticidal effect, i.e. insect mortality and weight reduction in surviving insect larvae. Similar results were disclosed in document P11, wherein plants containing the full-length Bt gene were shown to express the Bt toxin and to yield insecticide positive reactions above control plants. There was no evidence on file showing that the bioassays of document P11 were not available at the priority date of the patent in suit. The patent explicitly referred to and envisaged the use of both the full-length Bt gene and a truncated Bt gene encoding respectively the full-length Bt protoxin and a Bt fragment thereof.

Evidence was on file showing that *Agrobacterium* could be used for transforming monocotyledonous plants. As shown by document D1, alternative methods for transforming monocotyledons were also known in the prior art. The facts and evidence considered in decision T 612/92 of 28 February 1996 were not the same as in the present case.

XVIII. The respondent's arguments in writing and during oral proceedings, insofar as they are relevant to the present decision, may be summarised as follows:

Respondent I

None of the examples of the patent in suit disclosed the use of fully disarmed Ti-vectors. The prior art referred to the deletion of all tumour-inducing genes as essential for obtaining normal plants. Even if the patent referred to "micro-Ti" plasmids, none of these plasmids was used in the examples and thus, for an essential part of the patent - regeneration of normal plants - the skilled person was left with no guidance.

Apart from very general references, the patent did not disclose any insecticide structural gene other than the Bt gene. The expression of bacterial genes in plants was in its infancy and of an unpredictable nature. It could not be foreseen whether insecticidal activity could be obtained for these (undisclosed) insecticide genes since their expression and activity were dependent on plant environment and this activity could be toxic to transformed plants.

Example 11 of the patent in suit was the only example using a full-length Bt gene. However, there were no data on transgenic plants. This information was found in document D17 (whose authors were the inventors of the patent), which allegedly disclosed transformed plants with insect resistance, in particular plant 100. However, it did not detect full-length Bt mRNA but only a truncated Bt mRNA too short to encode an active insecticidal protein and it failed to show a

correlation between integrated Bt gene, Bt gene transcription, presence of Bt protein and insect toxicity. Document D17 further stated that many plants containing the Bt gene had no insect resistance and that it was important to rule out possible effects of altered secondary characteristics.

Document D15 (also written by the inventors) stated that, although transformed plants were toxic in insect bioassays, no Bt toxin protein could reliably be detected and it referred to the instability of Bt RNA transcripts as a possible reason therefor. These problems were apparent in all the technical evidence on file, in which there was always something missing, either the presence of Bt mRNA or Bt protein. Thus, the presence of any toxicity, if at all, could not be associated with the full-length Bt gene.

Similar results were reported in other post-published documents, such as document D22, which reported no insecticidal activity with the full-length Bt2 gene. Declaration E14 showed the same problems in detecting Bt RNA transcripts, Bt toxin and measuring insect toxicity, confirming the absence of any correlation among them. Moreover, there was no information on the protocols used, in particular the transformation procedures and insect bioassays.

This information was also missing in the patent in suit, which only referred to a very general bioassay. Thus, the skilled person could not reliably verify whether the transformed plants were insect resistant. Similarly, the transformed plant referred to in declaration E11 was mishandled, RNA analyses were inconsistent and

evidence for expression of full-length Bt gene was missing. The technical evidence on file, in particular declaration E17, showed the absence of insecticidal activity for the truncated Bt fragment exemplified in the patent in suit.

No specific comments were made in respect of the transformation and regeneration of monocotyledonous plants.

Respondent II

All post-published documents and technical evidence (declarations) on file used fully disarmed vectors, which were essential for regenerating normal, healthy plants. However, the patent in suit failed to indicate the relevance of these disarmed vectors. None of the deposited vectors was fully disarmed and the vectors explicitly mentioned in the claims were not disarmed vectors. The presence of the tumour-inducing tml gene could have a drastic influence on the ability of the transformed plant cells to give rise to morphological normal plants.

In the light of the unpredictability of the insecticidal activity in the plant environment and possible problems of expression of bacterial toxin genes in transformed plants, the patent in suit failed to provide a general teaching for insecticide structural genes.

Apart from a very general reference to regenerated plants (example 3.8), there was no disclosure of any transformed plant in the patent in suit. Post-published

documents, in particular documents D15, D17 (written by the inventors) and D22, referred to the absence of insecticidal activity in plants transformed with full-length Bt gene. Similar results were reported in document P11, which showed no insecticidal activity for leaves of plants transformed with constructs comprising the full-length Bt gene. There were also declarations on file showing the difficulties encountered in using the full-length Bt gene, in particular the instability of Bt RNA transcripts, the absence of any correlation between these RNA transcripts, the presence of Bt protein and the toxicity in insect bioassays.

The patent in suit failed to disclose these difficulties and to provide any guidance for overcoming them. It further failed to teach that insecticidal activity could be obtained with truncated Bt genes. The exemplified Bt fragment, which was shown in declaration E17 to have no activity, could not be a basis for a generalization to other Bt genes, let alone guidance for identifying and isolating appropriate fragments of other insecticide structural genes.

Reference was made to decision T 612/92 (cf. *supra*) which found that the *Agrobacterium* system was not available for transforming monocotyledonous plants. None of the other techniques available at the priority date of the patent in suit (microinjection, protoplasts fusion, etc.) allowed the transformation and regeneration of monocotyledonous plants in a successful manner.

Respondent III

The transformation and regeneration of monocotyledonous plants was not possible with the techniques available at the priority date. It was necessary to find the right balance between the extent or breadth of the claims - such as stated in decision T 116/95 (cf. *supra*) - and the actual technical contribution of the patent in suit, which failed to disclose any transformed plant, appropriate bioassay, etc.

XIX. The appellant (patentee) requested in writing that the decision under appeal be set aside and that the patent be maintained in accordance with the main request or one of the auxiliary requests 1 to 6 considered by the opposition division in the decision under appeal.

XX. The respondents (opponents) requested that the appeal be dismissed.

Reasons for the Decision

Article 83 EPC

1. The patent in suit relates to the production of insect resistant plants by transforming and regenerating plants with an insecticide structural gene, such as the exemplified *Bacillus thuringiensis* (Bt) crystal protein gene or a truncated fragment thereof. Three main issues arise for assessing the requirements of Article 83 EPC, namely (A) the availability of plant vectors without tumour-inducing genes, (B) the availability of insecticide structural genes and, particularly, the

functionality of the Bt crystal protein gene or a truncated fragment thereof and (C) the transformation and regeneration of monocotyledonous plants.

(A) Availability of plant vectors without tumour-inducing genes - micro-Ti or disarmed Ti-vectors.

2. The patent in suit identifies three tumour-inducing genes - tmr, tms and tml - in the T-DNA (transferred-DNA) of Ti-plasmids as well as their effects on shoot and root growth and on the regeneration of transformed plants (cf. *inter alia* page 6, lines 1 to 5, page 7, line 50 to page 8, line 8). It further states that Ti-transformed tissues are most easily regenerated if these tumour-inducing genes are inactivated (cf. *inter alia* page 17, lines 2 to 25). This teaching is also directly derivable from the prior art cited in the patent in suit, which explicitly refers to "mini-Ti" plasmids - lacking all non-T-DNA sequences of the Ti-plasmid - and "micro-Ti" plasmids as well as to a method of constructing "micro-Ti" plasmids, namely "*resectioning the mini-Ti with SmaI to delete essentially all of T-DNA but the nopaline synthase gene and the left and right borders*" (cf. *inter alia* page 11, lines 3 to 20, page 16, lines 37 to 47 and Figure 2). Both "mini-Ti" and "micro-Ti" plasmids are successfully transferred into plant cells when complemented with a Ti-plasmid in which its own T-DNA has been deleted - a binary plant vector strategy based on the separation of the (vir) virulence-region and the T-DNA region of the Ti-plasmid.

3. Document D76, cited in example 11 of the patent in suit (cf. page 30, line 7), discloses the nucleotide

sequence of the T-DNA region from *Agrobacterium tumefaciens* octopine Ti-plasmid pTi15955 and the physical map of this region. This document further refers to the successful use of "micro-Ti" plasmids (cf. page 345, paragraph bridging left- and right-hand columns). Similar results are mentioned in the prior art, such as *inter alia* documents D1, D3 and D37, which refer to the advantageous use of non-virulent binary Ti-plasmids for transforming plants with heterologous genes and regenerating normal, healthy plants (cf. page 178, left-hand column, lines 15 to 50 in document D1, page 672, paragraph bridging middle and right-hand column in document D3 and page 169, right-hand column, last full paragraph to page 170, left-hand column in document D37).

4. Thus, the "micro-Ti" plasmids referred to in the patent in suit were already well-known and available at the priority date and the advantages associated with their use were also clearly appreciated by the skilled person. The fact that these plasmids are not exemplified in the patent in suit does not - and cannot - change the teachings of this prior art. The genetically modified plants claimed in all requests on file (cf. paragraphs IX to XV *supra*) embrace both normal, healthy plants regenerated using Ti-plasmids without tumour-inducing genes - such as the known "micro-Ti" plasmids or disarmed Ti-vectors - as well as abnormal (crown gall-tumour) plants regenerated using Ti-plasmids with tumour-inducing genes - such as those exemplified in the patent in suit.

5. Thus, the board considers that the requirements of Article 83 EPC are fulfilled in respect of this issue, namely the regeneration of normal, healthy transformed plants with disarmed Ti-vectors.

(B) Availability and functionality of insecticide structural bacterial genes.

6. All the requests, except the first and second auxiliary requests, require that the "*expression of said (insecticide structural) gene renders said plant insect resistant*" (cf. paragraphs IX to XV *supra*). The first auxiliary request refers to genetically modified plants that "*contain and express an insecticide structural gene*" (cf. paragraph X *supra*), whereas the second auxiliary request reads only "*an insecticide structural gene...under control of a plant expressible promoter*" (cf. paragraph XI *supra*). Both requests thus require the expression of the insecticide structural gene. The expression of a gene is understood as the complete use of the information present in the gene, via transcription and translation, leading to the production of the corresponding encoded protein and hence, to the appearance of a specific phenotype determined by that gene, in the present case and in the light of the description, the appearance of a plant resistant to insect infection (insect toxicity and resistance). Thus, resistance and toxicity to insects is considered to be - either explicitly or implicitly - required in all requests on file.

(B.1) Availability of insecticide structural bacterial genes.

7. Insecticide structural genes - and insecticidal proteins - from bacteria are defined in the patent in suit in a general manner (cf. page 13, line 51 to page 14, line 27), referring in particular to bacterial phospholipases, hyaluronidases, phosphatases and proteases (cf. page 4, lines 24 to 25 and page 14, lines 22 to 23). Apart from a reference to a protease produced by *Pseudomonas aeruginosa* (cf. page 4, lines 25 to 27), all other references concern insecticidal toxins from *Bacillus* species, particularly from *Bacillus thuringiensis* (Bt) (cf. page 3, line 12 to page 4, line 27 and Table 3). The only references to the cloning of insecticide structural genes are to the genes coding for the crystal insecticidal proteins of Bt var. *kurstaki* strains HD-1-Dipel and HD-73 as well as Bt var. *berliner* strain 1715 (cf. page 3, line 40 to page 4, line 20).

8. Similarly, the references in the prior art on file to bacterial insecticide structural genes mainly concern Bt crystal toxin genes. Document D49 refers to bacterial, viral and fungal insecticides. The importance of insecticide toxins from *Bacillus* species, particularly from Bt, is clearly emphasized (cf. page 715, right-hand column, last paragraph to page 717, middle column, page 720, left-hand column, first full paragraph) and, as in the patent in suit, the sole bacterial insecticide structural genes referred to are the ones from Bt var. *kurstaki* (cf. page 717, middle column). General references to Bt insecticide structural genes are also found *inter alia* in documents D1 (cf. page 179, left-hand column, third full

paragraph) and D3 (cf. page 674, middle column, first full paragraph).

9. Thus, it appears that, at the priority date, the insecticide structural genes from Bt were the only ones (partially) characterised and available to the skilled person. However, in the light of the negative results obtained with these specific genes - Bt var. *kurstaki* HD-73 exemplified in the patent in suit - and in the absence of any indication as to how to overcome this failure (cf. B.2 and B.3 *infra*), the scarce information - both in the patent in suit and in the prior art - about other alternative bacterial insecticide structural genes made the selection, characterisation and use of any other possible alternative gene difficult if not impossible. To accomplish this task would therefore require undue burden and the exercise of inventive skill.

10. It follows from the foregoing that requests on file directed to a "*bacterial insecticide structural gene or a modified bacterial insecticide structural gene*" (i.e. the main request and the first, second, third and fourth auxiliary requests, cf. paragraphs IX to XIII *supra*) do not fulfil the requirements of Article 83 EPC.

(B.2) *Functionality of Bacillus thuringiensis crystal protein gene (full-length Bt gene).*

11. Example 11 of the patent in suit discloses plasmid p403B/BTB#3 with a full-length insecticide structural gene from Bt var. *kurstaki* HD-73 placed between the "1.6" promoter and polyadenylation site (cf. page 30, lines 1 to 4, Figures 2 and 4). Triparental mating (cf.

example 9, page 29) with *A. tumefaciens* RS2014 strain - containing a mutated pTi15955 with tumour-inducing genes tmr and tms deleted - results in the isolation of the R3-11 strain - with the p403/BTB#3 plasmid co-integrated into the mutated pTi15955 by a single homologous recombination event into the polyadenylation site side. This R3-11 strain is used to transform plant tissues which are cultured, single cell cloned and regenerated into plants (cf. page 30, lines 5 to 23). Although not exemplified in the patent in suit, "micro-Ti" plasmids may be used in a similar manner so as to regenerate normal, healthy transformed plants (cf. paragraphs 2 to 5 *supra*). The patent fails, however, to disclose any information on the transformed plants and, in particular on the very specific effect claimed, i.e. whether plants transformed with the full-length insecticide structural gene are insect resistant. This information is allegedly provided by several declarations and post-published documents, in particular declarations E11 and E14 and documents D17, D22 and P11 (all cited as expert opinions).

12. Post-published document D17 (cited as expert opinion) discloses the presence of a full-length insecticide crystal protein gene of Bt strain HD-73 (Bt HD-73 gene) - a 3.7 kb BamHI fragment similar to the one used in example 11 of the patent in suit - in the micro-Ti vector pH450 (cf. page 347, Figure 1). This vector is mated into an *A. tumefaciens* strain having a plasmid with the information required for plant transformation, i.e. complemented with a functional vir region of a normal Ti-plasmid, and plant cells and leaf segments are transformed and regenerated into plants. The presence of the Bt HD-73 gene integrated into the plant

genome is shown by Southern hybridisation assays (cf. page 348, Figure 2), transcription of the gene (mRNA) is analyzed by Northern blots (cf. page 349, Figure 3) and the insecticide Bt HD-73 protein is detected in transgenic leaf tissue by an optimised ELISA (detection of antigenic peptides) (cf. page 348, lines 15 to 17 and page 350, Table 1). Results of insect toxicity and resistance are also reported (cf. page 351, lines 3 to 13).

13. Post-published document D22 (cited as expert opinion) discloses the Ti-plasmid pGS1161 comprising a full-length insecticide Bt2 gene from Bt var. *berliner* strain 1715 (cf. page 33, Figure 1), which is used to produce transformed plants. Reference is made to levels of mRNA and protein (cf. page 35, right-hand column and page 36, Table 2) as well as to the insecticide effect - mortality and weight reduction - in transgenic plants containing the full-length Bt gene (cf. page 35, Figure 3 and page 36, Table 2).

14. Whereas there is no doubt that, using the method disclosed in the patent in suit and referred to in the post-published prior art, a full-length insecticide Bt gene is integrated into the plant genome, problems arise in the transcription of the full-length Bt gene. Document D17 refers to low levels of insecticide mRNA, the absence of a full-length mRNA and the presence of a short (1.7 kb) 3' truncated mRNA only (cf. page 350, first paragraph), which, in the light of the prior art (cf. page 54, line 19 to page 56, line 3 and Figures 20 and 22 of document P11), appears to be too short to encode an active insecticide protein. Reference is also made to the presence of possible premature termination

or cleavage/polydenylation signals (cf. paragraph bridging pages 351 and 352). Similarly, document D22 refers to low levels of insecticide mRNA (cf. page 35, right-hand column), in particular of full-length Bt gene, with a possible (low) differential RNA stability and translation efficiency (cf. page 37, left-hand column, first full paragraph). Post-published document D15 (cited as expert opinion) also refers to the specific instability of Bt RNA transcripts in transgenic plants and the presence of possible instability elements and premature termination codons, which result in abnormal degradation and metabolism of these mRNAs. It further refers to the inefficiency of these RNA transcripts due to a possible codon usage biased for expression in bacterial cells, not optimal for translation in plant cells.

15. These problems in the transcription of the full-length insecticide Bt gene explain the very low levels of insecticide Bt protein detected in transgenic plants, which are below or only slightly above the detection limit of all the detection assays used. Document D17 refers to the detection with Western blot of truncated Bt peptides - in some tissues - but of no full-length Bt protein (cf. page 352, lines 14 to 20). Low levels of Bt protein with very variable results are detected using an optimised ELISA (cf. page 348, lines 15 to 17, page 350, Table 1 and paragraph bridging pages 350 to 351). However, it is not clear whether the detected Bt protein corresponds to full-length Bt protein or to truncated fragments thereof (cf. page 352, first full paragraph). Similarly, document D22 discloses very low levels of the full-length Bt2 protein, slightly above the detection limit of the ELISA assay (cf. page 36,

Table 2 and page 37, left-hand column, first full paragraph).

16. In the light of these results, the authors of document D22 conclude that the insect toxicity of transgenic plants with the full-length Bt gene is not significant since the level of variability (insect mortality and weight reduction) is similar to control plants (cf. page 35, Figure 3, page 36, Table 2 and page 37, left-hand column, first paragraph). Document D17 refers to a single transgenic plant (plant 100) with significant insect resistance (53%) and to three other clones with higher mortality than non-transformed plants (25% over 7%) (cf. page 351, first full paragraph). However, since other plants transformed with the full-length Bt gene have no toxic effect on insects, it only draws cautious conclusions and refers to the possible importance of inherent plant resistance (cf. page 352, last paragraph). Similarly, document D15 refers to the presence of naturally occurring substances in the leaves of tobacco plants with toxic effects on the very specific insects - Manduca sexta, tobacco hornworm - used in document D17 (cf. page 1036, right-hand column, second full paragraph of document D15).

17. In fact, a high inherent variability of insect response to plant material at all levels (insect mortality, larval weight, leaf damage, whole plant, etc.) is shown in declaration E5, which refers to mortality levels for non-transformed plants similar to - and even higher than - those of document D17 (cf. *inter alia* paragraph 9 of declaration E5). It is in the light of these observations - high inherent variability of

non-transformed plants and of insect response thereto ***versus*** a very low insect toxicity of plants transformed with the full-length Bt gene - that a correlation between the integration of the full-length Bt gene (Southern blot), the transcription of this Bt gene (Northern blot), the presence of the corresponding Bt protein (Western blot, ELISA), and insect toxicity (bioassay) becomes essential for a clear distinction between these two possible effects.

18. Inherent variability is also shown in declaration E14, wherein values are indicated for experiments with the T_1 progeny of tobacco 532 plants transformed with the full-length Bt HD-73 gene (cf. Tables 5a to 5e, see also pages AD-2537 to AD-2545 of Appendix I). It refers to the low levels of Bt toxin in transformed plants, which require measurement of the toxicity effects in a careful and thoughtful manner (cf. page 11, lines 2 to 4). Low levels of Bt protein - at or below the limits of detection - are found in tobacco and alfalfa (cf. page 14, first full paragraph) and no correlation between Southern (genomic Bt integration) and Western blots (presence of Bt protein) is found in transformed alfalfa plants (cf. Table 6). Positive results in both Southern and Western blots are indicated for two tomato plants only - 2313-7-KT1-5B and 2313-7-T3-11-1A (cf. Table 9). However, insect mortality and growth inhibition is reported only for the former (cf. Table 10) and the results of insect bioassays for several transformed plants are not significant (cf. Tables 3 and 5e as well as the Tables in Appendix I). Moreover, there is no information as to the actual methods used, such as the specific insect bioassay tests (cf. page 5, first paragraph and page 6,

paragraph 12), improved protocols for effective (tissue) transformation (cf. page 5, paragraph 11 and page 7, paragraph 13), and regeneration of transformants (cf. page 11, last paragraph), etc.

19. Similar problems are found in declaration E11. In particular, reference is made to inconclusive results of Northern blots and to equivocal data on ELISA assays (cf. paragraphs 10 and 11), which require the development of an acceptable insect growth bioassay (cf. paragraph 12). Exhibit 14 to this declaration shows the difficulties encountered in differentiating the toxicity of full-length Bt toxin from the inherent toxicity of control plants (cf. pages 46, 49, 51, 68 and 73). In fact, the appellant itself refers to the importance of several parameters for an appropriate insect bioassay, such as the type of leaves (top or lower), the age of the plants at the time of the assay (8-10 weeks after transformation), and the leaf damage rating system. However, the patent in suit only refers to a general bioassay which incorporates extracts of Bt protein directly onto the surface of the insect feeding diet (cf. page 28, example 8 of the patent in suit).

20. Document P11 also outlines the problems encountered in the expression of Bt2 protein, which requires rigorous extraction and concentration procedures using selected transgenic calli for a reliable detection with immunological methods (cf. paragraph bridging pages 97 and 98, page 99, lines 13 to 15 and page 104, lines 17 to 27). However, these methods (ELISA, Western blot) do not differentiate the full-length Bt protein from inactive truncated fragments thereof. Levels of Bt2 protein detected in transgenic leaves are also said to

vary considerably depending on plant age, growth conditions, etc. and no Bt2 protein is detected for some constructs with a full-length Bt gene (cf. page 106, lines 10 to 13 and examples 11.3.2 and 11.3.3). Although for one construct toxicity is detected in callus tissue (cf. page 109, lines 20 to 23), no toxicity (mortality and growth inhibition) is detected using transgenic leaves of plants transformed with constructs containing the full-length Bt2 gene (cf. paragraph bridging pages 111 to 112, page 112, lines 22 to 23 and page 113, lines 8 to 9). The document further refers to the instability of the full-length insecticide Bt protein and/or the corresponding mRNA (cf. page 116, lines 12 to 21).

21. None of these problems referred to in this post-published prior art and in the technical evidence on file, is addressed by the patent in suit, which, as stated in paragraph 11 *supra*, fails to disclose any information on the actual transformed plants. In particular, there is no information concerning Bt mRNA transcripts and the associated levels of full-length Bt toxin or the specific requirements of a suitable insect toxicity bioassay. In the absence of all this information - both in the patent in suit and in the prior art - and in the light of the uncertain toxicity results shown in this post-published prior art, the board comes to the conclusion that, using the full-length insecticide Bt gene, the skilled person could not reliably achieve the envisaged result (insect resistant plants) without undue burden.

(B.3) Functionality of a modified Bacillus thuringiensis crystal protein gene (truncated Bt gene).

22. It remains to be assessed whether the patent in suit provides the skilled person - confronted with uncertain results when using the full-length Bt gene - with clear and straightforward guidance as well as the appropriate means for achieving certainty. Claims on file refer to an insecticide structural gene which is "*a modified bacterial gene*" or "*a modified Bacillus thuringiensis crystal gene*" (cf. paragraphs IX to XV). These modifications are mentioned in the description of the patent but only in a very general manner (cf. page 14, lines 1 to 6). However, examples 2, 3 and 4 disclose a very specific modification of the Bt gene, namely a truncated (2.8 kbp) form, which is in between the 67 kDa Bt toxin and the 130 kDa full-length Bt protoxin. Thus, the question arises whether using this modified (truncated) Bt gene - which in example 1.3 is identified as encoding an insecticidal active protein - the skilled person could achieve the claimed insect resistant transformed plants.

23. The patent itself, as stated in paragraph 11 *supra*, fails to disclose any information on plants transformed with either the full-length gene or the truncated fragment. However, declaration E17 - apparently made in connection with proceedings relating to document P11 - refers to the truncated Bt fragment of examples 2, 3 and 4, which is identified as "*the IAC106 chimeric gene*" (cf. paragraph 3). Figures 1 and 2 show that, in insect bioassays, both plants transformed with the IAC106 gene and control plants (untransformed) display similar results, and that transformed plants have a

variability which is similar in all to the one shown by control plants. The IAC106 gene is said to have similar properties to the full-length Bt gene in contrast to the insect toxicity shown by shorter truncated Bt fragments - IAC060 and IAC080 (cf. Figures 1 and 2 and points 4 to 6). However, there is no indication in the patent in suit of Bt fragments other than the exemplified one - IAC106. The skilled person - confronted with the negative results of IAC106 - could not have envisaged that shorter truncated Bt fragments, such as the ones disclosed in document P11 (cf. page 54, line 19 to page 56, line 3 and Figures 20 and 22) and D22 (cf. Figures 1 and 3, Table 2), would have insecticidal activity.

24. Thus, the board concludes on the basis of the evidence on file that, at the priority date neither the full-length Bt gene nor the truncated Bt form exemplified in the patent in suit could be used in a successful manner to transform plants and confer on them insect resistance. The absence of insecticidal activity in the exemplified specific embodiments of the patent leaves the skilled person completely at a loss as to the reasons for this failure. There is no other guidance, explicit or implicit, in the patent in suit that allows the skilled person to overcome this without undue burden.

(C) Transformation and regeneration of monocotyledonous plants.

25. Whereas the claims of the patent underlying decision T 612/92 (cf. *supra*) relate to a process for the incorporation of foreign DNA into the genome of monocotyledonous plants based on a Ti-plasmid of the

Agrobacterium or the *Rhizobium* bacteria (cf. paragraphs XVII and XVIII *supra*), in the present case none of the independent claims of the requests on file is actually limited to any particular transformation system. In fact, the patent in suit refers to the Ti-plasmid system for transforming dicotyledons and gymnosperms, whereas "*systems based on alternate vectors or means for vector delivery may be used to transform all gymnosperms and all angiosperms, including both monocots and dicots*" with reference to "*the use of vectors based upon viral genomes, minichromosomes, transposons*" and their delivery into plant cells by "*direct uptake of nucleic acid, fusion with vector-containing liposomes, microinjection and encapsidation in viral coat protein followed by an infection-like process*" (cf. *inter alia* the paragraph bridging pages 16 to 17).

26. At the priority date, methods for transferring genes directly into plant cells, including cells of monocotyledonous plants, were known to the skilled person (cf. *inter alia* document D37, page 170, left-hand column, last paragraph). Shortcomings and drawbacks of these methods were also known in the art, such as a low transformation frequency and, particularly, difficult regeneration of normal, fertile plants due to a lengthy development and regeneration with associated risk of somatic chromosomal aberrations. Improvements were, however, continuously developed for overcoming all these deficiencies (cf. *inter alia* pages 820 and 821 in post-published document D46, cited as expert opinion). Thus, the question arises whether the use of these methods and the improvements needed for achieving a successful transformation and

regeneration of monocotyledonous plants require inventive skill or undue burden from the person skilled in the art.

27. However, in the light of the conclusions reached above with respect to issue (B) (cf. paragraph 24 *supra*), which are relevant for all requests on file, it appears that the assessment of issue (C) - relevant only for a limited number of requests on file (cf. paragraphs IX to XI and XIV *supra*) - is not essential for arriving at a decision in the present case. Therefore, the board refrains from making any further comments with respect to this issue.

Conclusion

28. For all the foregoing reasons set out in B.1, B.2 and B.3 above, none of the requests on file is considered to fulfil the requirements of Article 83 EPC.

Order

For these reasons it is decided that:

The appeal is dismissed

The Registrar: The Chairman:

A. Wolinski C. Rennie-Smith