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**Datasheet for the decision
of 13 July 2018**

Case Number: T 1985/12 - 3.3.08

Application Number: 07703522.8

Publication Number: 1984496

IPC: C12N15/10

Language of the proceedings: EN

Title of invention:

NON-DESTRUCTIVE PROCEDURE FOR THE ISOLATION OF DNA FROM PLANTS

Applicant:

Rijk Zwaan Zaadteelt en Zaadhandel B.V.

Headword:

Root border cells/RIJK ZWAAN

Relevant legal provisions:

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

Keyword:

Main request - amended claims admitted (yes)
Requirements of the EPC met (yes)

Decisions cited:

T 0254/86

Catchword:



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Case Number: T 1985/12 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 13 July 2018

Appellant: Rijk Zwaan Zaadteelt en Zaadhandel B.V.
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Decision under appeal: **Decision of the Examining Division of the
European Patent Office posted on 3 April 2012
refusing European patent application No.
07703522.8 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chairman B. Stolz
Members: M. R. Vega Laso
J. Geschwind

Summary of Facts and Submissions

- I. The appeal of the applicant (appellant) lies from a decision of an examining division posted on 3 April 2012, refusing the European patent application No. 07703522.8 with the title "Non-destructive procedure for the isolation of DNA from plants". The application had been filed under the Patent Cooperation Treaty and published as WO 2007/093448 (in the following "the application as filed").
- II. In the decision under appeal, the examining division found that the subject-matter of the claims then on file did not involve an inventive step in view of document (6) or document (7) as closest state of the art and the common general knowledge of the skilled person at the relevant date as apparent from document (2) and document (3).
- III. Together with the statement setting out the grounds of appeal, the appellant submitted six sets of claims as main request and 1st to 5th auxiliary requests, and requested oral proceedings if the board envisaged maintaining the decision of the examining division.
- IV. The appellant was summoned to oral proceedings. In a communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal attached to the summons, the board provided observations on procedural and substantive issues. In particular, the board raised issues concerning Articles 84 and 56 EPC in respect of the main request, and drew attention to various issues in respect of the 1st to 5th auxiliary requests.
- V. In response to the board's communication, the appellant replaced the claims according to the main request then

on file by a new set of claims, withdrew its auxiliary requests and submitted additional arguments in support of an inventive step.

VI. Oral proceedings were held on 13 July 2018. After the discussion of issues concerning Articles 123(2) and 84 EPC, the appellant filed a new set of claims that replaced the previous claims as its main request.

VII. Claim 1 according to the main (and sole) request reads as follows:

"1. Method for screening populations of plants for genetic variation for the selection of plants during plant breeding, comprising harvesting root border cells in medium that surrounds growing roots of seedlings, or roots emerging from germinating seeds, or adventitious roots of *in vitro* plants, wherein the medium is a fluid or a tissue culture medium, and analysing DNA isolated from the root border cells."

Dependent claims 2 to 5 are directed to various embodiments of the method of claim 1.

VIII. The following documents are referred to in this decision:

(2): L. A. Brigham *et al.*, *Methods in Cell Biology*, 1995, Vol. 49, Chapter 26, pages 377 to 387;

(3): L. A. Brigham *et al.*, *Plant Physiol.*, 1995, Vol. 109, pages 457 to 463;

(6): A. Kumar *et al.*, *Plant Molecular Biology Reporter*, September 2003, Vol. 21, pages 309a to 309d; and

(7): I. Brunner *et al.*, *Molecular Ecology*, 2001,
Vol. 10, pages 2079 to 2087.

IX. The submissions made by the appellant concerning issues relevant to this decision, were essentially as follows:

Article 123(2) EPC - added matter

The amendments introduced into the claims did not offend against Article 123(2) EPC.

Article 56 EPC - inventive step

The examining division erred in finding that the method of the invention lacks an inventive step over document (6) or (7) and the common general knowledge of a person skilled in the art. Document (6) related to the identification of plants for medicine and described a simple protocol for DNA isolation from dry roots, rather than from the medium surrounding them. The document provided no indication that a non-damaging, efficient manner of sampling plant material was desirable, nor an incentive to seek for a screening method. Documents (2) and (3) did not describe sampling medium containing root border cells. Thus, contrary to the finding in the decision under appeal, the claimed method was not obvious to a person skilled in the art in view of document (6) combined with document (2) or (3). The same applied starting from document (7) as the closest state of the art.

X. The appellant (applicant) requested that the decision under appeal be set aside and a patent be granted on the basis of the main request filed during the oral proceedings.

Reasons for the Decision

Admission of the main request into the proceedings

1. During the oral proceedings before the board, the appellant filed a new set of claims as its main (and sole) request. The amendments introduced into this set of claims are aimed at overcoming objections concerning Article 84 EPC raised by the board either in its communication or during the discussion at the oral proceedings. Since the amendments do not raise any issues which the board cannot reasonably be expected to deal with without adjournment of the oral proceedings (see Article 13(3) RPBA), the main request is admitted into the proceedings.

Article 123(2) EPC - added matter

2. The application as filed discloses a method for obtaining DNA from a plant by extracting the DNA from root border cells collected from a growing root (see claim 1 of the application as filed), and the use of such method for screening populations of plants for genetic variation for the selection of plants during plant breeding (see page 4, lines 20 to 23 and page 11, lines 27 and 28). The root border cells are harvested by removing the medium that surrounds the roots (see page 3, lines 13 to 17). The DNA can be obtained from root border cells from a root which is part of a germinating seed (see claim 7 of the application as filed and page 3, lines 26 to 28 of the description), from the root of a seedling (see claim 8 and page 3, lines 32 and 33) or from adventitious roots of *in vitro* plants (see claim 9 and page 4, lines 1 to 4). The medium surrounding the growing roots can be a fluid

(see page 3, lines 15 and 16) or a tissue culture medium (see page 4, line 2). The DNA isolated from the root border cells can be analysed using different nucleic acid analysis technologies (see page 4, lines 9 to 11 and Examples 1, 5, and 7 to 9). Hence, the subject-matter of claim 1 can be directly and unambiguously derived from the application as filed.

3. The additional features in claims 2 and 3 are derivable from, respectively, claims 11 and 12 of the application as filed. The feature of claim 4 is disclosed on page 4, lines 25 to 28. The various plants specified in claim 5 to which the claimed method can be applied, are disclosed in Examples 1 to 5 and 7 to 11 of the application as filed.
4. In view of the above, the subject-matter of amended claims 1 to 5 does not extend beyond the content of the application as filed. Thus, Article 123(2) EPC is complied with.

Article 84 EPC - clarity and support

5. The board is persuaded that claims 1 to 5 are clear and concise. Their subject-matter is supported by the description and the examples. Hence, the requirements of Article 84 EPC are met.

Article 83 EPC - sufficiency of disclosure

6. No objections concerning Article 83 EPC were raised in examination proceedings, and the decision under appeal does not include any findings in this respect. Thus, it must be assumed that the examining division considered the requirement of sufficiency of disclosure to be

fulfilled. The board sees no reasons for a different finding.

Article 54 EPC - novelty

7. The examining division acknowledged the novelty of the subject-matter of the claims then on file (section 3 of the decision under appeal). Having considered the documents cited in examination proceedings, the board has no reason to doubt that the subject-matter of the claims of the present main request is novel.

Article 56 EPC - inventive step

The invention

8. The claimed invention relates to a method for screening plant populations obtained during plant breeding in order to select desired genetic variants. According to the application, screening methods known in the art at the relevant date require that progeny resulting from a crossing or mutagenized seeds be grown to the stage of young plants which were sampled by taking leaf samples of each individual plant and preparing DNA therefrom for analysis. These methods are expensive, labour and time consuming because they require manual dissection of tissue samples or harvesting seeds from each individual plant of the population under investigation.
9. The method of the invention reduces the required effort and allows screening of large plant populations with a high efficiency. This is achieved by sampling the medium surrounding growing roots of seedlings, roots emerging from germinating seeds, or adventitious roots of *in vitro* plants. Root border cells contained in this medium, which are detached from the young growing roots

by gentle agitation of the container, are used for isolating DNA for analysis. The method is non-destructive and amenable to automation. Since border cells can be harvested when the roots are very young, the results of DNA analysis become available within a short time. Only seedlings, germinated seeds or *in vitro* plants selected after the DNA analysis need to be grown in a greenhouse, thus saving space and labour.

Document (6) as the closest state of the art

10. Document (6) describes a protocol for the isolation of high-molecular weight DNA from dry roots of the medicinal plant *Berberis lycium*. The authors of document (6) state that lack of purity for herbal raw materials is one of the major problems faced by herbal drug manufacturers. Hence, their stated goal was "*... to develop a technology that uses molecular tools to accurately identify root samples of Berberis lycium from market samples*" (see page 309b, first full paragraph). In a first step of the technique, DNA is isolated by buffer extraction from **ground dried roots**, followed by two precipitation steps, treatment with RNase and purification using low-melting-temperature agarose (see passage under the heading "Materials and Methods" on pages 309b and 309c). Although the DNA obtained from 4-year-old dry roots of *B. lycium* using this method was partially degraded, it was suitable for random amplification (RAPD) experiments and restriction enzyme digestion (see Figure 1 of document (6)). The DNA was said to be free of polysaccharides and secondary metabolites "*... which have been observed to interfere with DNA isolation procedure and inhibit the activity of a wide range of DNA-modifying enzymes, such as restriction enzymes, polymerases, and ligases*" (see

page 309c, first paragraph under the heading "Results and Discussion").

11. The method described in document (6) differs from the method of claim 1 not only in its purpose (assessment of quality and purity in document (6) vs. screening populations of plants for selection during plant breeding in claim 1), but also in the starting material for the isolation of DNA (dry roots vs. root border cells) and how this material is obtained (grinding dry roots vs. harvesting the medium that surrounds growing roots of seedlings, roots emerging from germinating seeds, or adventitious roots of *in vitro* plants). Moreover, while the technical effect achieved by the method described in document (6) is an increased degree of purity of the DNA preparation which allows random amplification (RAPD) experiments and restriction enzyme digestion to be performed, the isolation of DNA from root border cells contained in the medium surrounding growing roots as specified in claim 1 results in a much more efficient method as regards time, space and labour, and allows screening large plant populations obtained by plant breeding to select plants with particular genetic variations.

12. In view of the above, it is highly questionable whether in the present case document (6) qualifies as the "most promising springboard" for an objective assessment of inventive step (see decision T 254/86; OJ EPO 1989, 115), as the method described therein neither is directed to the same purpose nor relies on the same effect as the claimed invention. Already for this reason, the content of document (6) cannot lead a person skilled in the art in an obvious way to the claimed invention.

13. In the decision under appeal, the examining division defined the problem to be solved as the "*... provision of a method for molecular marker based selection of genetic variants in plant populations*" (see page 5, third paragraph of the decision). This definition is, however, tainted by hindsight. While it is stated in document (6) that, with the increasing use of DNA fingerprinting in plant research, "*DNA extraction from tissue needs to be simple, rapid, inexpensive, and effective when many samples are used, such as in population studies*" (see page 309b, lines 13 to 16), there is no indication whatsoever in document (6) that would prompt a person skilled in the technical field of quality control of herbal samples to seek a method for screening populations of plants for genetic variants during plant breeding. Hence, starting from document (6) the problem to be solved has to be defined more generally as the provision of a simple and efficient method of isolating DNA from plant roots which can be used in population studies.
14. It was acknowledged in the decision under appeal that this problem is solved by the claimed method. However, the examining division held that the solution proposed in the claims was obvious in view of the common general knowledge "*... that root border cells can be released and collected from young seedling[sic] simply by agitating the root in a water solution*" (see lines 6 to 8 of the last paragraph on page 5 of the decision). As evidence, the examining division referred to documents (2) and (3).
15. In the board's view, the examining division's finding is the result of an *ex post facto* analysis with the benefit of hindsight knowledge of the invention. First, it should be noted that, since document (3) is a

publication in a specialized scientific journal, its content cannot be regarded as part of the common general knowledge of a skilled person at the relevant date. Secondly, even though document (2) - a chapter of a basic methodology book which according to the jurisprudence of the Boards of Appeal is considered to be part of the common general knowledge - relates to root border cells as tools in plant cell studies, it contains no suggestion whatsoever that such cells may be used as a DNA source in a method for screening populations of plants for genetic variation during plant breeding. Hence, a person skilled in the art would not have objectively inferred from document (6) supplemented with the common general knowledge as apparent from document (2) a method as claimed, without knowledge of the present patent application and the invention that it concerns.

16. For these reasons, an inventive step over document (6) read by a skilled person in the light of the common general knowledge in the art, is acknowledged.

Document (7) as the closest state of the art

17. Document (7) describes a method for reliable and fast extraction of DNA from fine roots in order to identify fine roots of tree species from the Alps. Fine roots of trees are used as indicators to assess soil alterations, e.g. those owing to atmospheric inputs of acidifying substances. The DNA extraction method is said to overcome the problems associated with the previous extraction methods which limited the amount of successful PCR amplifications or required time and cost intensive DNA purification steps (see page 2085, paragraph bridging the left- and right-hand column). Fine roots were isolated from soil samples from a

certain area which had been sampled using a spade. To obtain the fine roots, the soil samples were sieved through a 1-cm mesh sieve, and the remains were washed out under running water. The fine roots were gently rinsed again, pooled according to morphological criteria and lyophilized (see page 2082, left-hand column, paragraph under the heading "Field Study", lines 11 to 26). DNA was extracted using four different protocols, one of which was modified to obtain higher quality DNA in terms of success of PCR amplification and reproducibility (see page 2082, left-hand column, paragraph under the heading "DNA extraction").

18. The method of document (7) differs from the claimed method not only in its purpose, but also in the starting material (fine roots) used for DNA isolation and how this material is obtained. Like the method of document (6), the technical effect achieved by the method of document (7) is a higher degree of purity of the DNA preparation that allows molecular techniques to be used for further analysis, in particular for the identification of fine tree roots. Hence, essentially the same reasons given in connection with document (6) (see paragraphs 10 to 13 above) apply *mutatis mutandis* to document (7).

19. In sum, in view of document (7) supplemented with the content of document (2) as common general knowledge, the claimed method involves an inventive step.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.
2. The case is remitted to the examining division with the order to grant a patent on the basis of claims 1 to 5 filed as main request during the oral proceedings and a description to be adapted thereto.

The Registrar:

The Chairman:



L. Malécot-Grob

B. Stolz

Decision electronically authenticated