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D E C I S I O N
of 18 June 2002

Case Number: T 0984/00 - 3.3.4

Application Number: 88105808.5

Publication Number: 0290799

IPC: C12N 15/00

Language of the proceedings: EN

Title of invention:
Process for the introduction of expressible genes into plant-cell genomes and agrobacterium strains carrying hybrid ti plasmid vectors useful for this process

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

Opponent:
-

Headword:
Ti-plasmid vectors/MAX-PLANCK-GESELLSCHAFT

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

Keyword:
"Added subject-matter - no"
"Inventive step - yes"
"Clarity - yes"
"Sufficiency of disclosure - no"

Decisions cited:
T 0694/92, T 0292/85, T 0612/92

Catchword:
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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0984/00 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.1.1
of 18 June 2002

Appellant: Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V.
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Decision under appeal: **Decision of the Examining Division of the European Patent Office posted 20 July 2000 refusing European application No. 88105808.5 pursuant to Article 97(1) EPC.**

Composition of the Board:

Chairman: S. C. Perryman
Members: A. L. L. Marie
R. E. Gramaglia

Summary of Facts and Submissions

I. An appeal was filed by the applicant against the decision of the examining division refusing European Patent application 88 105 808.5 pursuant to Article 97(1) EPC. This application was divided out of European Patent application 83 112 985.3.

II. The decision of the examining division was based on a set of 15 claims, claims 1, 14 and 15 of which read:

"1. A cell of a plant which contains stably integrated into its genome a foreign DNA which is characterized in that:

(a) it does not contain T-DNA genes that control neoplastic growth and it is substantially free of internal T-DNA sequences of a wild-type Ti-plasmid, and

(b) it comprises at least one gene of interest containing:

(i) a coding sequence, and

(ii) a promoter region that contains a promoter sequence other than the natural promoter sequence of said coding sequence, and wherein said promoter sequence regulates transcription of downstream sequences containing said coding sequence to produce an RNA in said cell."

"14. A plant composed of the cells of any one of claims 1 to 13."

"15. A seed of the plant of claim 14 which is composed of the cells of any one of claims 1 to 13."

III. The following documents are mentioned in this decision:

(1) J. Leemans et al., The EMBO Journal, 1982, Vol. 1, No. 1, pages 147 to 152

(2) M.W. Bevan and M.-D. Chilton, Ann. Rev. Genet., 1982, Vol. 16, pages 357 to 384

(3) H. De Greve et al., Nature, 1982, Vol. 300, pages 752 to 755

(11) J. Leemans et al., "Molecular Biology of Plant Tumors", Academic Press Inc., 1982, pages 537 to 545

(12) M.-D. Chilton et al., Stadler Symposium, 1981, Vol. 13, pages 39 to 51

(14) J. Leemans et al., J. Mol. Appl. Genet., 1981, Vol. 1, No. 1, pages 149 to 164

(28) G. Ooms et al., Plasmid, 1982, Vol. 7, pages 15 to 29

(29) R.F. Barker et al., Plant Molecular Biology, 1983, Vol. 2, pages 335 to 350

(31) Comparison of Figure 5 of document (1) and Figure 1 of document (29) submitted on 6 June 2002.

IV. The reasons given by the examining division in its decision for refusing the application can be summarized as follows:

Clarity and Interpretation

– The expression "*...substantially free of internal T-DNA sequences of a wild-type Ti-plasmid...*" in claim 1 was not in itself clear and required interpretation on the basis of the description.

– No example of the patent showed a cell which actually combined both features (a) and (b) of claim 1. The actual examples referred to plasmids still containing substantial parts of internal T-DNA sequences.

– The meaning of "substantially free" as being "only the T-DNA borders" as put forward by the applicant was not accepted. Rather only a functional interpretation of "substantially free" as meaning merely the absence of genes which control neoplastic growth was appropriate.

– Claim 1 in essence thus comprised two features, namely the absence of tumour genes derived from T-DNA and the stable integration of a gene into the genome, said gene being under the control of a non-natural promoter functional in plants.

Balance between Article 83 and 56 EPC requirements
Contribution to art

- The balancing required when considering both Articles 83 and 56 became particularly relevant, given that the feature of the gene being under the control of a non-natural promoter had only been emphasized at a late stage in the examining procedure, the application stating at eg. page 20 "..which contains either its natural or an exogenous promoter".

- Document (1) already showed that none of the transcripts of TL-DNA, including those which were responsible for tumour growth, were necessary for the transfer of T-DNA, and in addition plant cells were produced which did not give rise to tumour production.

- Moreover the methods described in document (1) were suitable for introducing any foreign and non-selectable DNA into the T-DNA, this document in principle allowed the transfer of any desired DNA to be introduced into a plant cell genome and the generation of non-tumorigenic plants from said transformed plant cells.

- It was irrelevant that the plasmids used in document (1) still contained genes that controlled neoplastic growth and/or the TR-DNA, given that the essence of both the patent in suit and document (1) was that plants could be produced containing foreign DNA but not producing tumours,

i.e. the importance of deleting those genes involved in neoplastic growth.

– In addition document (11) had already proposed chimeric constructs wherein a foreign gene was put under the control of the promoter for the opine synthases, ie. the ocs or nos promoter.

– At the priority date such constructs could only be introduced into a plant cell by the use of Ti-plasmids, as described in both documents (1) and (11), which meant a clear incentive to combine the teaching of both documents.

– Thus, when using this theoretical approach the disclosure of documents (1) and (11) could be combined directly in order to produce plant cells without tumour genes wherein a foreign gene was put under the control of an opine synthase promoter.

– Although this approach was admittedly merely a theoretical one, the means for carrying out said process were all available in the art and also the sequences of the nos and ocs genes were known.

– In the application too products were only described in a theoretical manner, and no "normal" plant with a chimeric construct had been described as actually being made. The experimental disclosure in the application was limited to describing the successful expression of the ocs gene under the control of the nos promoter, i.e.

the confirmation of an experiment already described in the prior art.

– The examining division interpreted the Applicant's arguments on document (11) as meaning that even when using one of the two possible promoters proposed in document (11), achievement of expression was not possible without undue experimentation or even the use of inventive skill.

– But then, on the basis of the single example provided by the Applicant, namely the expression of the ocs gene under the control of the nos promoter, no guidance was given to the skilled person how to express any other gene under the control of any other possible promoter. If as alleged by the applicant the guidance in the prior art was insufficient even for the promoters suggested in document (11), then for all the other possible promoters embraced by claim 1, the situation would be as difficult, and the application gave no additional information on what to do.

– This was a situation comparable to that in case T 694/92 (EPO OJ 1997, 408), and when making a balance between the contribution of the application over the prior art, and the teaching of the prior art as represented by documents (1) and (11), the experimental evidence and technical details the description did not provide sufficient support for a claim directed to any plant cell containing in its genome in an expressible form any foreign gene under the control of any promoter

functional in plants, so that Claim 1 had to be refused under the provisions of Article 83 EPC.

Further possible objection under Article 83 EPC

– A further point which had yet to be considered with respect to the breadth of the claim, was that the claims encompassed also monocotyledonous plants, which at least at the filing date could not be successfully transformed by the use of Ti-plasmids (cf. decision T 612/92 28 February 1996).

V. The Board issued a communication under Article 11(2) of the rules of procedure of the boards of appeal giving the preliminary and non-binding opinion of the Board.

VI. Oral proceedings were held on 18 June 2002.

VII. The appellant filed during the oral proceedings a new main request with four claims, claim 1 of which read:

"1. A cell of a dicotyledonous plant, obtainable by Agrobaterium transformation, which contains stably integrated into its genome a foreign DNA which is characterised in that:

(a) it does not contain T-DNA genes that control neoplastic growth and it is substantially free of internal T-DNA sequences of a wild-type Ti-plasmid except for promoter sequences; and

(b) it comprises at least one gene of interest containing:

(i) a coding sequence; and

(ii) a promoter region that contains a promoter sequence other than the natural promoter of said coding sequence, and wherein said promoter sequence regulates transcription of downstream sequences containing said coding sequence to produce an RNA in said cell."

VIII. The arguments of the appellant can be summarized as follows:

Inventive step

– The application described a pioneer invention teaching the skilled person that every DNA placed between the borders of T-DNA was transferred into the genome of a plant, which could not have been derived from the prior art.

– The contribution to the art by the invention was the provision of a morphologically normal plant having integrated into its genome a gene comprising a coding sequence coding for a desired product and a promoter region that contains a promoter sequence other than the natural promoter of said coding sequence: the prior art had not made this available.

– This was a contribution of wide application, not linked to any particular chosen pair of coding sequence and promoter.

– The application contained adequate teaching on how this exogenous DNA could be introduced into dicotyledonous plant cells using modified Ti-plasmids, containing **only the T-border regions but not the internal T-DNA sequences of wild-type Ti-plasmid**, and inserted between the border regions the foreign coding sequence of interest and a promoter region other than the natural promoter sequence for the gene of interest.

– This plant would be "normal" in the sense that it did not contain T-DNA genes that control neoplastic growth and thus no tumorous growth would occur. The prior art had not shown that only the T-border regions were sufficient for integration of the Ti-plasmid.

– Indeed, the most extremely deleted plasmid of document (1), pGV2217 no longer contained the left border of TL-DNA, but still had TR-DNA, whereas the other plasmids still contained TL-DNA neoplastic genes. Further, document (1) was silent about the possible function(s) of TR-DNA and did not suggest to further delete TR-DNA.

– Document (11), on page 538, expected a DNA inserted between the "ends" of T-DNA to be transferred, but with the critical proviso that no essential function for T-DNA transfer and stable integration was inactivated by the insertion. Document (11) thus told the reader that further research on what might be critical was needed.

– Document (11) on page 543 defined properties that the modified T-DNA should exhibit and, in particular, in item 3 referred to possible enzymes for integration, the genes for which should not be deleted from the T-DNA. But document (11) gave the reader no information on the enzymes, the genes for these enzymes or where to look for them.

– Therefore, the mere combination of documents (1) and (11) was not sufficient to lead the skilled person to the subject-matter of the present application. The skilled person was left to do research whose outcome he would have been unable to predict.

– Documents (2) and (14) were even further remote from the subject-matter of the present application than documents (1) and/or (11). Document (2) merely referred on page 378 to document (14), as "reference (56)", for defining extreme forms of disarmed Ti-plasmids. According to document (14), however, these still contained parts of TL-DNA.

Sufficiency

– The invention was not concerned with identifying a match between any particular foreign gene and a suitable promoter, but rather with how to get these into the plant. The appropriate legal considerations were thus those stated in decision T 292/85 (EPO OJ 1989, 275) that at least one way had to be disclosed of carrying out the invention and this was the case.

– There was no reason to doubt that the vector system described in the application would serve to introduce into a dicotyledonous plant cell a chosen gene and promoter: certainly there was no evidence that this aspect might be difficult. The legal considerations set out in Decision T 694/92 (cf supra, paragraph IV) relied on by the examining division, were not applicable, as in contrast to the present case, there the contribution was not something generally applicable, but merely the successful practical implementation of what had already been postulated in theory, for which only a narrow claim to a specific combination of gene and promoter could be considered both enabled and inventive.

Support and clarity

– Feature (a), although expressed in negative terms, did not convey a negative teaching, since it resulted in the positive teaching concerning the structure that only the T-DNA borders were relevant. The word "***substantially***" was necessary because its deletion would result in an undue narrowing of the protection for the appellant as the precise isolation of the T-DNA borders was not critical. The "functional" interpretation of the examining division tying it to mere inactivation of oncogenes was inappropriate. Both the language of the claim and the description made clear that a structural interpretation was appropriate to the effect that substantially only the border regions and any desired promoter sequence remained.

– The feature "obtainable by Agrobacterium transformation" was consistent with and based on the description, and was introduced to meet the concern of the Board that otherwise the claim might cover plant cells transformed otherwise than using Agrobacterium. Plant cells transformed by the method of the invention would be characterized by part of the T-border region.

IX. The appellant requested that the decision under appeal be set aside and that a patent be granted on the basis of claims 1 to 4 submitted at the oral proceedings on 18 June 2002, amended pages 1, 8, 11, 12, 13, 15, 16, 19 to 21, 24, 26, 38, 41, 50 and 51 submitted at the oral proceedings on 18 June 2002, pages 2 to 7, 9, 10, 14, 17, 18, 22, 23, 25, 27 to 37, 39, 40, 42 to 49 as originally filed, the figures as originally filed.

Reasons for the Decision

Article 123(2) EPC

1. A basis for the introduction of "*dicotyledonous*" into claim 1 can be found on page 8, lines 13 to 15 of the application as filed.

2. The introduction of "...*except for promoter sequences...*" into claim 1 is based on Example 1, which describes pGV3850 containing the T-DNA nopaline synthase gene promoter and on Example 4, in which the chimeric gene containing either the octopine synthase structural gene or the sequence encoding dihydrofolate reductase are placed under the control of the nopaline

synthase gene promoter, which has its origin in the T-DNA of Ti-plasmid.

3. The phrase "obtainable by Agrobacterium" now introduced in claim 1, is based on the description as filed as a whole.

4. Otherwise claim 1 corresponds to the claim 1 considered by the Examining Division, which had no objections under Article 123(2) EPC. The Board sees no reason to raise any objections of its own under this article to claim 1 or the dependent claims. The request as a whole meets the requirements of Article 123(2) EPC.

Article 84 EPC

5. The expression "...*substantially free of internal T-DNA sequences...*" in claim 1 was objected to by the examining division and given by them a **functional** interpretation equating said expression to the absence of genes controlling the neoplastic growth.

6. The Board however agrees with the appellant that the language of the claim itself makes clear that a structural interpretation is appropriate to the effect that substantially only the border regions and any desired promoter sequence remain. The description is consistent with this structural interpretation.

7. Given that in this art the skilled person would isolate the T-DNA borders using restriction enzymes, which enzymes may not exactly cleave at the exact limit between the desired border and the T-DNA, some nucleotides belonging to internal T-DNA might remain

associated with the border regions, but without any deleterious effect. The use of "substantially" in the claim will be understood by the skilled person in this sense, and the Board is of the opinion that the use of "*substantially*" is thus justified in the present case, and that claim 1 of the main request thus meets the requirements of Article 84 EPC.

8. Regarding the interpretation by the Examination Divison of "substantially free internal T-DNA sequences of a wild-type Ti-plasmid" as meaning merely the absence of genes which control neoplastic growth, this appears to the Board inappropriate because it ignores the wording of the claim. The feature does indeed ensure the absence of genes which control neoplastic growth, but it also is a teaching of how to achieve this, and is a restriction on the scope of the claim.

9. Construing the above feature as merely the absence of genes which control neoplastic growth, would simultaneously make the scope of the claim broader by covering the case where other genes of the T-region remain, and the teaching more difficult to carry out as the skilled person would have to know which genes control neoplastic growth. The internal T-DNA genes included not only genes controlling the neoplastic growth, but also nopaline synthase and/or octopine synthase structural genes. At the priority date certainty did not exist as to the number or the function of all genes of the T-region.

10. Construing the feature "...*substantially free of internal T-DNA sequences...*" as structural makes a critical difference when it comes to considering sufficiency and inventive step.

Sufficiency (Article 83 EPC)

11. The restriction of the scope of claim 1 to "*dicotyledonous plant*" reflects the knowledge of the skilled person at the priority date of the present application, as judged by the disclosure of document (11), which states on page 542 that monocotyledonous plants lack *Agrobacterium* adherence sites and hence cannot be transformed with this organism. This avoids the Board having to consider the possible further objection already mentioned in the decision under appeal, namely that the application was not enabling for monocotyledonous plant cells.

12. Claim 1 is directed to a new product, namely a cell of a dicotyledonous plant containing stably integrated into its genome foreign desired DNA but substantially free of internal T-DNA sequences of a wild-type Ti-plasmid (except for promoter sequences) and thus free from T-DNA genes that control neoplastic growth. The foreign DNA includes a coding sequence for a desired product and a promoter sequence other than the natural promoter sequence of this coding sequence.

13. In the prior art it was desired to insert a foreign desired DNA into the cell of a dicotyledonous plant cell. On the documents on file, at the priority date the only practical way of achieving this was to use Ti-plasmids from Agrobacterium, by inserting the foreign

DNA into the T-region of the plasmid, and using Agrobacterium infection to integrate the T-region plus foreign DNA into the plant genome. This had the disadvantage that also the whole T-region, containing several genes causing tumour growth, was integrated into the plant genome not just the desired foreign DNA. The contribution to the art of the invention is based on the inventors having found out that the internal sequences of the wild type Ti-plasmid were not necessary to achieve integration, so that it would be possible to insert only the T-region and the desired DNA into the plant by Agrobacterium infection.

14. The application does not describe a single example of the whole invention put into practice, but it does give precise instructions on what to do to cut down the T-region. During the examination procedure the appellant filed evidence that the method has subsequently been successfully put into practice. There is no evidence before the Board that successful integration depends on the particular promoter/coding sequence, though of course this might affect the degree of expression obtained.

15. The Board here sees the contribution of the invention to the art as being the provision of cells in general containing a desired foreign DNA, but free from all the genes of the T-region of wild type Ti-plasmids and thus certainly free from any genes of the T-region which are deleterious.

16. The application itself suggests using the nos or ocs promoters from Ti-plasmids, known to work in dicotyledonous plants, for use with the foreign coding region. *Prima facie* there seems no reason to suppose that these would not work for any foreign coding region. How well the expression would function is a different matter, but the claim is not tied to achieving any particular level. Also other promoters known to work in the plant concerned would be obvious candidates. The Board does not consider the invention as being concerned with identifying a match between any particular foreign gene and a suitable promoter, or how well the gene is expressed in the plant cell. There may well be considerable scope for further research and possible invention in identifying optimal triple combinations of plant/desired foreign coding region/promoter, while still benefiting from the contribution of the invention now claimed, but the application cannot be expected to list all possibilities.

17. The restriction to a non-natural promoter is taken by the Board as being for the purpose of avoiding an Article 83 EPC objection, rather than a feature contributing to inventive step, as for a known complete gene including natural promoter and coding region, known to be expressed in something other than a plant, the natural promoter might well not work in a plant.

18. From the point of view of the legal principles to be applied, for assessing sufficiency under Article 83 EPC that stated in Decision T 292/85 (cf. supra, section VIII) that at least one way has to be disclosed of carrying the invention into effect seems most

appropriate. On the evidence in the case, the Board has no reason to doubt that for any dicotyledonous plant and desired foreign coding region, the skilled person should be able to select a suitable non-natural promoter and produce a modified plant cell.

19. The legal considerations set out in Decision T 694/92 (cf. supra, section IV) relied on by the examining division are applicable in a different type of situation, where something already suggested as a theoretical possibility was difficult to put into practice in each particular case, so that describing a solution for one particular case, gave no useful teaching for other cases. In such circumstances no enabling teaching would have been given for other cases, so that a claim broad enough to cover such other cases would not be enabled throughout its scope.

20. On the material before it the Board sees no basis for considering that the requirements of Article 83 are not met for the subject matter of the claims.

Novelty (Article 54 EPC)

21. No novelty objection was raised by the examining division in view of the cited prior art and the Board sees no reason to differ from this view.

Inventive step (*Article 56 EPC*)

22. The Board considers document (1), a research article published by a group of researchers including four of the inventors, to represent the closest prior art. It

is concerned with introducing foreign DNA into plant cells using Ti-plasmids, and is particularly concerned with the structural/functional requirements and the design of so-called "disarmed" Ti-plasmids (ie deleted to some extent in the T-DNA region so as not to be tumour inducing) for transferring and stably integrating foreign DNAs inserted in the T-DNA region into the plant genome.

23. The problem to be solved in view of this document is to obtain a dicotyledonous plant cell having a desired foreign DNA inserted into its genome with no foreign tumour inducing DNA.

This problem has been solved in the present application by the provision of a plant cell as defined in claim 1, ie devoid of genes controlling the neoplastic growth and substantially free of internal T-DNA sequences, which also provides a structurally simple solution.

24. The question to be answered in view of the assessment of inventive step over document (1) and others is whether the skilled person at the priority date would have derived the particular solution described in the present application in an obvious manner from the prior art.

25. In document (1) the most extensively deleted plasmid, pGV2217 has no longer the left border of the T-DNA, but still contains parts of the TR-DNA, the function(s) of which is(are) unknown, as demonstrated by document (28) on page 16 (right column, first sentence). Furthermore, the other plasmids mentioned in document (1) contain parts of the TL-DNA. Therefore, document (1) does not

itself lead the skilled person to the solution claimed in the present main request.

26. Document (2) on page 378 evokes the possible use of "disarmed" Ti-plasmids as vectors and considers that the extreme form of disarming consists in "*...the deletion of all the oncogenes leaving the signal sequences and the octopine or nopaline synthase gene intact*" and makes reference, in this context, to present document (14). However, the three plasmids of document (14) are far from being deleted of all the oncogenes, since pGV2201 has only antibiotic resistance genes inserted into the nopaline synthase gene, whereas pGV2208 has one of the T-DNA border deleted and pGV 2206 a substitution of *EcoRI* fragment 32 by the 5.8Md pGV1106 plasmid. Even if the reference to document (14) were assumed to be wrong, but should in fact be a reference to document (1), then documents (1) and (2) would still not lead to the solution of the present application, since, as demonstrated above (cf supra point 29), document (1) does not suggest the solution of the present application.

27. Document (11) expresses on page 538 the expectation that a foreign gene inserted between the "ends" (ie the borders) of the T-DNA should be transferred to the plant genome. However, document (11) could only with hindsight to be taken as suggesting that only the T-border regions are critical for transfer, since it states the proviso that no function essential for transfer and integration into the plant genome should be destroyed by this insertion. This suggests the possibility that essential functions (ie essential genes) for the transfer and the integration into the

plant genome could be destroyed by the insertion of the foreign gene and implies that **not all the genes of the internal T-DNA** should be deleted. Document (11) thus basically suggests that a research programme be initiated to find out what internal T-DNA is necessary and what not, and the solution now claimed cannot be derived from it, even when taken in combination with documents (1) and (2).

28. Some of the reasoning in the decision under appeal, given above under the heading "contribution to the art" in Point IV goes far to making out a case of obviousness based on documents (1) and (11) for the subject matter of a claim to any solution characterized by the absence of genes which control neoplastic growth. But claim 1 is not directed to any such solution in general, but is directed only to the more specific solution of being substantially free of all internal T-DNA sequences of wild-type Ti-Plasmid, and this solution is not rendered obvious by the documents on file.

29. The Board is thus of the opinion that the solution proposed in the present application cannot be deduced in an obvious manner from documents (1), (2) and (11) either considered alone or in combination and that the claims of the main request fulfil the requirements of Article 56 EPC.

Order

For these reasons it is decided that:

1. The decision is set aside.

2. The case is remitted to the first instance with the order to grant a patent on the basis requested by the appellant.

The Registrar: The Chairman:

M. Patin S. Perryman