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Datasheet for the decision of 18 January 2011

Case Number: T 0632/08 - 3.3.08

Application Number: 01913116.8

Publication Number: 1263976

IPC: C12N 15/82

Language of the proceedings: EN

Title of invention:
Expression of an antimicrobial peptide via the plastid genome to control phytopathogenic bacteria

Applicants:
Auburn University, et al

Opponent:
-

Headword:
Expression antimicrobial peptides in plastids/AUBURN

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

Relevant legal provisions (EPC 1973):
-

Keyword:
"Main request - inventive step (no)"
"Auxiliary request - added subject-matter (yes)"

Decisions cited:
G 0010/93

Catchword:
-

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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0632/08 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 18 January 2011

Appellants: Auburn University et al
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Auburn University, AL 36849 (US)

Representative: Predazzi, Valentina
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Decision under appeal: **Decision of the Examining Division of the European Patent Office posted 27 July 2007 refusing European patent application No. 01913116.8 pursuant to Article 97(1) EPC 1973.**

Composition of the Board:

Chairman: C. Heath
Members: P. Julià
M. R. Vega Laso

Summary of Facts and Submissions

I. European patent application no. 01 913 116.8, published as WO 01/64927 (hereinafter "*the application as filed*"), was refused by the examining division on the grounds that the requirements of Article 56 EPC were not fulfilled. The examining division considered that the set of claims filed with letter dated 30 September 2005 and received by fax on 3 October 2005 did not fulfil the requirements of Article 56 EPC.

II. The applicants (appellants) lodged an appeal against the decision of the examining division. Together with the statement setting out their grounds of appeal, the appellants filed two sets of claims as, respectively, main request and auxiliary request, the former being identical to the set of claims underlying the decision under appeal. As a subsidiary request, oral proceedings were requested.

III. The appellants were summoned to oral proceedings. In a communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA) attached to the summons, the appellants were informed on the board's preliminary, non-binding opinion on some of the issues to be discussed at the upcoming oral proceedings, in particular regarding Articles 84, 83 and 56 EPC for both the main request and the auxiliary request, and Article 123(2) EPC for the auxiliary request. In view of this opinion, the board was inclined to dismiss the appeal.

IV. No substantive reply was received from the appellants which, however, informed the board of their intention not to attend the oral proceedings.

V. Oral proceedings were held on 18 January 2011 in the absence of the appellants. At the end of the proceedings, the board announced the decision that the appeal was dismissed.

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

D1: WO 99/10513, published on 4 March 1999;

D3: WO 99/06564, published on 11 February 1999;

D4: N.P. Everett, "Design of Antifungal Peptides for Agricultural Applications", Chapter 20 in "ACS Symposium Series. Natural and Engineered Pest Management Agents." Ed. P.A. Hedin et al., 1994, pages 278 to 291.

VII. As stated in point II *supra*, the appellants' main request was identical to the set of 18 claims underlying the decision under appeal. Claim 1 read as follows:

"1. A stable plastid transformation and expression vector which comprises an expression cassette comprising, as operably linked components in the 5' to the 3' direction of translation, a promoter operative in said plastid, a selectable marker sequence, a heterologous DNA sequence coding for one or more cytotoxic antimicrobial peptides selected from the

groups of defensins, PGLA (frog skin), cecropins, apidaecins, melittin, bombinin and magainin, transcription termination functional in said plastid, and flanking each side of the expression cassette, flanking DNA sequences which are homologous to a DNA sequence of the target plastid genome, whereby stable integration of the heterologous coding sequence into the plastid genome of the target plant is facilitated through homologous recombination of the flanking sequence with the homologous sequences in the target plastid genome."

Claims 2 to 8 were embodiments of claim 1. In particular, claims 4 and 5 defined the antimicrobial peptide (AMP) as being, respectively, magainin I or II or an analogue thereof. Claim 6 defined the analogue as MSI-99. Claim 8 further characterized the vector of any of claims 1 to 7 as a universal vector, wherein the flanking DNA sequences were homologous to a spacer sequence of the target plastid genome and the sequence was conserved in the plastid genome of different plant species. Claims 9 to 16 were directed to stably transformed plants which comprised a plastid stably transformed with the vector of any of claims 1 to 8 and the progeny or subsequent generations thereof, including seeds. Claims 17 and 18 related to a method for stably transforming a target plant to control a phytopathogenic bacteria which comprised transforming plastids of said target plant with a vector according to any of claims 1 to 8, selecting transformed plant cells and allowing the transformed plant cells to grow.

VIII. Claim 1 of appellants' auxiliary request read as claim 1 of the main request (*supra*) except for the

addition at the end of the claim of the following feature:

"1. ... and whereby expression for said cytotoxic antimicrobial peptides is at least 21% of the total soluble proteins."

IX. In the decision under appeal, the examining division considered the claimed subject-matter not to be inventive over a combination of documents D3 and D1 with further reference to *inter alia* document D4.

The examining division stated that document D3, the closest prior art, disclosed the expression of genes encoding antimicrobial peptides (AMPs) in the nucleus of plants and also suggested their expression in plastids of plants. The technical problem to be solved was the provision of an alternative system for the production of AMPs in plants. The solution was the production of a selection of AMPs, singly or in combination, in plastids of plants. This solution was obvious and straightforward in the light of the prior art. Document D1 disclosed vectors for the integration of foreign genes into the genome of plastids, and Example 1 suggested the integration of AMP genes into the genome of plastids. Although the application was the first to teach the stable transformation of plant plastids with AMP genes, the skilled person wishing to act on the obvious suggestion in the prior art to produce AMPs in plastids merely had to clone the AMP genes into the (conventional) universal integration vector of document D1 and would arrive at the vectors of claim 1. The more so since the claims did not require any particular level of expression for the AMP

genes or AMPs production and, therefore, the alleged surprising achievement of a high expression of AMP genes in plastids could not be regarded as a special technical feature making a contribution over the prior art. There was no evidence in the prior art, including document D4, to support the allegation of an existing prejudice against the expression of AMP genes into plant plastids based on the lytic properties of the AMPs and their toxicity to plant plastids.

X. The submissions in writing by the appellants, as far as they are relevant to this decision, may be summarised as follows:

Document D3, the closest prior art, was concerned with the expression of AMP genes in plants. The application differed from that prior art in that a high production of AMPs was obtained in plant plastids. The objective technical problem to be solved was the provision of an alternative expression system for AMP genes in plants. The solution was a direct plastid transformation which then needed the exportation of the produced AMPs from said cellular compartment to the cytosol where phytopathogens colonized, to confer disease resistance.

Although document D3 mentioned that two or more expressed AMPs were preferably compartmentalized, it did not suggest direct plastid transformation or exportation of the produced foreign proteins from one cellular compartment to another. Document D3 only exemplified AMP gene constructions for cytosolic and for extracellular localization (Examples 2 and 3). There was no disclosure nor an indication of the means

required to achieve a plastid (such as chloroplast) transformation vector to confer disease resistance.

Document D1 disclosed a stable plastid transformation and expression vector competent for the transformation and stable integration into the plastid genome as well as the use of that vector for producing protein-based polymers. However, this vector did not comprise a DNA heterologous sequence encoding one or more AMPs selected from the group of AMPs cited in the claims. Although there was a reference to the production of a factor conferring pathogen resistance such as an AMPs (lytic peptides or chitinase), this reference was found among a long, non-exhaustive list of unrelated proteins, it was not exemplified and its feasibility was not demonstrated. Moreover, since the prior art considered the production of AMPs to be highly toxic for plastids of plants, the skilled person had no reasonable expectation of success.

Thus, there was no motivation to combine the teachings of document D3 with those of document D1. AMPs having a high antibacterial activity were considered in the prior art to have a large potential for toxic activity against plant chloroplasts because of the charge on the chloroplast membranes. The antimicrobial activity of these lytic peptides was known to be associated with the disruption of the membrane of the microorganism. Moreover, no signal protein sequences to export proteins from chloroplasts were known in the prior art, which was why there were no reports of directly engineering the chloroplast genome for disease resistance. Still further, short peptides were known to

be highly susceptible to proteolytic degradation in chloroplasts.

Although several foreign genes had been expressed within plastids, such as for introducing herbicide or insecticide resistance, all these genes encoded proteins that functioned within plastids so that when the engineered plastids were consumed by target insects, insecticidal proteins were released inside the insect gut. The use of the plastid compartment to engineer disease resistance required exportation of the foreign proteins from the plastids to the cytosol where phytopathogens colonize. However, neither document D3 nor document D1 taught or suggested engineering of the plastid genome to export the foreign protein to the cytosol to confer disease resistance. Rather, the plastids of the present invention lysed at the site of infection and released AMPs. These AMPs were protected from proteases in the chloroplast and contained so that they were not toxic to chloroplast membranes until lyse at the site of infection. The lysis of the plastids was previously unknown.

XI. The appellants requested that the decision under appeal be set aside and that a patent be granted on the basis of the main request or the auxiliary request, both filed with the grounds of appeal.

Reasons for the Decision

Procedural issues

1. In the communication annexed to the summons to the oral proceedings (cf. point III *supra*) and with reference to decision G 10/93 (OJ EPO, 1995, page 172) ruling that in *ex parte* proceedings the board has the power to examine the application or the invention to which it relates for requirements of the EPC which the examining division did not take into consideration, the board referred *inter alia* to some of the objections for lack of clarity raised in the International Preliminary Examination Report (IPER). Moreover, in view of the arguments submitted by the appellants in support of inventive step, the board also pointed out some issues concerning Article 83 EPC.

2. However, in view of the fact that the decision under appeal dealt exclusively with the issue of inventive step (Article 56 EPC) and the adverse conclusion reached by the board on that issue (*infra*), the board does not deem it necessary to discuss in this decision issues under Articles 84 and 83 EPC with respect to the main request and to the auxiliary request.

Main request
Article 56 EPC

3. Document D3, which is concerned with the expression of AMP genes in plants, fulfils the requirements defined in the case law of the Boards of Appeal for the closest prior art, namely it discloses a method conceived for the same purpose or aiming at the same objective as the

claimed invention and having the most relevant technical features in common (cf. "Case Law of the Boards of Appeal of the EPO", 6th edition 2010, I.D.3.1, page 163). Document D3 explicitly discloses the expression of AMP genes in plant plastids as well as the combined expression of two AMP genes in plastids and in a different cellular compartment, such as cytosol (cf. page 5, lines 3 to 10 and page 9, lines 9 to 13). Document D3 mentions as preferred AMPs the PGL and the magainin classes of peptides, including among others the MSI-99 analogue (cf. *inter alia* page 4, lines 2 to 4 and page 6, lines 5 to 26). However, as rightly argued by the appellants (cf. point X *supra*), there is no actual example in document D3 showing any of these particular embodiments. The teachings of document D3 are only exemplified by using plasmids for cytosolic and/or for extracellular localization of AMPs.

4. Starting from this prior art, the objective technical problem may be formulated as putting into practice the teachings of document D3 concerning the expression of AMP genes in plastids. The invention as claimed appears to solve this technical problem.

5. However, in the board's judgement this solution does not involve an inventive step. The board is convinced that, in view of the indications given in document D3, a skilled person wishing to put into practice the teachings of this document with regard to the expression of AMPs in plant plastids would certainly be drawn to methods for carrying out plastid transformation and in particular to the advantageous universal (chloroplast) vector of document D1.

6. Document D1 discloses "*a universal (chloroplast) vector*" having all the features specified in the claims of the main request, in particular those cited in claim 8 (cf. *inter alia* page 10, line 4 to page 11, line 8 and page 21, lines 10 to 29), and it explicitly mentions the advantages of chloroplast transformation over nuclear transformation, such as higher levels of expression and genetic containment (cf. *inter alia*, page 2, line 8 to page 3, line 29 and page 16, lines 6 to 14). Document D1 also contemplates the use of the disclosed universal vector for chloroplast transformation and expression of genes conferring resistance to "*pathogen resistance, such as antimicrobial (lytic peptides, chitinase)*" (cf. page 41, lines 14 to 24). The availability of methods for carrying out plastid transformation is also acknowledged by the references found in the application as filed (cf. paragraph bridging pages 3 and 4).

7. Thus, in the board's opinion, the skilled person would certainly have combined the teachings of document D3 with those of document D1, rendering thereby the claimed subject-matter obvious. Moreover, in the light of the prior art on file, the board cannot share the appellants' view that the skilled person had no reasonable expectation of success.

8. In the statement of grounds of appeal, the appellants take the view that an inventive step is to be acknowledged because documents D3 and D1 do not exemplify the production of AMPs in transformed plastids (chloroplasts) nor do they demonstrate its feasibility. The appellants mainly rely on two lines of argument. First, the skilled person considered the

expression of AMP genes to be highly toxic for plant plastids. Second, for AMPs to confer disease resistance, they must be exported from the plastid to the cytosol where phytopathogens colonize. In the appellants' view, none of the cited prior art suggested that exportation, nor did they mention the lysis of plastids and the release of AMPs. Thus, if the skilled person had contemplated carrying out the teachings of the prior art, he/she had no reasonable expectation of success (cf. point X *supra*).

9. The board does not, however, share the appellants' view for the following reasons:

10. As to the appellants' first argument, it is observed that the authors of document D3 did not expect any problem when AMP genes are expressed in plastids. Moreover, the evidence submitted by the appellants in support of the alleged technical prejudice is insufficient and does not fulfil the criteria defined in the case law (cf. "Case Law", *supra*, I.D.9.2, page 214). It is true that the application as filed (cf. page 8, lines 7 to 10) describes AMPs as having a high potential for toxic activity against the plastid with reference to "*the prior knowledge in the art*", but refers only to a single scientific publication, namely document D4 (cf. point VI *supra*).

10.1 The major goal of the study described in document D4 is the design of AMPs that are stabilized against protease degradation, retain potent activity and exhibit minimal phytotoxicity (cf. page 280, second paragraph). The class of peptides selected for the initial studies reported in that document are the antimicrobial frog

skin peptide class known as magainins. In view of the results of these studies, it is concluded in document D4 that the association between antibacterial activity and potential phytotoxicity "*does not represent an absolute correlation*" and "*(f)ive peptides with significant antibacterial activity (>50) showed modest potential pyhtotoxicity (<50%)*" (cf. page 282, first full paragraph).

10.2 In view of these statements, the board is not persuaded by appellants' first argument. The less so in view of the fact that there is no particular quantitative requirement (level of expression and/or activity, etc.) in any of the claims of the main request.

11. As to the appellants' second argument, the board observes that in document D3 itself, when referring to the expression of several AMP genes in different cellular compartments (explicitly including plastids), it is stated that "*if during pathogen attack, the cell membrane**s** lose their integrity, the compartmentalized peptides are then free to interact (synergistically) and kill the invading pathogen*" (bold added by the board) (cf. page 5, lines 3 to 7). Although it may be arguable whether the reference to cell membranes may include that of plastids, it certainly shows that no particular (export) mechanism was expected to be required when using plastids as an alternative cellular compartment for the production of AMPs. Indeed, the early plant hypersensitive response (HR) to a pathogen attack, which leads to cellular death and rupture of cellular (nuclear, organelle and cytoplasmic) membranes, was already well-known in the art at the priority date of the application, and it could thus be well expected

by the skilled person to occur (cf. page 2, lines 18 to 28 of the application as filed and bibliographic references cited therein).

12. In view of the above considerations, the board does not see any reason to deviate from the adverse finding of the examining division with regard to Article 56 EPC. Therefore, the claims of the main request cannot form a basis for the grant of a patent.

Auxiliary request
Article 123(2) EPC

13. Claim 1 of the auxiliary request differs from claim 1 of the main request in that the feature "*whereby expression for said cytotoxic antimicrobial peptide is at least 21% of the total soluble proteins*" has been introduced (cf. point VIII *supra*). The application as filed refers to transgenic plants in which 21% of the total soluble protein corresponds to the expressed AMP (cf. page 9, lines 4 to 6 and page 11, lines 3 to 5). However, this result has been obtained with the exemplified production of the specific magainin II analogue MSI-99 in chloroplasts. There is nothing in the application which allows the generalization of this specific result to each and every group of antimicrobial peptides specified in claim 1, let alone to each and every possible member thereof, or to each and every possible type of plant plastid, let alone to each possible combination thereof.

14. Therefore, the subject-matter of the auxiliary request is considered not to fulfil the requirements of Article 123(2) EPC.

Order

For these reasons it is decided that:

The appeal is dismissed

The Registrar: The Chairman:

C. Eickhoff C. Heath