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D E C I S I O N
of 28 February 1996

Case Number: T 0612/92 - 3.3.4

Application Number: 84200792.4

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Title of invention:
A process for the incorporation of foreign DNA into the genome of monocotyledonous plants

Patentee:
RIJKSUNIVERSITEIT LEIDEN, et al

Opponent:
Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V.

Headword:
monocotyledonous plants/Rijksuniversiteit Leiden

Relevant legal provisions:
EPC Art. 54, 83

Keyword:
"Novelty - (yes)"
"Sufficiency of disclosure - (no)"

Decisions cited:
T 0292/85, T 0409/91, T 0019/90

Catchword:
-

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Case Number: T 0612/92 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 28 February 1996

Appellant: (Opponent)
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Decision under appeal: **Interlocutory decision of the Opposition Division of the European Patent Office dated 16 April 1992 concerning maintenance of European patent No. 0 159 418 in amended form.**

Composition of the Board:

Chairman: L. Galligani
Members: F. L. Davison-Brunel
S. C. Perryman

Summary of Facts and Submissions

I. European patent No. 0 159 418 with the title "A process for the incorporation of foreign DNA into the genome of monocotyledonous plants" was granted with four claims based on European patent application No. 84 200 792.4 filed on 4 June 1984.

Claim 1 read as follows:

"A process for the incorporation of foreign DNA into the genome of monocotyledonous plants by infecting the monocotyledonous plants for incubating the protoplasts thereof, with Agrobacterium or Rhizobium bacteria containing a virulence region and at least one T-region originating from a Ti-plasmid or a Ri-plasmid or both, which T region is provided with said foreign DNA."

Claims 2 to 4 further related to specific embodiments of the process according to claim 1.

II. A notice of opposition was filed requesting the revocation of the patent under Article 100(a) EPC (lack of novelty and inventive step) and under Article 100(b) EPC (insufficiency of disclosure).

The documents cited during opposition proceedings which were considered most relevant by the Opposition Division are the following:

(3): EP-A- 0 116 718;

(7): Zambryski P. et al., EMBO J., 1983, volume 2, No. 12, pages 2143 to 2150;

(8): De Cleene, M. and J. De Ley, Bot. Review, 1976, volume 42, No. 4, pages 389 to 466;

(10): Bytebier B. et al., Proc. Natl. Acad. Sci. USA, 1987, volume 84, pages 5345 to 5349;

(18): Schäfer W. et al., Nature, 1987, volume 327, pages 529 to 532.

III. By the interlocutory decision within the meaning of Article 106(3) EPC dated 16 April 1992, the Opposition Division maintained the patent in amended form according to Article 102(3) EPC on the basis of claims 1 to 4 as filed on 19 September 1991 which were identical to claims 1 to 4 as granted except for the correction of the typographical error in claim 1 where the word "for" was replaced by "or".

It was determined that the description of the patent in suit disclosed the invention in an enabling manner. No scientific arguments existed to conclude that members of other monocotyledonous families were so much different from the ones exemplified that they could not be transformed by *Agrobacterium*. Documents (10) and (18) constituted **a posteriori** proof that the Ti DNA was incorporated into the plant genome following the infection of wounded plants.

Novelty was acknowledged over documents (3) and (7) as both these documents were concerned with dicotyledonous, not monocotyledonous plants.

The closest prior art was identified as document (8) which provided some indication that some monocotyledons are susceptible to *Agrobacterium*, as shown by the formation of crown gall-like tumors after infection. The combination of this with document (7) which

disclosed the Ti transformation of dicotyledons was found insufficient to negatively affect inventive step as it would not make it obvious that Ti could transform monocotyledons without the formation of crown gall-like tumors.

IV. The Appellants (Opponents) lodged an appeal against the decision of the Opposition Division and filed the statement of grounds of appeal.

V. Submissions were received from both parties including six new documents.

VI. A communication was issued by the Board according to Article 11(2) EPC of the Rules of Procedure of the Boards of Appeal setting out the Board's preliminary position.

VII. Further submissions were received from both parties together with seven additional references.

VIII. The following documents on file, are mentioned in the present decision (numbering as used by the Opposition Division):

(7),(8),(10) and (18): supra;

(14): Hooykaas-Van Slogteren G. M. S. et al., Nature, 1984, volume 311, pages 763 to 764;

(16): Graves A. C. F. and S. L. Goldman, Plant Mol. Biology, 1986, volume 7, pages 43 to 50;

(26): Graves A. C. F. and S. L. Goldman, J. Bact., 1987, volume 169, No. 4, pages 1745 to 1746;

(30): Potrykus I., Ann. Rev. Plant Physiol. Plant Mol. Biol., 1991, volume 42, pages 205 to 225;

(41): Deng W. et al., Science in China (Series B), 1990, volume 33, No. 1, pages 27 to 34;

(42): Wilmink A. et al., Plant Cell Reports, 1992, volume 11, pages 76 to 80;

(43): May G.D. et al., Biotechnology, 1995, volume 13, pages 486 to 492;

(45): Ritchie S.W. et al., Transgenic Research, 1993, volume 2, pages 252 to 265;

(46): Hiei Y. et al., The Plant Journal, 1994, volume 6, No. 2, pages 271 to 282.

IX. Oral proceedings were held on 28 February 1996. During these proceedings, the Respondent (Patentee) filed Auxiliary requests I and II.

Claim 1 of Auxiliary request I differs from granted claim 1 (corrected for the typographical error) in that the expression "..or incubating the protoplasts thereof.." has been deleted. Claims 2 to 4 remain unchanged.

Claim 1 of Auxiliary request II differs from granted claim 1 (corrected for the typographical error) in that the expression "...,and wherein said DNA is transiently expressed..." is added at the end of the claim. Claims 2 to 4 remain unchanged.

X. The submissions in writing and during oral proceedings by the Appellants can be summarized as follows:

- Claim 1 lacked novelty over document (8) which disclosed the formation of tumors in some monocotyledons when they are infected by *Agrobacterium tumefaciens*.

- The patent specification was not enabling in three respects:

  (a) The description did not approach the problem of incorporating Ti DNA into the genome of monocotyledonous plants other than *Liliaceae* and *Amaryllidaceae*. There existed serious doubts substantiated by numerous post-published documents that T-DNA incorporation could be achieved by following the method of claim 1.

  (b) The patent did not show that T-DNA incorporated into the plant genome of even *Liliaceae* and *Amaryllidaceae*. Post-published work (documents (10) and (18)) had not been carried out in the same experimental conditions as in the patent and, thus, could not serve as proof for enablement.

  (c) The process of claim 1, which was to be interpreted as the provision of transgenic monocotyledonous plants, could not be carried out as insufficient information had been given on how to perform the regeneration of wounded cells or protoplasts.

- It was known from document (7) that T-DNA could be incorporated into the genome of dicotyledonous plants when they were infected with *Agrobacterium*. Document (8) disclosed that some monocotyledonous plants were susceptible to infection by *Agrobacterium*. The combination of these two documents made it obvious that T-DNA could also be incorporated into the genome of monocotyledonous plants.

XI. The Respondent replied as follows:

- Document (8) could not be seen as novelty-destroying because the swellings observed on very few of the monocotyledons tested after *Agrobacterium* infection were not necessarily due to the incorporation of the T-DNA into the plant genome. Moreover, the Ti plasmid of document (8) was wild-type whereas claim 1 was to be understood as requiring a Ti plasmid including DNA which it does not normally carry.

- The patent specification was enabling because:

  (a) The patent specification provided examples of the inoculation of *Agrobacterium* into two monocotyledonous species of the families *Amaryllidaceae* and *Agaraceae*, followed by the introduction and expression of the Ti DNA in the plant cells. This established sufficiency of disclosure for the whole area of monocotyledonous plants. Several post-published references supported the fact that the teaching of the invention could be applied to cereals.

  (b) There were no reasons to believe that the differences in the experimental conditions used in document (10) or (18) and in the patent in suit would have any influence on the T-DNA incorporation into the plant genome. It was, thus, justified to consider these two documents as *a posteriori* proof of said incorporation.

(c) The specification left no doubts that the techniques generally in use for regeneration of dicotyledons were likewise applicable to monocotyledonous plants.

- Document (8) disclosed that only 5 out of 79 inoculations of different monocotyledons species by *Agrobacterium* resulted in tumor formation. Thus, document (8) did not lead the skilled person to the present invention, but, on the contrary, it taught away therefrom. At the priority date, there existed a definite prejudice in the art against the ability of *Agrobacterium* to infect monocotyledons. Overcoming this prejudice was the merit and the crux of the invention.

XII. The Appellants requested that the decision under appeal be set aside and that the European patent be revoked. The Respondent requested that the appeal be dismissed and that the patent be maintained on the basis of the main request submitted on 19 September 1991 or of the first or second Auxiliary request submitted at oral proceedings on 28 February 1996.

Reasons for the Decision

1. The appeal is admissible.

Late filed documents

2. Two documents, including document (30), were filed late in opposition and their introduction into the proceedings was refused by the Opposition Division. Thirteen further documents were also filed by both parties during the written part of the appeal

proceedings. All these documents concern the state of the art with regard to *Agrobacterium* or to Ti transformation of monocotyledons before and after the priority date of the patent. In view of their relevance for the assessment of sufficiency of disclosure (see points 18 to 24, infra), the Board decides to allow them into the proceedings pursuing to Article 114(1) EPC.

Main request

Amendments (Article 123(2)(3)) EPC)

3. No objection has been raised that the patent in suit contained added subject-matter or that the amendment in claim 1 extended the protection conferred. The claims are identical to the granted claims except for the correction of the typographical error. Thus, no questions concerning Article 123(2)(3) EPC arise for consideration.

Novelty (Article 54 EPC)

4. Document (8) has been cited as relevant to the assessment of novelty. It describes a study of the host range of *Agrobacterium* B6 performed by the authors on 48 species from 39 genera in 14 monocotyledonous families and also reviews all results made available on *Agrobacterium* tumorigenicity from 1911 onwards. In all cases, the appearance of swellings at the site where the plants have been inoculated is taken as evidence of the invasive effect of the micro-organism.

5. The Appellants argued that the process described in document (8) was identical to that of claim 1 because the *Agrobacterium* strains used for host-range testing must have contained the Ti plasmid. The observed

swelling effect could not but be due to the introduction of said plasmid into the monocotyledonous plants .

6. The Board accepts that although no direct evidence is provided in document (8) of the presence of the Ti plasmid in the *Agrobacterium strains* used for infection, these strains most probably contained said plasmid, the existence of which in wild-type *Agrobacterium* was known since 1974. This seems to be undoubted at least for the *Agrobacterium* strain B6, which is acknowledged by both parties as carrying Ti.

7. However, careful scrutiny of document (8) reveals that none of the inoculations which were performed by the authors resulted in tumor formation, except that of **Cordillyne stricta**. Yet, in this latter case, the authors indicate that the swelling occurred once, on one plant only and that its nature remained to be established. Also reported in document (8) are earlier experiments described in four publications dating from 1949, 1938, 1936 and 1928 which disclosed the formation of swellings with *Allium cepa*, *Aloe densiflorus*, *Aloe ferox* and *Agave salmiana*. It must, however, be noted that:

- Repeated attempts to reproduce the experiment with *Allium cepa* failed (1961 and 1973);

- In the case of *Aloe* and *Agave*, no information is available on the bacterial strain used.

8. Accordingly, in view of the paucity of the positive results and the uncertainty attached to their significance, the Board considers that document (8) does not teach the person skilled in the art that

monocotyledonous plants can be infected with *Agrobacterium*, let alone that the T-DNA would be incorporated into the genome of said plants. The argument presented in point 5 (supra) must, therefore, fail.

9. The subject-matter of claim 1 is, thus, considered novel.

Sufficiency of disclosure (Article 83 EPC)

10. The claimed invention is defined as a process for the incorporation of foreign DNA into the genome of monocotyledonous plants. As it is apparent from the specification (page 3, column 2, paragraph 4), the patent in suit does not disclose a technique new in itself, but rather makes the suggestion that a technique already known for the incorporation of foreign DNA into the genome of dicotyledonous plants will also work for monocotyledonous plants. Therefore, the contribution to the state of the art by the patent in suit is the suggestion of a new application for a known technique. It must, thus, be of particular relevance for the assessment of sufficiency of disclosure that the process can indeed be carried out over the whole range of the claimed application, and that the skilled person will not find himself/herself in a situation where, despite using reasonable endeavours, he or she cannot carry out the process in relation to the particular monocotyledonous plant, he or she is interested in.

11. The established case law of the European Patent Office gives guidance as to the circumstances in which sufficiency of disclosure may or may not be acknowledged (e.g. T 0292/85, OJ EPO 1989, 275;

T 0409/91, OJ EPO 1994, 653; and T 0019/90, OJ EPO 1990, 476).

12. It is stated in T 0292/85 (supra) that: "...an invention is sufficiently disclosed if at least one way is clearly indicated enabling the skilled person to carry out the invention. Consequently, any non-availability of some particular variants of a functionally defined component feature of the invention is immaterial to sufficiency as long as there are suitable variants known to the skilled person through the disclosure or common general knowledge, which provide the **same** effect for the invention. The disclosure need not include specific instructions as to how all possible component variants within the functional definition should be obtained" (cf. point 3.1.5 of the Reasons).

13. However, it is necessary that the skilled person is given sufficient guidance for performing the invention in the whole range claimed without undue burden to give effect to the legal principle that the scope of the patent should be justified by the technical contribution to the art (cf. in this respect T 0409/91, supra).

14. As stated in T 0019/90 (supra): "...the mere fact that a claim is broad is not in itself a ground for considering the application as not complying with the requirement for sufficiency of disclosure under Article 83 EPC. Only if there are serious doubts, substantiated by verifiable facts, may an application be objected to for lack of sufficient disclosure" (cf. point 3.3 of the Reasons).

15. In the Respondent's view, the rationale of T 0292/85 (supra) should apply to the present case because the patent specification provides examples of the inoculation of *Agrobacterium* into two monocotyledonous species of the families *Amaryllidaceae and Agavaceae*, followed by the introduction and expression of the Ti DNA in the plant cells and these establish sufficiency of disclosure for the whole area of monocotyledonous plants.

16. As stated in decision T 0292/85 point 3.3.2 (supra) the character of the invention which was the subject of that patent, was one of general methodology which was fully applicable with any starting material. By contrast here the subject is concerned with applying a known method to a new area of application, defined as monocotyledonous plants. Unlike the feature in the claims under consideration in T 0292/85 (supra), this is not a feature defined in functional terms, for which one variant which can be carried out by the skilled person may be sufficient in some circumstances. The feature in the claim now under consideration relates to known plants, and the novelty of the process is applying known methods to these known plants. But then the information in the patent and common general knowledge at the priority date must also enable the skilled person to carry out the method throughout the novel field of application claimed, that is for all monocotyledonous plants. There is no justification for allowing the claim to cover the application of the process to monocotyledonous plants which the skilled person could not with the information in the patent transform using a Ti-plasmid. The claim here will thus be invalid for non-compliance with Article 83 EPC, if

the appellant can show that for one type of monocotyledonous plant the process could not be carried out on the basis of the information in the patent and common general knowledge.

17. The Board notices that the class of the monocotyledons regroups as many as fifty-three widely diversified families such as *Orchideae, Gramineae* and *Agavaceae* (cf. document (8)) and that considering the results obtained with *Amaryllidacae* and *Agavacae* the Respondent himself expressed the view that: "Further research is needed to establish whether crops from other monocot families (for example, Gramineae) can be transformed by the Ti plasmid" (cf. later document (14) as an expert's opinion).

18. Furthermore, a close survey of the available literature, brings out the following information:

18.1 From 1984 to 1990, the transforming ability of *Agrobacterium* was demonstrated with one species of *Dioscoreaceae* (document (18)). Evidence was also provided that the infection with *Agrobacterium* could lead to opine synthase production in one species of *Iridaceae* (document (26)) and also in seedlings of *Zea mays* (document (16)). The findings with *Zea mays* were later challenged in document (30).

18.2 Document (30), published in 1991, reviews the state of the art in the field of *Agrobacterium*-mediated gene transfer until 1990. It describes the transformation of cereal plants with *Agrobacterium* as a complete failure "despite the enormous effort so far invested in this approach" (see page 210, last paragraph) and goes on to discuss the potential reasons why monocotyledons in general are resistant to transformation.

18.3 Research on transformation of monocotyledons with *Agrobacterium* seems to have taken some impetus in the early 1990. Between 1990 and 1995, the transformations of wheat and barley (document (41)), tulip (document (42)), maize (document (45)), rice (document (46)) and banana (document (43)) were thus achieved, none however, by the protocol described in the patent in suit. In each case, special conditions had to be found such as the use of specific *Agrobacterium* strains (documents (45), (46)), the inoculation of specific tissues (document (43)), the addition of hormones (document (41)).

18.4 Document (43) published in 1995, states (second column on page 486) "A consensus opinion developed at a recent conference on banana and plantain improvement concluded that the transformation of *Musa* spp. with *Agrobacterium* would be ineffective because this monocot is not susceptible to infection by *Agrobacterium tumefaciens*...". The authors go on to report that they had succeeded using a particular method involving inter alia bombarding cut surfaces of corm slices with gold micro particles to cause wounding of the cells underlying, but then still states that: "There is insufficient data available to predict the extent to which meristematic tissues of other monocotyledonous species can be made susceptible to *Agrobacterium* [...]. This situation has further complexity because of the differences in infection efficiency between different Ti plasmid vectors and levels of virulence between *Agrobacterium* strains. Because of this uncertainty, the bacterial gene transfer in other untested monocotyledonous species cannot be predicted." (cf. page 491, left-hand column, second paragraph).

18.5 Document (46) stresses that the optimization of the conditions of co-cultivation and the choice of tissue are of critical importance (cf. page 277, right-hand column, last paragraph and page 278, left-hand column second paragraph).

19. The Respondent argues that if research on the transformation of monocotyledons by *Agrobacterium* took so long to be implemented, it is because the transformation of plant tissues was tried rather than that of plants, and tissue culture is a difficult technique to handle. In his view, the statement in document (14) (point 17, supra) does not imply that further inventions are necessary to make the claimed process repeatable but rather that optimal conditions should be found by standard laboratory practice. In fact, the situation should be compared to that encountered with dicotyledons in that the transformation of each species requires specific measures. Thus, the choice of meristematic tissues disclosed in document (43) was already known from work done on that latter class of plants. In the same manner, whereas it is true that the choice of a specific *Agrobacterium* strain or the addition of hormones may improve the efficiency of the claimed process, transformation can, nonetheless, be expected to occur in the absence of these improvements. Finally the Respondent draws the Board's attention to the statement in document (46) that "the controversy (on whether monocotyledons may be transformed with Agrobacterium) now appears to be resolved, at least for rice.."(locution added).

20. The Board is willing to accept that the lack of an efficient protocol for cultivating plant tissue may have slowed down the progress of research. It would seem, however, that even at the time when the technique

of tissue culture had been mastered (e.g. document (43)), the problems remained in relation to the infection process (cf. point 18.3 supra).

21. Yet, the specification of the patent in suit never mentions the need for adapting the claimed process to each specific monocotyledonous species. Neither, of course, does it suggest which parameters should be changed.

22. The skilled person might infer from the work done on dicotyledons which measures ought to be tried in order to set up conditions favourable for transformation. However, this approach implies a substantial amount of work. In view of the number of features which may have to be altered (alone or in combination) to obtain transformation, the Board finds that this work would amount to an undue burden of experimentation.

23. Furthermore, the Board derives the impression from studying the documents on file that even up until the present time, any successful, new transformation of another monocotyledonous species with *Agrobacterium* is perceived as an achievement in its own right (e.g. document (43)). It is clear that, as late as 1990, the transformation of cereal plants could not be achieved (document (30)).

24. Thus, the Board comes to the conclusion that the facts presented in points 17 to 18.5, 20 to 23 supra, show that the information in the patent was insufficient to allow the invention to be carried out with the majority of monocotyledonous plants and this, quite independently of whether it is the plants or the protoplasts which are put into contact with the T-DNA.

25. For these reasons, in the light of the established case law as discussed in points 11 to 16 above, the main request must be refused under the provisions of Article 83 EPC for lack of sufficient disclosure.

Auxiliary request I

26. Auxiliary request I differs from the main request in that the expression "or incubating the protoplasts thereof" has been deleted from claim 1. Claims 2 to 4 remain unchanged.

27. In the Board's view, the deletion of this alternative technical feature neither extends the subject-matter beyond the content of the application as filed nor extends the protection conferred. Moreover, it does not alter the clarity of the claim. With regard to novelty, the same reasoning applies as given for the allowability of the main request. Thus, Auxiliary request I fulfils the requirements of Articles 123(2)(3), 84 and 54 EPC.

28. The Board is, however, of the opinion that the finding of non-compliance with Article 83 EPC applies equally to claim 1 of this request, now limited to the first of the two alternatives offered by claim 1 of the main request, as it does to claim 1 of the main request. This claim is still addressed to a process for incorporating foreign DNA into monocotyledons which would require an undue burden of experimentation to be reproduced over the whole width of the field of application (cf. in particular point 24 supra).

29. Accordingly, Auxiliary request I is refused under the provisions of Article 83 EPC.

Auxiliary request II

30. Auxiliary request II differs from the main request in that the expression "..., and wherein said DNA is transiently expressed." is added at the end of the claim. Claims 2 to 4 remain unchanged.

31. Said expression is not to be found in the application as filed. The Board is unable to attach any definite meaning to "transient". It is inconsistent to claim the incorporation of the T-DNA into the plant genome, and yet only require transient expression to occur.

33. Auxiliary request II is thus refused because it does not meet the clarity requirements of Article 84 EPC.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The patent is revoked.

The Registrar: The Chairman:

L. McGarry L. Galligani