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Bezeichnung der Erfindung:
Regeneration von fertilen Gramineen-Pflanzen aus der Unterfamilie Pooideae ausgehend von Protoplasten

Patentinhaber:
Syngenta Participations AG

Einsprechender:
Monsanto Company
Bayer BioScience N.V.

Stichwort:
Regeneration von Graminee-Pflanzen/SYNGENTA PARTICIPATIONS AG

Relevante Rechtsnormen:
EPÜ Art. 123(2)
EPÜ R. 67

Schlagwort:
"Hauptantrag und erster bis fünfter Hilfsantrag: nicht ursprünglich offenbart (ja)"

Zitierte Entscheidungen:
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Orientierungssatz:
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E N T S C H E I D U N G
der Technischen Beschwerdekammer 3.3.04
vom 16. September 2007

Beschwerdeführer: (Patentinhaber)
Syngenta Participations AG
Schwarzwaldallee 215
CH-4058 Basel (CH)

Vertreter:
Fisher, Adrian John
CARPMAELS & RANSFORD
43-45 Bloomsbury Square
London WC1A 2RA (GB)

Beschwerdegegner: (Einsprechender 01)
Monsanto Company
P.O. Box 7020
St.Louis, Missouri 63166-7020 (US)

Vertreter:
UEXKÜLL & STOLBERG
Patentanwälte
Beselerstrasse 4
D-22607 Hamburg (DE)

(Einsprechender 02)
Bayer BioScience N.V.
Technologiepark 38
B-9052 Gent (BE)

Vertreter:
Almond-Martin, Carol
ERNEST GUTMANN - YVES PLASSERAUD S.A.S.
88, Boulevard des Belges
F-69452 Lyon Cedex 06 (FR)

Angefochtene Entscheidung: **Entscheidung der Einspruchsabteilung des Europäischen Patentamts, die am 12. Oktober 2004 zur Post gegeben wurde und mit der das europäische Patent Nr. 0332581 aufgrund des Artikels 102 (1) EPÜ widerrufen worden ist.**

Zusammensetzung der Kammer:

Vorsitzender: U. Kinkeldey
Mitglieder: R. Gramaglia
D. S. Rogers

Summary of Facts and Submissions

I. European Patent No. 0 332 581 (application No. 89 810 162.1) was filed in German language and was granted on the basis of 33 claims for the Contracting States AT, BE, CH, DE, FR, GB, IT, LI, LU, NL and SE and 24 claims for the Contracting State ES.

II. Notices of opposition were filed by opponents O1 and O2 requesting the revocation of the European patent on the grounds of Article 100(a), (b) and (c) EPC. During the opposition proceedings the opposition division with agreement of all the parties changed the language of the proceedings from German to English. The opposition division revoked the patent for non-compliance with the requirements of Article 123(2) EPC of the claims of the main request and of the claims of the first and second auxiliary requests then on file.

III. The appellant (patentee) filed an appeal against the decision of the opposition division. The Statement of Grounds of Appeal included a main request and a first to fifth auxiliary requests.

IV. Claims 1, 6, 10, 15, 19, 21, 22 and 25 of the main request for all designated Contracting States except ES read as follows:

"1. Eine embryogene Zellkultur, die sich von Gramineen-Pflanzen aus der Unterfamilie Pooideae ableitet und von der Protoplasten isoliert werden können, die Zellwände regenerieren, sich teilen und schliesslich einen Kallus ausbilden, der zu ganzen Pflanzen regeneriert werden kann, dadurch erhältlich, dass man

(a) Gewebe aus geeigneten Teilen von Pflanzen aus der Unterfamilie Pooideae isoliert;
(b) dieses Gewebe in einem Medium kultiviert, das in der Lage ist, die Ausbildung eines embryogenen Kallus oder von Embryonen zu induzieren;
(c) von besagtem Kallus und von besagten Embryonen periodisch Folgekulturen in einer Suspensionskultur anlegt, indem man kleine Zellkluster mit cytoplasmareichen Zellen in frisches Medium überträgt, das in der Lage ist, eine kontinuierliche Proliferation in Gang zu halten; und
(d) nach 4 bis 20 Übertragungen (Transfers) embryogene Zeilkluster mit einer Grösse von 150µm bis 2000µm isoliert."

"6. Verfahren zur Herstellung von Protoplasten, die zu ganzen Pflanzen regeneriert werden können, ausgehend von Gramineen-Pflanzen der Unterfamilie Pooideae, dadurch gekennzeichnet, dass man
(a) Gewebe aus geeigneten Teilen von Pflanzen aus der Unterfamilie Pooideae isoliert;
(b) dieses Gewebe in einem Medium kultiviert, das in der Lage ist, die Ausbildung eines embryogenen Kallus oder von Embryonen zu induzieren;
(c) von besagtem embryogenen Kallus und von besagten Embryonen periodisch Folgekulturen in einer Suspensionskultur anlegt, indem man kleine Zellkluster mit cytoplasmareichen Zellen in frisches Medium überträgt, das in der Lage ist, eine kontinuierliche Proliferation in Gang zu halten;
(d) nach 4 bis 20 Übertragungen (Transfers) embryogene Zellkluster mit einer Grosse von 150µm bis 2000µm isoliert; und

(e) mit Hilfe geeigneter Enzyme die Zellwände entfernt und die resultierenden Protoplasten isoliert."

"10. Verfahren zur Herstellung einer Zellkultur, die zu ganzen Pflanzen regeneriert werden kann, ausgehend von Gramineen-Pflanzen der Unterfamilie Pooideae, dadurch gekennzeichnet, dass man
(a) Gewebe aus geeigneten Teilen von Pflanzen aus der Unterfamilie Pooideae isoliert;
(b) dieses Gewebe in einem Medium kultiviert, das in der Lage ist, die Ausbildung eines embryogenen Kallus oder von Embryonen zu induzieren;
(c) von besagtem embryogenen Kallus und von besagten Embryonen periodisch Folgekulturen in einer Suspensionskultur anlegt, indem man kleine Zellkluster mit cytoplasmareichen Zellen in frisches Medium überträgt, das in der Lage ist, eine kontinuierliche Proliferation in Gang zu halten;
(d) nach 4 bis 20 Übertragungen (Transfers) embryogene Zellkluster mit einer Grösse von 150µm bis 2000µm isoliert;
(e) mit Hilfe geeigneter Enzyme die Zellwände entfernt und die resultierenden Protoplasten isoliert;
(f) Protoplasten gemäss Verfahrensschritt (e) bis zur Ausbildung von Zellkolonien in einem geeigneten Kulturmedium kultiviert;
(g) besagte Zellkolonien oder Teile davon auf einem geeigneten Medium, das die Bildung von Zellkulturen fördert, kultiviert; und
(h) die resultierenden Zellkulturen isoliert."

"15. Verfahren gemäss Anspruch 10, dadurch gekennzeichnet, dass besagte Protoplasten oder Zellen einen vorher ausgewählten Abschnitt exogener DNA stabil

in ihr Genom eingebaut enthalten, die nicht auf natürlichem Weg in das Genom integriert werden kann."

"19. Verfahren gemäss einem der Ansprüche 17 oder 18, dadurch gekennzeichnet, dass besagte Pflanzenzellen, die den Kallus aufbauen, einen vorher ausgewählten Abschnitt exogener DNA stabil in ihr Genom eingebaut enthalten, die nicht auf natürlichem Weg in das Genom integriert werden kann."

"21. Verfahren zur Herstellung transgener Gramineen-Pflanzen aus der Unterfamilie Pooideae, dadurch gekennzeiclmet, dass man
(a) Protoplasten gemäss Anspruch 6 mit Hilfe bekannter Transformationsverfahren mit einem vorher ausgewählten Abschnitt exogener DNA transformiert, die nicht auf natürlichem Weg in das Genom integriert werden kann;
(b) die transformierten Protoplasten gemäss Anspruch 10 kultiviert; und
(c) ganze Pflanzen gemäss einem der Anspruche 17 oder 18 regeneriert."

"22. Verfahren gemäss Anspruch 21, dadurch gekennzeichnet, dass es sich bei besagter exogener DNA um ein chimäres Gen oder um mehrere chimäre Gene handelt, die den transformierten Protoplasten sowie den sich daraus entwickelnden Geweben und insbesondere den Pflanzen vorteilhafte Eigenschaften verleihen."

"25. Gramineen-Pflanzen aus der Unterfamilie Pooideae sowie deren Vermehrungsgut, dadurch gekennzeichnet, dass es sich bei besagter Pflanze um eine fertile

Pflanze handelt, welche ausgehend von einer embryogenen Zellkultur gemäss einem der Anspruche 1 bis 5 durch Regeneration erhältlich ist und dass besagte Planzen und deren Vermehrungsgut einen vorher ausgewählten, stabil ins pflanzliche Genom integrierten Abschnitt exogener DNA enthält, die in Pflanzen oder pflanzlichen Zellen exprimierbar ist und nicht auf natürlichem Weg in das Genom integriert werden kann."

The claims of the first auxiliary request differed from those of the main request in that the expressions "DNA ... die nicht auf natürlichem Weg in das Genom integriert werden kann" and "einen vorher ausgewählten Abschnitt" have been deleted from claims 15, 19, 21 and 25.

The claims of the second auxiliary request further differed from those of the first auxiliary request in that the feature of claim 22 ("chimäres Gen oder mehrere chimäre Gene") had been incorporated into claim 21, and the same feature had been incorporated into former claim 25 (now claim 24).

The claims of the third auxiliary request differed from those of the main request in that the wording "von dem Suspensionskulturmedium" had been inserted before "isoliert" in step (d) of claims 1, 6 and 10.

The claims of the fourth auxiliary request combined the amendments of the first and third auxiliary requests

The claims of the fifth auxiliary request combined the amendments of the second and third auxiliary requests.

The claims of the main and first to fifth auxiliary requests for the Designated Contracting State ES were drafted as corresponding process claims.

V. With the letter dated 23 March 2006 the appellant informed the board that "he has been instructed not to attend oral proceedings".

VI. The oral proceedings were cancelled.

VII. The appellant's arguments in writing, insofar as they are relevant to the present decision, may be summarized as follows:

Main request and first auxiliary request
Article 123(2) EPC

Claims 1, 6 and 10

Feature "embryogene Zellkluster mit einer Grösse von 150 µm bis 2000 µm isoliert"

- This feature did not mean that cell clusters having the recited size between 150 µm to 2000 µm were isolated from clusters having sizes outside this range. Rather, it meant that cell clusters obtained in step (c), the vast majority of which had the recited size, were taken as a whole from the medium in which they were grown, irrespective of their size, and were retained for further use in the regeneration/transformation process.

- The application as filed (see column 10, lines 43 to 49, column 30, lines 46-50 and column 30, lines 54-

57 of the published application) and claim 32 as originally filed ("isoliert") provided a basis for the above interpretation of the claim.

Claims 15, 19, 21 and 25

Feature "DNA ... die nicht auf natürlichem Weg in das Genom integriert werden kann"

- This technical feature had no effect on the scope of the claims because the context of this feature in the claims made clear that it related to DNA introduced into protoplasts. Thus since protoplasts were not found in nature, there could be no "natural means" of introducing DNA into them, and consequently the feature was meaningless.

- This feature was supported by the application as filed because it was synonymous with the term "heterologous" as defined in the application as filed (see e.g. at column 23, lines 53 to 54 of the application as published).

Claims 15, 19, 21 and 25

Feature "einen vorher ausgewählten Abschnitt exogener DNA"

- Although this expression had no literal support in the application as filed, it was implicit from the disclosure as a whole, particularly Section F (columns 23 to 28) of the description relating to the in vitro treatment of protoplasts with DNA. This part of the disclosure taught that the DNA had to be

pre-selected since human intervention was required, and this type of DNA was thus distinct from DNA which was integrated into the plant cell genome by natural means.

Second auxiliary request
Article 123(3) EPC

Claims 21 and 24

Feature "chimäres Gen oder mehrere chimäre Gene"

– There was no infringement of Article 123(3) EPC by claims 21 and 24 because chimäric genes were by definition non-natural. Accordingly there were no chimeric genes which could be integrated into a plant genome by natural means.

Third to fifth auxiliary request
Claims 1, 6 and 10

Feature "embryogene Zellkluster mit einer Grösse von 150 µm bis 2000 µm von dem Suspensionskulturmedium isoliert"

– The further introduction of "*von dem Suspensionskulturmedium*" made it clear that the "isolation" which was referred to was isolation from the cell culture medium, not from cell clusters outside the specified size range.

Reimbursement of the appeal fee

- Reimbursement of the appeal fee was justified in view of a substantial procedural violation by the opposition division in issuing the decision under appeal after a delay of one year and 9 months (see decisions T 390/86 (OJ EPO 1989, 30), T 243/87 of 30 August 1989 and T 346/92 of 29 July 1993).

VIII. The respondents' arguments in writing, insofar as they are relevant to the present decision, may be summarized as follows:

Main, first and second auxiliary requests
Article 123(2) EPC

- The feature "embryogene Zellkluster mit einer Grösse von 150µm bis 2000µm isoliert" in step (d) of claims 1, 6 and 10 of these requests had no support in the application as filed.

- The feature "DNA ... die nicht auf natürlichem Weg in das Genom integriert werden kann" in ***C***laims 15, 19, 21 and 25 of these requests had no support in the application as filed.

- The feature "einen vorher ausgewählten Abschnitt exogener DNA" in ***C***laims 15, 19, 21 and 25 of these requests had no support in the application as filed.

Third to fifth auxiliary requests
Article 123(2) EPC

- The further introduction into claim 1, 6 and 10 of these requests of the wording "von dem

Suspensionskulturmedium" had no support in the application as filed.

IX. The appellant requested that the decision under appeal be set aside, and that the patent be maintained on the basis of the claims of the main request or of one of the first to fifth auxiliary requests, all filed with the letter dated 17 February 2005.

Reimbursement of the appeal fee was also requested pursuant to Rule 67 EPC.

The respondents (opponents O1 and O2) requested that the appeal be dismissed.

Reasons for the Decision

Language of the proceedings

1. During the oral proceedings before the opposition division the parties agreed that the English language be used during the oral proceedings as well as in the minutes and in the decision under appeal. The appellant was however reminded that the claims had to be submitted in German (see paragraph 1 of the "Minutes"). The board adheres to this way to proceed in view of decision J 18/90, O.J. EPO 1992, 511 (see also decision T 788/91 of 24 November 1994), according to which the board may use in decisions an official language (here: English) other than the language of the proceedings (here: German), provided all the parties to the proceedings had given their agreement.

Main request

Article 123(2) EPC

Claims 1, 6 and 10

Feature "embryogene Zellkluster mit einer Grösse von 150µm bis 2000µm isoliert"

2. The board agrees with the appellant that the published application teaches that after 4 to 20 transfers, the embryogenic cell clusters have to be recovered from the medium in which they are grown, irrespective of their size, for further use in the regeneration/ transformation process. Column 10, lines 43 to 49 and column 30, lines 46-50 of the published application indeed merely state that "the majority of the embryonic cell cluster typically exhibit a size from 150 µm to 2000 µm" and "the majority of the embryogenic cell clusters is relatively small (150 µm to 2000 µm)", without requiring any step aiming at excluding the fraction of embryogenic cell clusters having sizes outside the 150 µm to 2000 µm range.

3. These passages are in line with column 30, lines 54-57 and column 15, lines 13-22 of the published application, showing that it is the whole suspension culture of embryogenic cell clusters which is subjected to filtration on a 0.2 µm Nalgene® filter for further use in the protoplast production. This way of recovering the cell clusters from the suspension culture is also consistent with claim 32 (d) as filed, where no size range of the embryogenic cell clusters is indicated.

4. The appellant argues that the wording "embryogene Zellkluster mit einer Grösse von 150µm bis 2000µm isoliert" in step (d) of claims 1, 6 and 10 would be read by the skilled person in the context of the description, and would be understood to mean that cell clusters obtained in step (c), the vast majority of which have the recited size, are taken irrespective of their size from the medium in which they are grown, and are retained for further use.

5. However, this appellant's interpretation runs against the claim language, which has the precise and unambiguous meaning of "setting apart cell clusters having sizes from 150 µm to 2000 µm from others not having those sizes". This understanding also makes technical sense since the isolation of cell clusters having a particular size range can easily be achieved in practice by filtering the suspension cultures on a series of filters having different mesh sizes (see for example Table 1 of document D12 (Yang Y.M. et al., Plant Cell Report, Vol. 13, pages 176-179, 1994). Therefore, the board concludes that the wording of the claims implies that the end product of step (d) can only be **isolated** cell clusters having sizes from 150 µm to 2000 µm. However, the application as filed (see points 2 and 3 supra) provides no basis for this technical situation, reflected by the present claim language, contrary to the requirements of Article 123(2) EPC.

6. The board also notes in passing that the appellant's interpretation of step (d) of claims 1, 6 and 10 in the context of Article 123(2) EPC is at odds with that

expressed during the opposition phase in the context of Articles 54 and 56 EPC (see the appellant's letter dated 14 July 1998, items 1.3.5, 1.3.10, 1.3.11 and 1.4.12 ("claim 1 directs the reader to isolate cell clusters with a size from 150μm to 2000 μm")).

Claims 15, 19 and 25

Feature "DNA..., die nicht auf natürlichem Weg in das Genom integriert werden kann"

7. It is the appellant's view that this technical feature has no effect on the scope of the claims because the context of this feature in the claims makes it clear that it relates to DNA introduced into protoplasts. But since protoplasts are not found in nature, in the appellant's opinion, there can be no "natural means" of introducing DNA into them, and consequently the feature has no effect on the claims.

8. However, unlike claim 21, claims 15, 19 and 25 do not require that the DNA be introduced into protoplasts but merely that the cells forming the callus or the plants and their propagules contain exogenous DNA, with the exclusion of any DNA introduced by natural means (T-DNA, viral DNA introduced via homologous recombination or DNA encoding sexually-introduced traits, etc). This is a technical feature relating to the status of the DNA contained within the cells regardless of when and how it has been introduced. The above wording thus has a clear ("exclusion") effect on the scope of claims 15, 19 and 25.

9. The appellant maintains that this feature is supported by the term "heterologous" (see column 23, lines 53 to 54 of the published application). The term "heterologous with respect to the transformed plant" as used in column 23, lines 53 to 54 means "DNA in a non-natural environment". In the light of the definition given in the published application, "heterologous" DNA in a genome thus includes T-DNA, viral DNA and sexually transmitted DNA, provided they are in a non-natural environment. In contrast to this definition, the expression in dispute "DNA which cannot be integrated into the genome by natural means" excludes said T-DNA, viral DNA and sexually transmitted DNA because these types of DNA can be integrated into cell genomes by natural means. In conclusion, the term "heterologous" cannot provide support for an implicit disclosure of the terms now appearing in claims 15, 19 and 25, which terms represent thus added subject-matter, contrary to the requirements of Article 123(2) EPC.

Claims 15, 19 and 25

Feature "einen vorher ausgewählten Abschnitt exogener DNA"

10. Although this expression has no literal support in the application as filed, it is the appellant's opinion that this expression is implicit from the disclosure as a whole, particularly Section F (columns 23 to 28) of the description relating to the in vitro treatment of protoplasts with exogenous DNA. This part of the disclosure teaches that the exogenous DNA has to be pre-selected since human intervention is required, and the appellant concludes that this type of exogenous DNA

is thus distinct from exogenous DNA which is integrated into the plant cell genome by natural means.

11. However, as emphasised under point 8 supra, claims 15, 19 and 25 do not refer to in vitro treatment of protoplasts. Therefore, the wording of these claims is such that the "pre-selected segment of exogenous DNA" may also be introduced into the cell before or after the protoplast stage. The disclosure of the published application in Section F therefore does not relate to the same process steps as those recited in claims 15, 19 and 25 and thus cannot provide a basis under Article 123(2) for the feature "pre-selected segment of exogenous DNA" used in the context of a different type of process.

First auxiliary request

Article 123(2) EPC

12. The claims of the first auxiliary request differ from those of the main request in that the expressions "DNA ... die nicht auf natürlichem Weg in das Genom integriert werden kann" and "einen vorher ausgewählten Abschnitt" have been deleted from the claims.

13. However, claims 1, 6 and 10 of this request still contravene Article 123(2) EPC for the same reasons as those set out above for the main request, i.e. the inclusion of the feature "embryogene Zellkluster mit einer Grösse von 150µm bis 2000µm isoliert" in step (d) of these claims (see points 2 to 6 supra).

Second auxiliary request

Article 123(2) EPC

14. The claims of the second auxiliary request further differ from those of the first auxiliary request in that the feature of claim 22 ("chimäres Gen oder mehrere chimäre Gene") has been incorporated into claim 21, and the same feature had been incorporated into former claim 25 (now claim 24).

15. However, claims 1, 6 and 10 of this request contravene Article 123(2) for the same reasons as those set out above for the main request i.e. the inclusion of the feature "embryogene Zellkluster mit einer Grösse von 150µm bis 2000µm isoliert" in step (d) of these claims (see points 2 to 6 supra).

Third auxiliary request

Article 123(2) EPC

16. The claims of the third auxiliary request differ from those of the main request in that the wording "von dem Suspensionskulturmedium" has been inserted before "isoliert" in step (d) of claims 1, 6 and 10. In the appellant's view, the further introduction of "von dem Suspensionskulturmedium" makes explicit that the "isolation" referred to in step (d) of these claims is isolation from the cell culture medium, not from cell clusters outside the specified size range.

17. However, the introduction into step (d) of claims 1, 6 and 10 of the further feature "von dem Suspensionskulturmedium" does not heal the deficiency

pointed out in point 5 supra, since the claim language implies that the end product of step (d), regardless of its source, must be isolated cell clusters having sizes from 150 µm to 2000 µm, and the application as filed does not provide any basis for this claim language, contrary to the requirements of Article 123(2) EPC.

18. Moreover claims 15, 19 and 25 of this request also contravene Article 123(2) EPC because they contain the two features which have been shown to offend against Article 123(2) EPC in claims 15, 19 and 25 of the main request, namely the feature "DNA ... die nicht auf natürlichen Weg in das Genom integriert werden kann" (see points 8 and 9 supra), and the feature "einen vorher ausgewählten Abschnitt exogener DNA" (see point 11 supra).

Fourth auxiliary request

19. The claims of the fourth auxiliary request combine the amendments of the first and third auxiliary requests. As a consequence, claims 1, 6 and 10 of this request contravene Article 123(2) EPC for the same reasons as those set out above for the third auxiliary request i.e. the inclusion of the feature "embryogene Zellkluster mit einer Grösse von 150 µm bis 2000 µm von dem Suspensionskulturmedium isoliert" in step (d) of these claims (see points 17 and 18 supra).

Fifth auxiliary request

20. The claims of the fifth auxiliary request combine the amendments of the second and third auxiliary requests. As a consequence, claims 1, 6 and 10 of this request

contravene Article 123(2) EPC for the same reasons as those set out above for the third auxiliary request i.e. the inclusion of the feature "embryogene Zellkluster mit einer Grösse von 150 µm bis 2000 µm von dem Suspensionskulturmedium isoliert" in step (d) of these claims (see points 17 and 18 supra).

21. In summary, none of the appellant's requests before the board, including those for the Designated Contracting State ES, can be allowed.

Reimbursement of the appeal fee

22. Since, according to Rule 67 EPC, a reimbursement of the appeal fee is only possible if the board of appeal deems the appeal allowable, which is not the case here, there is no legal reason to allow this appellant's further request.

Order

For these reasons it is decided that:

1. The appeal is dismissed.

2. The request for reimbursement of the appeal fee is refused.

The Registrar: Chair:

D. Meyfarth U.M. Kinkeldey