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Datasheet for the decision of 14 March 2019

Case Number: T 0464/15 - 3.3.04

Application Number: 08075865.9

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IPC: C12N15/82

Language of the proceedings: EN

Title of invention:
Improved targeted DNA insertion in plants

Patent Proprietor:
Bayer CropScience NV

Opponent:
Zacco GmbH

Headword:
Targeted DNA insertion/BAYER CROPSCIENCE

Relevant legal provisions:
EPC Art. 54, 56, 104(1)
RPBA Art. 12(4)

Keyword:
Novelty - main and auxiliary request 2 (no)
Inventive step - auxiliary requests 1, 3 and 4 (no)

Decisions cited:

Catchword:

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Beschwerdekammern

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Case Number: T 0464/15 - 3.3.04

D E C I S I O N
of Technical Board of Appeal 3.3.04
of 14 March 2019

Appellant I: (Patent Proprietor)	BASF Agricultural Solutions Seed US LLC 100 Park Avenue Florham Park, NJ 07932 (US)
Representative:	Herzog, Fiesser & Partner Patentanwälte PartG mbB Dudenstrasse 46 68167 Mannheim (DE)
Appellant II: (Opponent)	Zacco GmbH Bayerstrasse 83 80335 München (DE)
Representative:	Prusko, Carsten Dietmar Zacco GmbH Bayerstraße 83 80335 München (DE)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 22 December 2014 concerning maintenance of the European Patent No. 2025756 in amended form**

Composition of the Board:

Chairwoman	G. Alt
Members:	A. Chakravarty
	M. Blasi

Summary of Facts and Submissions

I. Appeals were filed by both the patent proprietor (appellant I) and the opponent (appellant II) against the interlocutory decision of the opposition division that European patent No. 2 025 756 in an amended form, based on auxiliary request 1, met the requirements of the EPC. The patent is based on European patent application No. 08 075 865.9 with the title "*Improved targeted DNA insertion in plants*".

II. The patent was opposed under Article 100(a) EPC on the grounds of lack of novelty (Article 54 EPC), lack of an inventive step (Article 56 EPC) and under Article 100(b) EPC for lack of sufficient disclosure of the invention.

III. With the statement of grounds of appeal appellant I filed sets of claims of auxiliary requests 1 to 3. A set of claims of auxiliary request 4 was filed with the reply to appellant II's statement of grounds of appeal.

IV. The board issued a communication pursuant to Article 15(1) RPBA setting out its preliminary opinion on the appeals. In this communication the parties were informed that, in relation to the novelty of claim 1 of the main request with respect to the disclosure in document D17, the board was inclined to agree with the opposition division that *Cre* should be regarded as a "rare-cleaving double stranded DNA break inducing enzyme".

V. In response to the board's communication, appellant I filed a replacement auxiliary request 2.

VI. Oral proceedings before the board were held on 14 March 2019. During these proceedings, appellant I filed a set of claims of auxiliary request 2, replacing the previous auxiliary request 2. In respect of those documents whose admission to or exclusion from the proceedings had been requested, the parties relied only on documents D20 and D23 which the board admitted into the proceedings. At the end of the proceedings, the chair announced the decision of the board.

VII. Claim 1 of the main request (patent as granted) reads:

"1. A method for introducing a foreign DNA of interest into a pre-selected preselected site of a nuclear genome of a plant cell comprising the steps of

(a) inducing a double stranded DNA break at a preselected site in said nuclear genome by introduction into said plant cell of [a] plant-expressible gene encoding a rare-cleaving double stranded DNA break inducing enzyme recognizing said preselected site;

(b) introducing the foreign DNA of interest into the plant cell by direct DNA transfer".

Claim 12 of auxiliary request 1 reads:

"12. A method for introducing a foreign DNA of interest into a preselected site of a nuclear genome of a plant cell comprising the steps of

(a) inducing a double stranded DNA break at a preselected site in said nuclear genome by introduction

into said plant cell of plant-expressible gene encoding a rare-cleaving double stranded DNA break inducing enzyme recognizing said preselected site;

(b) introducing the foreign DNA of interest into the plant cell by direct DNA transfer
whereby said plant cell is incubated in a plant phenolic compound prior to step (a)".

Claim 1 of auxiliary request 2 differs from claim 1 of the main request in that in step (a) the enzyme is defined as a rare-cleaving double stranded DNA break inducing **endonuclease** recognising said pre-selected site (emphasis added by th board).

Claim 1 of auxiliary request 3 is identical to claim 12 of auxiliary request 1.

Claim 1 of auxiliary request 4 differs from claim 1 of auxiliary request 2 in that it includes the additional feature "whereby said plant cell is incubated in a plant phenolic compound prior to step (a)".

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

D2: WO 98/37212

D3: WO 03/080809

D17: WO 02/077246

D20: WO 03/004659

D23: Guo F. et al, 1997, Nature, 389, 40-46.

Documents D19, D21 and D22 are also mentioned but they played no role in the board's considerations, thus their bibliographic data need not be provided here.

IX. The arguments of appellant I, relevant to the decision, are summarised as follows:

Admission of documents D20 and D23

Document D20 should not be admitted into the proceedings because it was submitted only in the appeal proceedings whereas it should have been filed in the proceedings before the opposition division.

Document D23 should be admitted into proceedings in case the board admitted document D20. Document D23 was filed at the oral proceedings before the opposition division to clarify that the Cre-lox system did not fall under the term "rare-cleaving double stranded DNA break inducing enzyme".

Main request - claim 1

Novelty - Article 54 EPC

The decision of the opposition division that the subject-matter of claim 1 lacked novelty over the disclosure in document D17 should be set aside and the patent should be maintained as granted.

Claim 1 as granted used the term "rare-cleaving double stranded DNA break inducing enzyme". However, throughout the description of the patent, multiple terms such as "rare cutting endonuclease", "rare-cutting double stranded break inducing endonuclease", "a rare-cutting endonuclease", "double stranded DNA

break inducing endonuclease", "rare-cleaving endonucleases", "DSBI (double stranded break inducing) endonuclease" and "rare cleaving double stranded DNA break inducing endonuclease" were used interchangeably. Thus, it was clearly and unambiguously derivable from the application as filed as a whole that the term "rare-cleaving double stranded DNA break inducing enzyme" as used in claim 1 as granted was in fact directed to a rare-cleaving endonuclease enzyme and not to a recombinase.

The skilled person understood that an endonuclease cleaved phosphodiester bonds within a nucleic acid chain. By contrast, site-specific recombinases such as Cre guided recombination between two DNA sequences at a specific site.

Indeed, Cre was not capable of performing the claimed method. Claim 1 as granted required that the double stranded DNA break was induced as a consequence of the introduction of the plant-expressible gene encoding the DSBI enzyme. However, the mere introduction (and expression) of a plant-expressible gene encoding Cre did not result in the induction of a double-stranded DNA break at a pre-selected (Lox) site and certainly no double-stranded cleavage.

Accordingly, the subject-matter of claim 1 was novel over the disclosure in document D17.

Auxiliary request 1 - claim 12
Auxiliary request 3 - claim 1

Inventive step - Article 56 EPC

As set out in the argumentation on novelty for claim 1 of the main request, document D17 did not disclose the use of a rare-cleaving DSBI enzyme in the sense of this claim. Thus, the skilled person would not have arrived at the claimed subject-matter even by combining the disclosure in document D17 with that in document D2.

If, for the sake of argument, it were assumed that document D17 disclosed the use of a rare-cleaving DSBI enzyme, the claimed subject-matter was still not obvious because the claim required that the foreign DNA of interest is introduced into the plant cell by direct DNA transfer and not by *Agrobacterium*-mediated transformation. The claim comprised the feature that "the plant cell is incubated in a plant phenolic compound prior to step (a)" which had the technical effect of further increasing the frequency of targeted insertion events, as demonstrated in Example 4 of the patent.

The skilled person would not have combined the disclosure of document D17 with that of document D2, as these documents were from different technical fields. Document D17 was from the field of targeted integration of a DNA fragment into a plant nuclear genome, while document D2 related to a process for integrating a foreign DNA fragment into the genome of a monocotyledonous plant cell, i.e. it was from the field of random insertion of a DNA fragment into a plant nuclear genome.

There was no indication in document D2 that a pre-treatment with a plant phenolic compound would also be beneficial in the context of DSBI-mediated targeted insertion of a foreign DNA into a pre-selected site. Moreover, document D2 focused on *Agrobacterium-mediated* transformation. It disclosed that pre-incubation with a phenolic compound in direct DNA transfer methods improved the transformation efficiency only about three times. In contrast, in *Agrobacterium-mediated* transformation improvement was about seven to ten times higher than without the phenolic compound. This difference would have dissuaded the skilled person from using direct delivery methods in the method disclosed in document D17. Instead, document D2 would have prompted the skilled person to use *Agrobacterium*-mediated transformation in the method disclosed in document D17.

Thus, the subject-matter of claim 1 was not obvious by a combination of the disclosure in documents D17 and D2.

Auxiliary requests 2 and 4 - claim 1

Novelty - Article 54 EPC

The disclosure in document D3 did not anticipate the claimed subject-matter because it related to zinc-finger endonucleases which were not embodied by the definition of the enzyme referred to in the claim. The claim required that the site of recognition and the site of insertion were the same, i.e. they were both at the site at which the double-stranded break was made by the enzyme. This was true for the enzyme referred to in the claim, but not for zinc-finger endonucleases where these sites were not the same.

Secondly, document D3 was focused on the generation of genetically modified animals and the methods exemplified therein only concerned this. Although plants were mentioned in Example 8 and the corresponding claim 35, these were only prophetic, speculative examples. The skilled person would not have seriously considered them.

Auxiliary request 4 - claim 1

No additional arguments beyond those provided for claim 1 of auxiliary request 2 were provided in relation to auxiliary request 4.

Apportionment of costs - Article 104 EPC; Article 16 RPBA

In case the board admitted any of documents D19 to D22, filed by appellant II during the appeal proceedings, a different apportionment of costs under Article 104 EPC was requested.

X. The arguments of appellant II, relevant to the decision, are summarised as follows:

Admission of documents D20 and D23

Document D20 had been submitted as early as possible in the appeal proceedings and should be admitted.

Document D23 on the other hand should not be admitted into proceedings. It had correctly been excluded by the opposition division due to late filing and was not *prima facie* relevant to the case.

Main request - claim 1

The claimed method had the following features:

i. a method for introducing a foreign DNA of interest;

ii. the foreign DNA is introduced into a pre-selected site;

iii. the pre-selected site being in the nuclear genome of a plant cell;

iv. said pre-selected site is recognised and cleaved by a rare-cleaving double-stranded DNA break inducing enzyme, whereby a double-stranded DNA break is induced

v. the double-stranded DNA break inducing enzyme is encoded by a gene which has been introduced into said plant cell;

vi. the foreign DNA of interest is introduced into said plant cell by direct DNA transfer.

The "preselected site" was according to the definition provided in paragraph [0021] of the patent "*a particular nucleotide sequence in the plant nuclear genome at which location it is desired to insert the foreign DNA*". No further details are given as to the size, structure and nucleotide composition of the nucleotide sequence. This definition neither limited the "preselected site" to a specific site between two nucleotides within the genomic DNA of a plant cell, nor required that the site of DNA cleavage was located within the sequence recognised by the cleaving enzyme. A "preselected site" according to this definition could be a nucleotide sequence within the genomic DNA of a

plant cell encompassing both a stretch of nucleotides recognised by the cleaving enzyme and an adjacent stretch of nucleotides harbouring the site of DNA cleavage, as in the case of the "preselected sites" for zinc-finger endonucleases. Together, such stretches formed a nucleotide sequence which was both recognised and cleaved by the cleaving enzyme, and at which location within the plant nuclear genome a foreign DNA was inserted. The term "preselected site" included loxP sequences, containing specific binding sites for the site-specific DNA recombinase Cre, as well as the recognition sequences for other endonucleases such as I-Sce I.

The expression "rare-cleaving double stranded break inducing enzyme" was not defined in the patent. The expression therefore was not synonymous with "rare-cleaving endonuclease" and encompassed any enzyme which could recognise a pre-selected site in the nuclear genome of a plant cell and was capable of cleaving both strands of a DNA. This included the Cre protein or a zinc-finger endonuclease.

The patent itself identified the Cre protein as being a "*rare-cleaving double stranded DNA break inducing enzyme*" in paragraph [0021] where reference was made to Table 1 of document D20. In this table, Cre was listed as a double-stranded DNA break inducing enzyme.

Novelty - Article 54 EPC

Document D17 disclosed a method for introducing a foreign DNA of interest into the nuclear genome of a plant cell (see e.g. claim 2). The plant cell contained a target site for site-specific recombination in its nuclear genome, i.e. a pre-selected site (see claim 2).

The cell was transfected or transformed with DNA comprising a sequence of interest, the transfection or transformation being achieved by non-biological delivery, i.e. direct DNA transfer (see claim 2 in combination with claim 7). These delivery means included microprojectile bombardment, electroporation and polyethylene glycol (PEG)-mediated treatment of protoplasts (see page 21, second full paragraph).

An enzyme for recombination was provided (see claim 2) either from an additional vector or from a gene previously incorporated into said plant cell (see page 9, lines 22 to 24).

Cells containing the sequence of interest integrated at the target site were subsequently selected (see claim 2).

Document D17 further disclosed that the Cre-lox system could be used for carrying out the integration of the sequence of interest (see page 9, lines 22 to 24 in combination with page 20, beginning of last paragraph). The Cre protein was the enzyme for recombination while the lox site was the target site in the plant nuclear genome. Because the lox site was not naturally occurring in plant cells, it was a pre-selected site in the sense of the present claim 1.

As set out above, Cre was a "rare-cleaving double stranded DNA break inducing enzyme" within the meaning of claim 1. Thus, document D17 disclosed all the features of claim 1 of the main request and the subject-matter of that claim lacked therefore novelty.

Auxiliary request 1 - claim 12
Auxiliary request 3 - claim 1

Inventive step - Article 56 EPC

The claimed method was obvious in view of the disclosure in documents D17 and D2. Document D17 disclosed all the features of claim 1 except the incubation of the plant cell in a plant phenolic compound (e.g. acetosyringone) prior to inducing a double-stranded DNA break.

The opposition division formulated the problem to be solved as providing an alternative method for the introduction of a foreign DNA in a plant cell genome. This problem was accepted. The opposition division concluded that, although document D2 disclosed the use of plant phenolic compounds for increasing efficiency of transformation, this only applied to *Agrobacterium*-mediated transformation, and that the skilled person would not have made the link between the teaching of document D2 and document D17.

This was incorrect. Firstly, document D2 came from the same technical area as both the opposed patent and document D17, namely the integration of foreign DNA into a plant nuclear genome. Secondly, document D2 was not only concerned with *Agrobacterium*-mediated transformation, but also with the use of plant phenolic compounds such as acetosyringone in direct DNA transfer methods such as electroporation, direct gene transfer using polyethylene glycol or micro-projectile bombardment (see document D2, page 4, lines 6 to 23).

Example 4 of document D2 provided experimental evidence that improved transformation efficiency could be achieved in electroporation transformation methods. This was further supported by the claims, in particular by claim 1 in combination with claim 16.

Thus, the person skilled in the art seeking to solve the problem formulated by the opposition division, would have been motivated by the disclosure in document D2 to employ pre-treatment using a plant phenolic compound in a method for introducing a foreign DNA of interest into a pre-selected site of a nuclear genome of a plant cell as disclosed document D17 to arrive at a method as claimed.

Auxiliary request 2 - claim 1

Novelty - Article 54 EPC

Document D3 disclosed a method for generating a genetically modified plant according to the claim. The main question to be answered was whether or not a zinc-finger endonuclease fell within the expression "rare-cleaving double stranded break inducing endonuclease" in claim 1.

A zinc-finger endonuclease comprised a zinc-finger domain that bound to an endogenous chromosomal target nucleotide sequence within a target sequence and an endonuclease domain. Because the zinc-finger endonuclease specifically bound to the endogenous chromosomal target sequence and generated a double-stranded cut within said target sequence, it was a rare-cleaving double-stranded DNA break inducing endonuclease in accordance with the opposed patent.

Auxiliary request 4 - claim 1

Inventive step - Article 56 EPC

The claimed method was obvious to the person skilled in the art. Document D3 represented the closest prior art for this claim and had been found to anticipate the subject matter of claim 1 of auxiliary request 2. The claimed subject matter differed from that of claim 1 of auxiliary request 2 in that it included the requirement "whereby said plant cell is incubated in a plant phenolic compound prior to step (a)". The skilled person starting from the disclosure in document D3 and seeking to provide an improved transformation method would have combined the teaching in document D3 with that in document D2 for the reasons given for the subject-matter of claim 1 of auxiliary request 3.

Apportionment of costs - Article 104 EPC; Article 16 RPBA

There were no grounds to allow the different apportionment of costs under Article 104 EPC as requested by appellant I.

XI. Appellant I requested that the decision under appeal be set aside and that the patent be maintained as granted (main request), or alternatively, that the patent be maintained in amended form on the basis of the claims of auxiliary requests 1 to 4, the sets of claims of auxiliary requests 1 and 3 having been filed with the statement of grounds of appeal, the set of claims of auxiliary request 2 at the oral proceedings before the board and the set of claims of auxiliary request 4 with

letter dated 18 September 2015. Furthermore, a different apportionment of costs was requested.

XII. Appellant II requested that the decision under appeal be set aside, that the patent be revoked, and that the request for a different apportionment of costs be rejected.

Reasons for the Decision

1. The appeals comply with Articles 106 to 108 and Rule 99 EPC and are therefore admissible.

Admission of documents D20 and D23

2. Appellant I requested that document D20, filed with the appellant II's reply, not be admitted into the appeal proceedings. Appellant II requested the same regarding document D23, filed at the oral proceedings before the opposition division and resubmitted with the respective statement of grounds of appeal.

3. The board decided to admit the above mentioned documents into the appeal proceedings. Document D23 played no role in the board's considerations in reaching the decision, thus the reason for its admission need not be given here. Document D20 was cited in the description of the patent and then filed by appellant II in reply to the appeal of appellant I.

4. Given that document D20 was filed as an appropriate reaction to the issues raised in the appeal of appellant I and to further support the finding of the opposition division in the decision under appeal (see point 21.16), the board cannot conclude that the

document should have been filed in first instance proceedings and sees no justification to hold it inadmissible (Article 12(4) RPBA).

Main request - claim 1

Novelty - Article 54 EPC

5. In the decision under appeal, the opposition division held that document D17 disclosed a method falling within the ambit of claim 1 of the main request. The opposition division agreed with the opponent that document D17 disclosed "*a process of causing a targeted integration of DNA of interest into a plant cell nuclear genome wherein the cell contains a target site, [...comprising] transforming the cell with a non-biological delivery system (direct DNA transfer) having a region for recombination with the target site; providing enzymes for recombination and selecting the cells which have integrated in their genome the DNA of interest*" (see point 21.13 of the decision under appeal; see also document D17, claims 1 and 7 and page 9, lines 22 and 23 as well as page 20, final paragraph).

6. Appellant I's main line of argument against this finding was that the expression "rare-cleaving double stranded DNA break inducing enzyme" used in claim 1 would be understood by the skilled person as synonymous with "rare-cleaving double stranded DNA break inducing **endonuclease**" (emphasis added by the board). In appellant I's view, Cre was not an endonuclease but a recombinase.

7. On this topic, the opposition division stated "[a]*s the application lacks a definition of what is meant by that*

term [rare-cleaving double stranded DNA break inducing enzyme], *any enzyme capable of cleaving both strands of a DNA at a specific site, i.e. a preselected site, that is of an uncommon sequence of nucleotides in that specific genome i.e. a rare site, and that is able to facilitate a recombination that leads to the insertion of a foreign DNA at that site, falls within the term of claim 1. There is no indication in the patent that the cleaving has to be effected simultaneously in both DNA strands. As the lox sites do not belong to a plant genome, they fall within the definition of rare preselected sites. It follows that the Cre recombinase is a "rare-cleaving double stranded DNA break inducing enzyme" as in claim 1 of the granted patent*" (see decision under appeal, point 21.16).

8. The board has seen no arguments that would persuade it that the opposition division's conclusions were wrong. In particular, appellant I's arguments that the patent used the terms "*enzyme*" and "*endonuclease*" interchangeably and that this was evidence of their equivalence is not persuasive. The board agrees with appellant II that the terms "*enzyme*" and "*endonuclease*" have non-equivalent meanings in the art that the skilled person would have understood, with the latter being a sub-category of the former and therefore concurs with the opposition division that the skilled person would have understood that the enzyme Cre falls within the meaning of "*rare-cleaving double stranded DNA break inducing enzyme*" as used in the claim.

9. The board's conclusion is supported by the reference in in the description of the patent at paragraph [0020] which reads "*A list of rare cleaving DSB inducing enzymes and their respective recognition sites is provided in Table I of WO 03/004659 (pages 17 to 20)*

(incorporated herein by reference)". WO 03/004659 is document D20 in the present proceedings. Table 1 has the title "*Erkennungssequenzen und Herkunftsorganismus von DSBI Enyzmen*" [translation by the board: "Recognition sites and organism of origin of DSBI enzymes"] and the heading of the first column of that table is "*DSBI-Enzym*" ["DSBI-enzyme"]. The first entry in this column is Cre.

10. In view of the above, the board concludes that document D17 discloses a method falling within the ambit of the claim, wherein the "*rare-cleaving double stranded DNA break inducing enzyme*" is Cre. The claimed subject-matter is therefore not novel.

Auxiliary request 1 - claim 12
Auxiliary request 3 - claim 1

Inventive step - Article 56 EPC

Closest prior art, difference and its effect, objective technical problem

11. Document D17 discloses a method which anticipates the subject-matter of claim 1 of the main request (see point 10., above). The subject-matter of this claim differs from the subject-matter of claim 1 of the main request only in that it includes the additional step of incubating the plant cell "in a plant phenolic compound prior to step (a)". Both parties consider that document D17 can represent the closest prior art for the subject-matter of the claim (see appellant I's statement of grounds of appeal, sections 4.2 and 5.3 and appellant II's statement of grounds of appeal, section VI).

12. The technical effect of this difference is to improve the recombination rate/frequency obtained by the method (see Example 4 of the patent: "*Acetosyringone pre-incubation improves the frequency of recovery of targeted insertion events*").

13. In view of the above mentioned difference between the method disclosed in document D17 and the claimed method and of the technical effect provided by this difference, the problem to be solved can be formulated as the provision of an improved method for introducing foreign DNA of interest into a pre-selected site of a nuclear genome of a plant cell.

Obviousness

14. Document D2 relates to methods of transformation of plants (see title). It discloses a process of integrating a DNA fragment into the genome of a cell of a monocotyledonous plant which comprises the step of incubating plant cells in a medium containing a plant phenolic compound such as acetosyringone prior to contacting them with the DNA to be inserted (see claims 1 and 4). The transformation may be done by direct DNA transfer, for example electroporation or bombardment with DNA coated micro projectiles (see claim 16). Example 4 of this document is entitled "*Pre-treatment of type I callus from corn with acetosyringone, improves transformation frequency by electroporation*". Here it is reported that transformation frequencies were about three times higher when finely cut type I callus pieces were pretreated with acetosyringone than when they were not pretreated.

15. The board considers that the skilled person seeking to solve the above mentioned technical problem and starting from document D17 representing the closest prior art, would have considered document D2, since it belongs to the same technical field as the claimed invention, namely plant transformation. They would have learned from this document that the transformation efficiency (rate) of direct DNA transfer methods can be improved by pre-incubating the cells to be transformed with acetosyringone. Thus the skilled person seeking to solve the above mentioned technical problem could and would have combined the transformation method disclosed in document D17 with a pretreatment step as disclosed in document D2.

16. Appellant I presented three main lines of argument as to why the skilled person would not have considered that the claimed method was obvious in view of the disclosure in document D17 when considered in the light of the disclosure of document D2. The first line of argument was that document D2 came from a different technical field, concerning methods in which DNA was integrated at a random site in plant genome, as opposed to a predetermined site. The second line of argument was that the skilled person learned from the disclosure in document D2 that an improvement of transformation frequency could be obtained by incubation of the plant material to be transformed with plant phenolic compounds only in the case of *Agrobacterium-mediated* transformation (see document D2, page 10, line 25). The third line of argument was that the skilled person learned from document D2 that the improvement in transformation frequency to be gained by pretreatment with acetosyringone was small in electroporation methods compared to that seen in *Agrobacterium-mediated* transformation methods. The former methods showed an

increase of about 3 times, while the latter showed an increase of 7 to 10 times (compare Example 1 and Example 4).

17. The board is not persuaded by these arguments for the following reasons. The first line of argument is dealt with in point 15., above. In relation to the second line of argument, it is noted that document D2 clearly discloses that improvements in transformation efficiency can be achieved both in *Agrobacterium-mediated* and in direct transformation methods. The disclosure of the latter can be found for example in the claims (see claims 1 and 16) and in Example 4. Thus, the argument that the skilled person would consider that the teaching in document D2 was restricted to *Agrobacterium*-mediated transformation fails. In relation to the third line of argument, the board agrees with the view of appellant II that no reason has been given as to why the skilled person would have dismissed the results presented in Example 4 that "*Pretreatment of type I callus from corn with acetosyringone, improves transformation frequency by electroporation*" merely because the improvement obtained using electroporation was smaller than that seen for *Agrobacterium*-mediated transformation.

18. The subject-matter of claim 12 of auxiliary request 1 and claim 1 of auxiliary request 3 therefore lacks an inventive step and does not meet the requirements of Article 56 EPC.

Auxiliary request 2 - claim 1

Novelty - Article 54 EPC

19. Document D3 discloses "*a method of generating a genetically modified plant in which a desired nucleic acid has been introduced, comprising: obtaining a plant cell comprising an endogenous target DNA sequence into which it is desired to introduce said nucleic acid; generating a double-stranded cut within said endogenous target DNA sequence with a zinc finger endonuclease comprising a zinc finger domain that binds to an endogenous target nucleotide sequence within said target sequence and an endonuclease domain; introducing an exogenous nucleic acid comprising a sequence homologous to at least a portion of said endogenous target DNA into said plant cell under conditions which permit homologous recombination to occur between said exogenous nucleic acid and said endogenous target DNA; and generating a plant from said plant cell in which homologous recombination has occurred*" (see claim 35).

The description has a corresponding disclosure in Example 8. The description also discloses that "*introducing an exogenous nucleic acid*" can be done by "*using microparticle bombardment or electroporation techniques*" (see paragraph [0103]). Thus, document D3 discloses a method as claimed, but using a zinc-finger endonuclease.

The question to be answered is therefore whether or not such an enzyme can be considered to fall within the definition in claim 1 "rare-cleaving double stranded DNA break inducing endonuclease recognizing said preselected site" which can introduce "a foreign DNA of

interest into a preselected site of a nuclear genome of a plant cell".

20. Appellant I argued that a zinc-finger endonuclease could not be an embodiment of the enzyme referred to in the claim because the site of recognition and the site of insertion, i.e. the site at which the double-stranded break is made by the enzyme, are not the same, as required by the claim.

21. The board notes that paragraph [0020] of the patent provides guidance as to the kind of enzymes that are to be understood as included by the expression "rare-cleaving double stranded DNA break inducing endonuclease recognizing said preselected site". The relevant part of this paragraph reads: "*Furthermore, methods are available to design custom-tailored rare-cleaving endonucleases that recognize basically any target nucleotide sequence of choice. Such methods have been described e.g. in WO 03/080809, WO94/18313 or WO95/09233 and in Isalan et al., 2001, Nature Biotechnology 19, 656- 660; Liu et al. 1997, Proc. Natl. Acad Sci. USA 94, 5525-5530)*".

22. The board notes that WO 03/080809 is in fact document D3 in the present proceedings, which relates to zinc-finger endonucleases, see point 19., above. There can therefore be no doubt that the phrase "rare-cleaving double stranded break inducing endonuclease recognizing said preselected site" was, in the context of the patent, intended to include zinc-finger endonucleases.

23. Appellant I also noted that document D3 largely relates to the generation of genetically modified animals since the methods exemplified therein mainly concern genetic

modification of animals. Genetically modified plants were only mentioned in Example 8 and in the corresponding claim 35, but these were prophetic. Thus, the skilled person would not have seriously considered the methods relating to plants disclosed in document D3.

24. Appellant I did not advance the argument that the disclosure in document D3 as it relates to the genetic modification of plants could not be carried out by the person skilled in the art in the sense of Article 83 EPC. Moreover, the fact that Example 8 contains no experimental data is, by itself, not a reason for the skilled person to doubt and thus disregard its teaching. In summary, the board has seen no persuasive reason why the skilled person reading document D3 would disregard the above mentioned disclosure of the genetic transformation of plants.

25. In view of the above considerations, the subject-matter of claim 1 of auxiliary request 2 is found to lack novelty over the disclosure in document D3.

Auxiliary request 4 - claim 1

Inventive step - Article 56 EPC

26. The subject-matter of this claim differs from that of claim 1 of auxiliary request 3 (also claim 12 of the main request) in that the enzyme in step (a) is "a rare-cleaving double stranded DNA break inducing **endonuclease** recognizing said preselected site" (emphasis added by the board).

27. The subject-matter of this claim is a combination of that of claim 1 of auxiliary request 2 with that of

claim 1 of auxiliary request 3, i.e. it combines the feature that the enzyme is an endonuclease with the feature of "incubating the plant cell in a plant phenolic compound prior to step (a)". The closest prior art can be taken as being represented by document D3 (which anticipates the subject-matter of claim 1 of auxiliary request 2, see point 25 above).

28. The claimed subject-matter differs from that disclosed in document D3 only in the feature of "incubating the plant cell in a plant phenolic compound prior to step (a)". The reasons given in points 11. to 18. above as to why the subject-matter of claim 1 of auxiliary request 3 lacks an inventive step apply to the subject-matter of the present claim insofar as they concern the obviousness of "incubating the plant cell in a plant phenolic compound prior to step (a)".

29. The subject-matter of claim 1 of auxiliary request 4 thus lacks an inventive step because the skilled person starting from document D3 would have combined the disclosure in that document with that in document D2.

Apportionment of costs - Article 104 EPC; Article 16 RPBA

30. Appellant I requested that, if the board were to admit any of documents D19 to D22, filed by appellant II during the appeal proceedings, a different apportionment of costs be ordered. No further substantiation of this request was provided by appellant I.

31. Under Article 104(1) EPC, each party must, as a rule, meet the costs it has incurred, unless reasons of equity justify to order otherwise. Pursuant to Article 16(1) RPBA, the board may, subject to

Article 104(1) EPC, on request order a party to pay some or all of another party's costs which shall, without limiting the board's discretion, include those incurred *inter alia* due to an amendment to a party's case as filed pursuant to Article 12(1) RPBA or an abuse of procedure.

32. The board admitted document D20 into the appeal proceedings. However, it can see no persuasive reasons of equity to order a different apportionment of costs because the document was filed by appellant II with the statement of grounds of appeal and, hence, forms part of the party's case under Article 12(1) RPBA. Moreover, nothing on file indicates that there are circumstances on the basis of which the board would have to conclude that appellant II's actions were unfair, let alone represented an abuse of procedure. The request for a different apportionment of costs is therefore rejected.

Order

For these reasons it is decided that:

1. Appellant I's appeal is dismissed.

2. The decision under appeal is set aside.

3. The patent is revoked.

4. Appellant I's request for a different apportionment of costs is rejected.

The Registrar: The Chair:

S. Lichtenvort G. Alt

Decision electronically authenticated