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Datasheet for the decision of 1 August 2017

Case Number: T 2163/13 - 3.3.04

Application Number: 09010216.1

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Title of invention:
Doubling of chromosomes in haploid embryos

Applicant:
Pioneer Hi-Bred International Inc.
E. I. du Pont de Nemours and Company

Headword:
Method for producing doubled haploid maize plants/PIONEER HI-BRED and E. I. DU PONT DE NEMOURS

Relevant legal provisions:
EPC Art. 56

Keyword:
Inventive step - (no)

Decisions cited:
T 2044/09

Catchword:

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Case Number: T 2163/13 - 3.3.04

D E C I S I O N
of Technical Board of Appeal 3.3.04
of 1 August 2017

Appellant: (Applicant 1)	Pioneer Hi-Bred International Inc. 7100 N.W. 62nd Avenue Box 1014 Johnston, IA 50131-1014 (US)
Appellant: (Applicant 2)	E. I. du Pont de Nemours and Company 1007 Market Street Wilmington, DE 19898 (US)
Representative:	J A Kemp 14 South Square Gray's Inn London WC1R 5JJ (GB)
Decision under appeal:	**Decision of the Examining Division of the European Patent Office posted on 26 March 2013 refusing European patent application No. 09010216.1 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chairwoman	G. Alt
Members:	M. Montrone
	P. de Heij

Summary of Facts and Submissions

I. The appeal was lodged by the applicants (hereinafter "appellants") against the decision of the examining division to refuse European patent application No. 09 010 216.1 (hereinafter the "application"). The application is a divisional application of the earlier European patent application No. 06 814 915.2, which was filed as an international application and published as WO 2007/038075 (hereinafter the "earlier application as filed") entitled "*Doubling of chromosomes in haploid embryos*".

II. In its decision, the examining division dealt with one main and seven auxiliary requests. It held that claim 12 of the main request, claim 11 of auxiliary requests 2 and 6 and claim 1 of auxiliary requests 4 and 5 contained subject-matter extending beyond the content of the application as filed (Article 123(2) EPC). Furthermore, it took the view that the subject-matter of at least claim 1 of auxiliary requests 1 to 7 lacked inventive step (Article 56 EPC).

III. With the statement of grounds of appeal, the appellants submitted a main request and seven auxiliary requests, which were identical to those underlying the impugned decision.

IV. The appellant was informed of the board's preliminary view in a communication pursuant to Article 15(1) RPBA. The board observed *inter alia* that, in the light of the teaching of document D1, it appeared doubtful that the subject-matter of claim 1 of auxiliary requests 1, 3 and 7 was based on an inventive step (Article 56 EPC) (see section V below).

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

D1: WO 02/085104

D7: Declaration of Dr. Z.-Y. Zhao, dated 31 January 2013

VI. Oral proceedings before the board were held on 1 August 2017. The appellants submitted two new main requests. They withdrew all pending claim requests except the new main request submitted at 13:15 hrs. At the end of the oral proceedings the chairwoman announced the board's decision.

Claim 1 of the new main (and sole) request reads:

"1. A method of obtaining a doubled haploid maize plant comprising:

(a) isolating an immature haploid embryo obtained by pollinating silks of a maize ear with a maize inducer line;

(b) contacting said isolated haploid embryo scutellum side up on an embryo culture medium containing a chromosome doubling agent to produce at least one doubled haploid embryo cell;

(c) culturing said doubled haploid embryo scutellum side down on an embryo maturation medium that does not contain a chromosome doubling agent to generate a doubled haploid maize plant from said doubled haploid embryo cell,

wherein step (b) occurs 4-21 days after pollination;

and wherein the inducer line contains a marker gene that is expressed in embryo tissue and said marker gene is expressed 4 or more days after pollination."

VII. The appellants' arguments may be summarised as follows:

Admission of the new main request into the appeal proceedings (Rule 13(1)(3) RPBA)

The new main request was submitted to overcome objections under Article 76(1) EPC raised by the board in its communication.

Amendments (Article 76(1) EPC)

The subject-matter of steps (b) and (c) in claim 1 had a basis in examples 2 and 3 of the earlier application as filed in conjunction with the disclosure on page 11, lines 15 to 20.

Inventive step (Article 56 EPC)

Document D1 represented the closest prior art. The method according to claim 1 essentially differed from claim 50 disclosed in document D1 by the feature "contacting said isolated haploid embryo scutellum side up" referred to in step (b) and the feature "culturing said doubled haploid embryo scutellum side down" referred to in step (c) (hereinafter the "inversion feature"). A further difference was that the method of the invention was directed to maize plants, while the method according to claim 50 in document D1 related to the generation of double haploid plants in general.

The effect associated with the "inversion feature" was that a higher number of fertile maize plants, i.e. doubled haploid plants, were obtained. Experimental data comparing the efficacy of obtaining fully fertile plants by the claimed method *vis-à-vis* the method of the closest prior art were not available. An increased recovery rate by the claimed method was, however, indicated by the values of 93.3% and 83.3% fertile plants reported in examples 2 and 3 of the application. These high values were consistent with the statements in document D7, the declaration by Dr. Zhao, that the penetration of the chromosome doubling agent into the plant embryo was facilitated by a direct contact between the embryo having its "*scutellum side up*" and the agent in the medium, and the subsequent inversion of the doubled haploid embryo to its "*scutellum side down*", which increased the air exchange of the embryo to improve its germination.

If the above line of argument was not accepted, an alternative line of argument was that a particular technical effect was not associated with the "inversion feature".

The technical problem was thus the provision of either an improved method for obtaining doubled haploid maize plants or an alternative method for obtaining such plants.

The "inversion feature" of claim 1 was not suggested by the teaching of document D1 either alone or in combination with the teaching of any of the other available prior art documents. Thus, the subject-matter of claim 1 constituted a non-obvious solution to the problem underlying the invention.

VIII. The appellants requested that the decision under appeal be set aside and that a patent be granted on the basis of the new main request submitted at 1 August 2017.

Reasons for the Decision

1. The appeal is admissible.

2. In view of its decision on inventive step (see below), the board sees no need to provide detailed written reasons for its decision at the oral proceedings to admit the new main request into the appeal proceedings and for its opinion announced at the oral proceedings that the new main request met the requirements of Article 76(1) EPC (both having been decided in the appellants' favour).

Introduction to the invention

3. The invention concerns a method for the production of doubled haploid maize plants generated by the duplication of a single set of chromosomes in haploid plant embryos (see paragraphs [0001] and [0004] of the application).

4. Doubled haploid plants are essentially homozygous, i.e. they have doubled haploid sets of chromosomes (see paragraph [0006] of the application). These plants are useful for obtaining a homozygous trait of interest and for applications dealing with functional genomics, including for example, the analysis of knock-outs of particular genes, the functional analysis of recessive genes, and studies dealing with homologous chromosomal

recombinations for gene targeting (see paragraph [0027] of the application).

5. The maize embryo has a flattened asymmetric shape with the scutellum surface on one side and the embryo axis containing the apical meristem with actively dividing cells contributing to the development of an entire plant on the other (see document D7, point 5.1). The scutellum is a thin structure of the embryo that facilitates the absorption of nutrients.

Inventive step (Article 56 EPC) - claim 1

6. The board agrees with the appellants that document D1 represents the closest prior art for the method according to claim 1. Document D1 discloses a method for the production of doubled haploid seeds, i.e. of plants, since "*the term "plant" includes [...] seeds*" (see page 2, lines 9 and 10). The method includes crossing a female parent with a male inducer line to produce a haploid embryo, treatment of the haploid embryo with a chromosome doubling agent and growing the treated embryo to produce a regenerated plant having homozygous seed (see claim 50). The document further reports in the section "*Detailed description of the invention*" that the method can be practiced with plants including maize (see page 8, lines 31 and 32) and that male inducer lines may contain a scorable marker gene for the selection of haploid embryos (see e.g. page 8, lines 16 to 30).

Furthermore, document D1 mentions in the context of example 1 that, based on the identification of the marker gene, haploid embryos are isolated from immature

maize ears at days 4 to 20 after pollination with male inducer lines for transformation purposes (see page 22, lines 5 to 12).

Technical problem and solution

7. The appellants argued that the claimed method differed essentially from the method referred to above as the closest prior art in that the chromosome doubling step was carried out on an embryo culture medium with the embryo's "*scutellum side up*" (see step (b)) and that the embryo was then inverted to carry out the culturing of the doubled haploid embryo on an embryo maturation medium with its "*scutellum side down*" (see step (c)). The board agrees with this view.

The changing of the position of the embryo for the chromosome doubling in step (b) and the regeneration of the maize plants in step (c), both referred to in claim 1, will hereinafter be referred to as the "inversion feature".

8. The appellants argued that the technical effect associated with the "inversion feature" was that a higher number of fertile maize plants, i.e. of doubled haploids (see paragraph [0006] of the application), was obtained. The appellants did not associate any other difference between the closest prior art and the claimed method with a particular technical effect.

9. The appellants conceded that comparative data supporting such an increased recovery rate of fertile plants by the claimed method *vis-à-vis* the method of the closest prior art were not available.

10. However, values of 93.3% and 83.3% fertile plants reported in examples 2 and 3, respectively, in the application were an indication that changing the position of the embryo had this effect. The high values were also consistent with the explanations given in the declaration D7 in points 5.(1) and (2):

1) "[...]*To facilitate rapid penetration of the doubling agent into the meristem, embryos are oriented on the doubling agent medium with the embryo axis in contact with the medium (i.e. scutellum side up). This insures that the apical meristem (which contains the actively dividing cells that contribute to the entire developing plant) is exposed to the doubling agent*".

2) *After adequate time for the doubling agent to penetrate into the meristem region; the embryos are inverted so that the scutellum (i.e. scutellum side down) is in contact with the medium and the embryo axis faces away from the medium. Embryo germination requires good air exchange, and when the embryo axis remains in contact with the medium, air exchange is inhibited resulting in reduced germination. Inverting the embryos after the doubling process exposes the meristem to the surrounding air, providing a more conducive environment for germination*" (see declaration D7, page 3, points 5.1 and 2).

11. The board notes that indeed, as acknowledged by the appellants, experimental evidence is not available in support of the alleged improved recovery rate of fertile maize plants by the claimed method compared with the closest prior art.

12. In the absence of such evidence, the board does on the one hand consider it conceivable that the technical

effect associated with the "inversion feature" may indeed be that the number of fertile plants obtained is increased, since, as is derivable from declaration D7, the "*scutellum side up*" orientation facilitates the penetration of the embryo with the chromosome doubling agent, while the "*scutellum side down*" orientation allows a good air exchange.

13. However, on the other hand, it is equally conceivable that the positioning of the embryo on the media may not have an effect on the number of recovered fertile plants.

Indications for this view are the embryo's small size of about 1.5 to 2 mm in combination with its relative long incubation time of 24 to 48 hours with the chromosome doubling agent (see examples 2 and 3 of the application), which both contribute to a complete saturation of the embryo with the agent, irrespective of the side of the embryo that is in contact with the doubling agent-containing medium. The appellants did not argue that the chromosome doubling agent cannot pass through the scutellum, if the scutellum side faces the medium.

A further consequence of the embryo's small size is, in the board's view, that it has a relatively large surface area in relation to its volume. This should allow a good air exchange of the embryo at all times, i.e. irrespective of which side is facing the medium.

14. Thus, in the present case, the board is not convinced that the technical effect achieved by the "inversion feature" according to the claimed method is an increase in the number of fertile maize plants compared with the

number of plants obtained by the method according to the closest prior art.

15. Therefore, in view of these considerations, the board concludes that the objective technical problem to be solved is the provision, not of an improved, but of an alternative method for the generation of doubled haploid maize plants.

16. The board is satisfied that the subject-matter of claim 1 solves this technical problem in view of the results disclosed in examples 2 and 3 of the application.

Obviousness

17. It remains to be assessed whether or not the skilled person, starting from the method of producing doubled haploid plants disclosed in document D1 and faced with the technical problem defined above, would arrive at the claimed subject-matter in an obvious manner.

18. The appellants argued that the subject-matter of claim 1 was inventive, since there were no pointers derivable for the "inversion feature" either from the teaching of document D1 alone or from its combination with any of the available prior art documents.

19. The board agrees with the appellants that the teaching in document D1 does not point to the "inversion feature" referred to in claim 1 and that this feature is also not hinted at in combination with the teaching of any of the available prior art documents. However, in the present case this is not sufficient to establish an inventive step, since the appellants have not

provided evidence that the "inversion feature" has a particular functionality in the claimed method, let alone that it provides a surprising effect. Thus, the board considers that the "inversion feature" is an arbitrary, non-functional additional step to the method of the closest prior art which cannot *per se* constitute the basis for acknowledging an inventive step (see e.g. decision T 2044/09, point 4.6 of the Reasons).

20. Consequently, the subject-matter of claim 1 and hence the new main request does not meet the requirements of Article 56 EPC.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairwoman:

P. Cremona G. Alt

Decision electronically authenticated