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Case Number: T 0068/13 - 3.3.04

Application Number: 04809955.0

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Language of the proceedings: EN

Title of invention:
Cry1F and cry1Ac transgenic cotton lines and event-specific identification thereof

Applicant:
Dow AgroSciences LLC

Headword:
Pest resistant transgenic cotton plants/DOW AGROSCIENCES

Relevant legal provisions:
EPC Art. 56

Keyword:
Inventive step - all requests (no)

Decisions cited:
T 0775/08, T 2239/08, T 0915/10

Catchword:

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Case Number: T 0068/13 - 3.3.04

D E C I S I O N
of Technical Board of Appeal 3.3.04
of 17 February 2017

Appellant: (Applicant)	Dow AgroSciences LLC 9330 Zionsville Road Indianapolis IN 46268-1054 (US)
Representative:	Zwicker, Jörk ZSP Patentanwälte PartG mbB Radlkoferstrasse 2 81373 München (DE)
Decision under appeal:	**Decision of the Examining Division of the European Patent Office posted on 26 July 2012 refusing European patent application No. 04809955.0 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chairwoman	R. Morawetz
Members:	M. Montrone
	M. Blasi

Summary of Facts and Submissions

I. The appeal was lodged by the applicant (hereinafter "appellant") against the decision of the examining division to refuse European patent application No. 04809955.0. The application was filed as an international application and published as WO 2005/103266 (hereinafter "the application") with the title "*Cry1F and cry1Ac transgenic cotton lines and event-specific identification thereof*".

II. The examining division held in the impugned decision that the subject-matter of claim 1 of the main request and of auxiliary requests 1 to 3 lacked an inventive step.

III. With the statement of grounds of appeal, the appellant relied on a main request which corresponded to the main request underlying the impugned decision and submitted an auxiliary request - subsequently re-numbered to auxiliary request II, corresponding to auxiliary request II underlying the impugned decision -, and document D10 (see section VI below).

Claims 1 and 2 of the main request read:

"1. A cotton seed comprising in its genome cry1F cotton event 281-24-236, wherein the cotton event 281-24-236 is a cry1F insert polynucleotide sequence consisting of nucleotides 2075-12748 of SEQ ID No.:1 and at least 20 contiguous flanking nucleotides on both sides of said insert sequence, wherein the 5'-flanking nucleotides are from nucleotide residues 1-2074 of SEQ ID No.:1 and the 3'-flanking nucleotides are from nucleotide residues 12749-15490 of SEQ ID No.:1.

2. The cotton seed of claim 1 further comprising in its genome cry1Ac cotton event 3006-210-23, wherein the cotton event 3006-210-23 is a cry1Ac insert polynucleotide sequence consisting of nucleotides 528-8900 of SEQ ID No.:2 and at least 20 contiguous flanking nucleotides on both sides of said second insert sequence, wherein the 5'-flanking nucleotides are from nucleotide residues 1-527 of SEQ ID No.:2 and the 3'-flanking nucleotides are from nucleotide residues 8901-9382 of SEQ ID No.:2."

Claim 1 of auxiliary request II reads:

"1. A cotton seed comprising in its genome cry1F cotton event 281-24-236, wherein the cotton event 281-24-236 is a cry1F insert polynucleotide sequence consisting of nucleotides 2075-12748 of SEQ ID No.:1 and at least 20 contiguous flanking nucleotides on both sides of said insert sequence, wherein the 5'-flanking nucleotides are from nucleotide residues 1-2074 of SEQ ID No.:1 and the 3'-flanking nucleotides are from nucleotide residues 12749-15490 of SEQ ID No.: 1, and cry1Ac cotton event 3006-210-23, wherein the cotton event 3006-210-23 is a cry1Ac insert polynucleotide sequence consisting of nucleotides 528-8900 of SEQ ID No.:2 and at least 20 contiguous flanking nucleotides on both sides of said second insert sequence, wherein the 5'-flanking nucleotides are from nucleotide residues 1-527 of SEQ ID No.:2 and the 3'-flanking nucleotides are from nucleotide residues 8901-9382 of SEQ ID No.:2."

IV. The board informed the appellant of it's preliminary view in a communication pursuant to Article 15(1) RPBA, *inter alia* observing that document D3 represented the closest prior art for the subject-matter of claim 1 of

auxiliary request II, and introducing document D11 into the appeal proceedings.

V. In reply to the board's communication the appellant submitted auxiliary requests I and III and *inter alia* document D15 (see section VI below), and re-numbered the auxiliary request filed with its statement of grounds of appeal to auxiliary request II.

Claim 1 of auxiliary request I differs from claim 1 of the main request in that the feature "*and having a representative seed deposited with American Type Culture Collection (ATCC) with Accession No. PTA-6233*" has been added at its end.
The wording of claim 2 of auxiliary request I is identical to that of claim 2 of the main request.

Claim 1 of auxiliary request III differs from claim 1 of auxiliary request II in that the feature "*and having a representative seed deposited with American Type Culture Collection (ATCC) with Accession No. PTA-6233*" has been added at its end.

VI. The following documents are cited in this decision:

D3: Perlak F. J. *et al.*, The Plant J., 27(6), 489-501, 2001

D5: Press release of Dow AgroSciences, dated 23 April 2003

D10: Kohli A. *et al.*, Plant Mol. Biol., 52, 247-258 2003

D11: US Environmental Protection Agency, review on WideStrike™ cotton, dated 17 February 2004

D15: US Environmental Protection Agency biopesticides registration action document on WideStrike™ cotton, dated May 2005

VII. Oral proceedings before the board were held on 17 February 2017. At the end of the oral proceedings the chairwoman announced the board's decision.

VIII. The appellant's arguments may be summarised as follows:

Main and auxiliary requests I to III

Inventive step (Article 56 EPC)

Document D3 represented the closest prior art for the subject-matter of claim 2 of the main request. The document disclosed the Bollgard® II transgenic cotton plant containing in its genome the cry1Ac and cry2Ab2 genes which made the plant resistant against insect pests.

The subject-matter of claim 2 of the main request differed therefrom in that the seeds contained in their genome the cry1Ac and cry1F genes defined by the events cry1Ac 3006-210-23 and cry1Fa 281-24-236. The effects associated with this difference were that both of the cry toxin genes in the claimed seeds were constantly expressed throughout the growth period of the plant, unlike the Bollgard® II plant disclosed in document D3, which exhibited a decline in the expression of one of the two cry toxin genes.

The application was silent with regard to the persistent expression of the two cry toxin genes in plants grown from the claimed seeds. However, the

application disclosed in the part relating to the background art that it was commonly known at the filing date of the application that the expression of foreign genes in transgenic plants was influenced by the particular location of the insert in the plant's chromosome. Therefore, hundreds or thousands of transgenic plants had to be generated and screened to identify those exhibiting an optimal expression of the newly introduced gene(s) (see paragraphs [0003] and [0004]). Thus, the teaching of the application already foreshadowed the effect of an improved expression level of the cry1F and cry1Ac genes in the seeds according to claim 2.

This effect was corroborated by document D11, which disclosed that cotton plants grown from the seeds according to claim 2, - unlike Bollgard® II plants - expressed the two toxin genes constantly throughout their growth season and that insect pests feeding on leaves of these plants were exposed to both toxins simultaneously at consistent levels throughout this period (document D11, page 8, last paragraph to page 9, first paragraph). The objective technical problem was thus the provision of transgenic seeds with an improved expression of insecticidal protein toxins.

The subject-matter of claim 2 was not an obvious solution to this problem, since the persistent expression of the two toxin genes throughout the growth period of the plants depended on the particular location of the two events in the chromosome, which was due to a random insertion and thus a chance event. Accordingly, there was no expectation that the skilled person would succeed in identifying plants containing these two events in a screening program.

Further surprising effects associated with the improved expression level of the cry1F and cry1Ac genes in the seeds according to claim 2 of the main request were (i) that the Cry1F or Cry1Ac toxin proteins were not expressed at detectable levels in the nectar of plants, (ii) that the plants exhibited superior agronomic performance due to improved cotton fibre characteristics, and (iii) that both toxins were expressed at high and stable levels. Although all of these effects were not explicitly disclosed in the application, they were foreshadowed by the influence of the location of an insert in the plant's genome on the expression of a gene of interest, which required screening for plants exhibiting optimal levels and patterns of gene expression as disclosed in paragraphs [0003] and [0004] of the application, or in document D10 (page 247, column 2, second paragraph). These effects were corroborated by the disclosure of document D11 (pages 17 and 18) or document D15 (page 7, second paragraph, page 15, first paragraph, Table 3 on page 19, pages 60 and 61). Since these improved properties of the claimed seeds were surprising they justified the acknowledgement of an inventive step.

IX. The appellant requested that the decision under appeal be set aside and that a patent be granted on the basis of the claims of the main request or, alternatively, on the basis of the sets of claims of one of auxiliary requests I to III, the main and auxiliary request II corresponding to the main and auxiliary request II underlying the decision under appeal, and auxiliary requests I and III as filed with letter dated 3 February 2017.

Reasons for the Decision

Main request

Introduction to the invention

1. The invention concerns cotton plants which have been genetically modified so as to make them resistant to *lepidopterous* insect pests. The insect protection is mediated by the expression of Cry1F and Cry1Ac protein toxins derived from *Bacillus thuringiensis* (*B. thuringiensis*) which were introduced into the plant genome by transformation. Cry1F and Cry1Ac proteins are toxic to lepidopteran insects in a species-specific manner. In susceptible species, they bind to specific receptors present on midgut epithelial cells, forming pores that disrupt the osmotic balance of the cells, thereby causing their lysis and death (see paragraph [00105] of the application).

2. Foreign DNA introduced by transformation processes may be inserted at any location in the plant genome, the chromosomal position of this insertion being random and hence unpredictable. In the art, each independent insertion is termed a transgenic "event", which is defined by the nucleic acid sequence of the insert, *i.e.* the gene(s) of interest - here the toxins cry1F and cry1Ac -, and the plant's chromosomal sequences immediately flanking the insert(s) on both sides - here the at least 20 contiguous nucleotides. The unique chromosomal location of the insert is defined by the sequence of the DNA spanning the junctions between the insert(s) and the plant genome, - here cotton.

Inventive step (Article 56 EPC)

Closest prior art

3. The subject-matter of claim 2 is directed to cotton seeds which contain in their genome the transgenic insertion events cry1F 281-24-236 (hereinafter "cry1F event") and the cry1Ac event 3006-210-23 (hereinafter "cry1Ac event").

4. The board concurs with the appellant that the disclosure of document D3 represents the closest prior art. The document reports on the insect-resistant Bollgard® II transgenic cotton plant which contains the two cry1Ac and cry2Ab genes in its genome, each expressing an insecticidal protein, *i.e.* Cry1Ac or Cry2Ab (see page 497, column 2, second paragraph). The expression of these two insecticidal Cry proteins provides a transgenic cotton plant exhibiting an "*increased activity and an expanded spectrum of insect control*" accompanied by "*positive implications for resistance management issues*" compared to plants containing only one cry toxin gene (see page 496, column 1, last paragraph and page 497, column 2, second paragraph).

Problem to be solved and solution

5. The cotton seeds according to claim 2 differ from the Bollgard® II plant disclosed in document D3 by the events cry1Ac and cry1F. Transgenic inserts introduced into a plant's genome by independent transformations are located at unique locations in the chromosomes (see point 2 above). Accordingly, the cry1Ac event referred to in the seeds of claim 2 is located at a different position in the plant's genome from that containing the

same cry1Ac gene in the Bollgard® II plant. The same applies to the cry1F event referred to in the seeds of claim 2, which is moreover structurally distinct from the cry2Ab gene in the Bollgard® II plant, since it is a different member of the cry toxin family.

6. The appellant argued that the technical effect associated with these differences was that the cry1Ac and cry1F genes were consistently expressed in the plant throughout its growth period.

6.1 This property of the two genes was foreshadowed by the teaching of the application in the part relating to the background art which disclosed that "*The expression of foreign genes in plants is influenced by where the foreign gene is inserted in the chromosome. [...] For example, the same gene in the same type of transgenic plant (or other organism) can exhibit a wide variation in expression level amongst different events*" (see paragraph [0003]). The application further reported that because of the known positional effect of the chromosome on the expression of the inserted transgenes "*it is necessary to create and screen a large number of events in order to identify an event that optimally expresses an introduced gene of interest. For commercial purposes, it is common to produce hundreds to thousands of different events and to screen those events for a single event that has desired transgene expression levels and patterns*" (see paragraph [0004]).

6.2 The disclosure of document D11 (see page 8, last paragraph to page 9, first paragraph) only corroborated that the cry1Ac and cry1F genes were indeed consistently expressed throughout the growth period of the plant. In the appellant's view, the use of this effect was thus allowable and the technical problem was

considered to be the provision of transgenic seeds with an improved expression of insecticidal protein toxins.

7. The case law of the boards of appeal concerning subsequently invoked technical effects, has consistently held that effects of described features which are not indicated in the application may be taken into account, when determining the technical problem underlying the invention for the purpose of assessing inventive step, only, if they can be deduced by the skilled person from the application considered in relation to the closest prior art (Case Law of the Boards of Appeal of the European Patent Office, 8th edition 2016 (hereinafter "CLBA"), I.D.4.4.2, first paragraph).

8. It has thus to be assessed in the present case whether or not it is inferable from the application that the cry1Ac and cry1F genes contained in the seeds according to claim 2 exhibited a consistent expression throughout the growth period of the plant.

9. It was uncontested by the appellant that the application did not explicitly disclose expression levels or patterns of the cry1Ac and cry1F genes in cotton plants grown from the seeds as claimed, let alone over their entire growth period.

10. As regards an implicit disclosure, the application only mentions in general terms in the part relating to the background art that "*the expression of foreign genes in plants is influenced by where the foreign gene is inserted in the chromosome*" and therefore "*can exhibit a wide variation in expression level*" (see paragraph [0003]), thereby necessitating the generation and screening of a "*large number of events in order to*

identify an event that optimally expresses an introduced gene of interest" (see paragraph [0004]).

11. In the board's view, it is derivable from these two paragraphs of the application that it was common general knowledge of the skilled person that the expression of foreign genes in transgenic plants depends on their particular location in the plant's genome.

11.1 It is further derivable from these passages that the particular location in a chromosome does not necessarily influence the expression of an inserted gene, since this effect is one that only might occur, as is inferable from the statement reading "*can exhibit a wide variation in expression level*" (see paragraph [0003], emphasis added). This is also confirmed by document D10 which discloses that "*the position of integration and the structure of the transgene locus can vary considerably among independent transformants, and each of these factors may have a profound effect on the level and stability of transgene expression*" (see page 247, column 2, second paragraph, emphasis added).

11.2 However, the mere possibility that the location of a foreign gene in the plant's genome influences the level or pattern of the expression of an inserted gene cannot foreshadow to the skilled person whether or not these positional effects are associated with (i) a specific location - here the two locations defined by the events referred to in the seeds of claim 2 -, or (ii) with a specific technical effect - here the consistent expression of cry1Ac and cry1F in the plant throughout its growth period.

12. In view of these considerations the board concludes that the general disclosure about potential positional effects of the insertion into the chromosome on the expression of an inserted transgene in paragraphs [0003] and [0004] of the application does not implicitly disclose to the skilled person that the unique chromosomal insertion sites of the two cry genes in the seed according to claim 2 has an effect on the duration of their expression, and in particular not throughout the growth period of the plants. Therefore, this effect cannot be relied on for the formulation of the technical problem as suggested by the appellant (see points 6 to 6.2 above).

13. It also follows from the board's conclusion in point 12 above that the evidence disclosed in document D11 cannot be taken into account (see point 6.2 above).

14. With regard to the other subsequently invoked technical effects of the cry1F or cry1Ac genes that have been submitted by the appellant, *i.e.* (i) the selective non-expression of the Cry1F or Cry1Ac toxin proteins in the nectar of the plant, or (ii) the superior agronomic performance of the plants, due to improved cotton fibre characteristics, or (iii) the higher and more stable expression of the cry1F or cry1Ac genes, the board notes the following:

14.1 It was uncontested by the appellant that these effects of the cry1F or cry1Ac genes in the seeds according to claim 2 were likewise not explicitly disclosed in the application. Moreover, for the reasons outlined above (see points 10 to 12), these effects are also not implicitly inferable by the skilled person from the disclosure in paragraphs [0003] and [0004] of the application.

14.2 Accordingly, the board concludes that these effects too cannot be relied on for the formulation of the technical problem, and the evidence disclosed in both document D11 and document D15 cannot be taken into account.

14.3 It further follows from this conclusion of the board that the present case differs from those underlying decisions T 775/08 of 1 February 2011, T 2239/08 of 10 January 2013 or T 915/10 of 11 June 2015, all of which acknowledged inventive step because surprising technical effects could be taken into account when assessing it (see decisions T 775/08, point 12.4 of the reasons, T 2239/08, point 13 of the reasons, T 915/10, points 18 to 21 of the reasons).

15. The application discloses in example 1 that the effects associated with the crylAc and crylF genes in plants grown from the seeds according to claim 2 are that they "*provide improved insect resistance because the two Cry proteins provide a greater spectrum of control than either does alone and have differential activity against the lepidopteran pests that they are effective against. More importantly, it may help delay the development of resistant insects* (see paragraph [00105] of the application). The same effects are disclosed with regard to the closest prior art Bollgard® II plant containing the cry1Ac and cry2Ab genes (see point 4 above).

16. The board therefore considers that since a particular technical effect of the two events contained in the seeds of the claimed invention *vis-à-vis* the ones contained in the closest prior art plant is not apparent, the technical problem to be solved is the

provision of transgenic cotton seeds containing an alternative combination of cry events.

17. The board is satisfied that this technical problem is solved by the subject-matter of claim 2.

Obviousness

18. The question to be assessed is whether or not the skilled person, starting from the Bollgard® II cotton plant containing the cry1Ac and cry2Ab genes as disclosed in document D3 and faced with the technical problem defined above, would be motivated to provide the combination of cry events according to claim 2.

19. Document D3 already discloses that the proteins Cry1Fa and Cry2Ab2 exhibit a toxicity towards the two *lepidoptera* species beet and fall armyworm which is absent from the Cry1Ac toxin (see Table 1, columns 1 to 3). Furthermore, document D5 discloses that the transgenic cotton plants containing both the cry1Ac and the cry1F gene provide "*season-long protection against a broad spectrum of lepidopteran pest*" (see page 1, third paragraph).

20. Accordingly, the teaching of document D3 combined with that of document D5 provides a motivation for the skilled person seeking to provide cotton plants containing an alternative combination of cry events, to replace the cry2Ab gene with a cry1F gene in combination with the cry1Ac gene.

21. It was further uncontested that the nucleic acid sequences of the cry1F or cry1Ac gene were known before the filing date of the application. The skilled person would then have generated transgenic cotton plants or

their seeds containing cry1F and cry1Ac genes by applying standard cloning and plant-transformation technologies (see e.g. document D3, page 491, column 2, third paragraph to page 492, column 1, third paragraph) and would have applied a standard screening programme to identify those plants exhibiting a desired expression level and pattern of the inserted transgenes. Lastly, the skilled person would have successfully determined the unique chromosomal locations of the inserts in the selected plants by applying standard DNA sequencing technologies.

22. The thus obtained combinations of cry events represent alternative solutions to the technical problem formulated above (see point 16). In the absence of any surprising technical effect linked to the events in the claimed invention and, distinguishing the claimed solution from all other possible solutions, it has to be considered an arbitrary selection of one of several equally available alternatives. The claimed seeds are therefore to be regarded as obvious (see also CLBA, I.D.9.18.7).

23. For the reasons set out above, the subject-matter of claim 2 and hence the main request do not fulfil the requirements of Article 56 EPC.

Auxiliary requests I to III

Inventive step (Article 56 EPC)

24. The subject-matter of claim 2 of auxiliary request I and that of claim 1 of auxiliary request III differs from that of claim 2 of the main request only in that the feature "*and having a representative seed deposited*

with American Type Culture Collection (ATCC) with Accession No. PTA-6233" has been added. However, this additional feature does not change the essence of the subject-matter claimed since it only refers to a deposited representative sample of the claimed seed. Furthermore, the subject-matter of claim 1 of auxiliary request II is identical to that of claim 2 of the main request.

25. Accordingly, the reasoning set out above with regard to the subject-matter of claim 2 of the main request applies *mutatis mutandis* to the subject-matter of claim 2 of auxiliary request I and that of claims 1 of auxiliary requests II and III. As a result, none of the three auxiliary requests fulfils the requirements of Article 56 EPC either.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairwoman:

P. Cremona R. Morawetz

Decision electronically authenticated