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Title of invention:
Marker free transgenic plants: engineering the chloroplast genome without the use of antibiotic selection

Applicants:
Auburn University
University of Central Florida

Headword:
Chloroplast transformation/AUBURN UNIVERSITY

Relevant legal provisions:
EPC Art. 56

Keyword:
"Main and auxiliary request - inventive step (no)"

Decisions cited:
T 0939/92

Catchword:
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Case Number: T 1657/08 **-** 3.3.04

D E C I S I O N
of the Technical Board of Appeal 3.3.04
of 1 February 2012

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Decision under appeal: **Decision of the Examining Division of the European Patent Office posted 17 March 2008 refusing European patent application No. 01918263.3 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chairman: C. Rennie-Smith
Members: G. Alt
R. Morawetz

Summary of Facts and Submissions

I. This is an appeal by the applicants (hereinafter "appellants") against the decision of the examining division whereby the European patent application No. 01 918 263.3, published as International application WO 01/64023, was refused. The title of the application is "Marker free transgenic plants: Engineering the chloroplast genome without the use of antibiotic selection".

II. The following documents are referred to hereinafter:

D1 WO 99/10513

D3 US 5,877,402

D4 US 5,633,153

D5 The Plant Journal, 1994, vol. 6, no. 5, pages 749-758, Holmström, K.-O. et al.

D6 Planta, 1994, vol. 193, pages 155-162, Rathinasabapathi, B. et al.

III. The decision of the examining division was based on a main and an auxiliary request.

Claim 1 of the main request read:

"1. Use of an integration and expression plastid vector competent for stably transforming the plastid genome of a plant where growth is inhibited by an antibiotic-free

phytotoxic aldehyde, said vector comprising as operably joined components:

- a 5' part of the plastid DNA sequence inclusive of a spacer sequence,
- a ribosome binding site and a 5' untranslated region (5'UTR),
- a DNA sequence encoding an aldehyde dehydrogenase acting as a selectable marker which is capable of detoxifying said antibiotic-free phytotoxic aldehyde in the cells to the corresponding nontoxic compound,
- at least one restriction site for the insertion of a heterologous target gene,
- a 3' untranslated region (3' UTR), and
- a 3' part of a plastid DNA sequence inclusive of the spacer sequence,

and wherein said phytotoxic aldehyde is selected from the group consisting of an acetaldehyde, formaldehyde, propronaldehyde, butyraldehyde and betaine aldehyde."

Independent claim 7 of the main request was directed to a method for stably transforming the plastid genome of a plant to confer resistance to antibiotic-free phytotoxic aldehyde.

Claim 1 of the auxiliary request differed from claim 1 of the main request only in that the list of phytotoxic aldehydes was restricted i.e. it referred to betaine aldehyde only.

Dependent claim 6 of the main and the auxiliary request read:

"6. Use of an integration and expression plastid vector according to any of claims 1 to 5 wherein the DNA sequence encoding a detoxifying enzyme encodes betaine aldehyde dehydrogenase (BADH) as a selectable marker which is capable of detoxifying said betaine aldehyde in the cells to glycine betaine."

IV. The examining division decided that neither the subject-matter of the main nor of the auxiliary request met the requirements of Article 56 EPC.

V. The appellants filed a statement setting out their grounds of appeal. The appellants requested "to quash the Decision to refuse the European Patent Application EP No. 01918263.3". Oral proceedings were requested as a subsidiary measure.

VI. In their statement the appellants commented on the decision under appeal. Their arguments may be summarized as follows:

Document D1 disclosed an integration vector for stable transformation of the plastid genome of plants to confer resistance to adverse environmental factors such as salt or drought. In particular, the document dealt with DNA constructs comprising DNA sequences encoding proteins for osmotolerance, such as betaine aldehyde dehydrogenase.

Also document D3 disclosed DNA constructs for the stable transformation of the plastid genome of plants. In particular, they comprised a marker gene for the selection of transformed plants under non-lethal conditions which preferably encoded aminoglycoside 3"-

adenyltransferase, an enzyme conferring resistance to the antibiotics spectinomycin and streptomycin.

Although both documents D1 and D3 could be considered as the closest prior art documents, document D3 was preferred.

The claimed invention differed from the disclosure in document D3 in that the plastid integration and expression construct contained a marker gene for selection under lethal conditions consisting of a DNA sequence encoding an aldehyde dehydrogenase capable of detoxifying a phytotoxic aldehyde. The preferred dehydrogenase is betaine aldehyde dehydrogenase.

The effects achieved by this difference were that the selection (a) took place under lethal conditions, (b) resulted in a 25 fold higher chloroplast transformation efficiency and (c) lead to a more rapid regeneration of chloroplast transgenic plants.

The objective problem to be solved was thus "(i) to provide an alternative to non-lethal (antibiotic) selection markers for plastid transformation (ii) which further allows a higher chloroplast transformation efficiency and a rapid regeneration of chloroplast transgenic plants obtained under antibiotic-free phytotoxic aldehydes selection, and preferably betaine aldehyde (BA) selection, in comparison with spectinomycin/streptomycin selection" (see page 2 of the statement).

The solution as claimed to this problem was not obvious.

Document D3 suggested that it was critical to perform chloroplast transformation under non-lethal selection conditions.

The main teaching of document D4 was directed to nuclear transformation using an aldehyde dehydrogenase, and in particular betaine aldehyde dehydrogenase, for production in the cytoplasm of transgenic plants. Document D4 stated that production of betaine aldehyde dehydrogenase in the cytoplasm may provide for higher selection efficiency. To this aim document D4 disclosed using, inter alia, betaine aldehyde dehydrogenase-encoding genes which were not targeted to the chloroplast. Thus, in view of document D4 the skilled person would have been prompted to select or modify betaine aldehyde dehydrogenase genes for the production of betaine aldehyde dehydrogenase exclusively in the cytoplasm of transgenic plants.

Document D5 disclosed nuclear expression of a betaine aldehyde dehydrogenase-encoding gene from Escherichia coli in plants. The corresponding protein was found either in the cytoplasm or in the chloroplast. The document disclosed that plants in which betaine aldehyde dehydrogenase was present in the chloroplast were not as resistant to betaine aldehyde as those in which the enzyme was located in the cytoplasm, and this was so, although the chloroplast-located betaine aldehyde dehydrogenase had higher activity. As an explanation for these findings the authors of document D5 suggested that betaine aldehyde was not effectively transported to the chloroplast.

Thus, the disclosures in documents D4 and D5 would discourage the skilled person from providing the claimed subject-matter as a solution to the underlying problem and therefore its provision involved an inventive step.

VII. On 25 October 2011 the appellants were summoned to oral proceedings to be held on 1 February 2012. A communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA) was annexed.

In the communication the board noted that it interpreted the appellants' request "to quash [the] Decision to refuse the European Patent Application EP No. 01918263.3" as a request that the decision of the examining division to refuse the application at issue be set aside and that a patent be granted on the basis of the main or the auxiliary request both filed on 25 January 2008.

Moreover, the board informed the appellants about its preliminary opinion on some of the substantive issues.

– In its view claims 1 and 7 of the main request (which corresponds to the main request dealt with by the examining division) lacked clarity for the following reasons:

Due to the term "competent", the expression in claim 1 that the vector was "competent for stably transforming the plastid genome of a plant" could not be regarded as the indication of the purpose of the use, but rather as a characterization of the vector. There was no other expression in claim 1 that could be interpreted as a "purpose".

Thus, since claim 1 was directed to a use without stating its purpose, it had to be considered as unclear.

Moreover, it was unclear what was meant in claim 1 by a plant "**where** growth is inhibited by an ..." (emphasis added).

Finally, the term "antibiotic-free phytotoxic aldehyde" in claims 1 and 7 was unclear because a phytotoxic aldehyde was always "antibiotic-free".

– Moreover the board observed that in its view the subject-matter of claim 1 lacked novelty.

Since claim 1 did not state a purpose for which the "integration and expression plastid vector" was used, the claim had to be interpreted as being directed to the use of the plastid vector for any purpose.

In claim 1 the "DNA sequence encoding an aldehyde dehydrogenase" was defined as "acting as a selectable marker" and as being capable of "detoxifying said phytotoxic aldehyde in the cells to the corresponding non-toxic compound". There was no structural difference between a "DNA sequence encoding an aldehyde dehydrogenase" defined as in claim 1 and one defined as in document D1 on page 41, lines 14 to 17 which conferred osmotolerance. Moreover, both had the capability to detoxify a phytotoxic aldehyde. Thus, document D1 disclosed subject-matter falling under the terms of claim 1.

– With reference to the appellants' submissions in their statement of the grounds of appeal the board remarked the following.

The board considered document D3 as the closest prior art document.

In view of the examples in the application and knowledge from the prior art the board was very hesitant to accept the problem formulated by the appellants in their statement, but rather it considered that the problem to be solved as the provision of means alternative to those disclosed in document D3.

In view of the teachings in document D1 it was not convinced by the appellants' argument that the skilled person would not have considered selection under lethal conditions for chloroplast transformation.

The board further informed the appellant that some of the observations made with regard to the main request also applied to the auxiliary request (which corresponds to the auxiliary request dealt with by the examining division) and raised one further objection of lack of clarity.

VIII. On 30 January 2012 the board received by telefax a letter from the appellants' representative stating that "we hereby inform you that neither the Applicants nor their representatives will attend the Oral Proceedings scheduled for 01.02.2012 at 9.00 hrs in Munich (DE)."

IX. Oral proceedings were held as scheduled. Nobody appeared for the appellant. At the end of the oral proceedings the chairman announced the board's decision.

Reasons for the Decision

Main request

1. The board has serious doubts that claims 1 and 7 are clear and that the subject-matter of claim 1 is novel (see section VII above). However, in view of the board's finding below that the subject-matter of claim 6 of both the main and auxiliary requests lacks an inventive step there is no reason to give a final decision on these issues.

Inventive step (Article 56 EPC)

Inventive step was the only issue dealt with in the decision under appeal.

2. In proceedings before the European Patent Office, the problem-solution-approach is generally applied to assess inventive step. It involves as a first step the identification of the closest prior art document (see Case Law of the Boards of Appeal, 6th edition 2010, I.D.2)

Closest prior art

3. According to established case law the closest prior art document is a document disclosing subject-matter

conceived for the same purpose or aiming at the same objective as the claimed invention and having the most relevant technical features in common (see Case Law of the Boards of Appeal, 6th edition 2010, I.D.3.1).

4. The present application provides a vector for the transformation of the plastid genome of a plant cell. The vector comprises as a marker for the selection of transgenic plant cells a gene which encodes an aldehyde dehydrogenase. This enzyme converts phytotoxic into non-toxic aldehydes. Such a marker system is referred to as "lethal" because only those plants which carry the marker gene, i.e. which are successfully transformed, survive in the presence of the phytotoxic aldehyde.

5. Documents D1 and D3 relate to vectors for the transformation of the plastid genome of plants. Yet, the invention disclosed in document D1 focuses on the provision of so-called "universal" chloroplast vectors, i.e. vectors which can be used to transform the chloroplast genome of multiple species of plants (see for example the paragraph bridging pages 6 and 7 and the second paragraph on page 8) whereas document D3 emphasizes the importance for the efficient transformation of plastids of using marker systems which allow selection under "non-lethal" conditions. According to document D3 (see the paragraph bridging columns 15 and 16) a "non-lethal" selection marker is one where, in contrast to a "lethal" selection marker (see point 4 above), both the transformed and non-transformed cells survive in the presence of the selective agent. Transformed cells are identified by other means, such as for example colour. As a specific

example of a non-lethal marker, document D3 discloses the bacterial aadA gene encoding the aminoglycoside 3"-adenyltransferase which converts the antibiotics spectinomycin and streptomycin into inactive forms (column 19, line 45 to 55; column 34, Example 2).

6. Thus, since both the present application and document D3 focus on the type of marker system to be used for plastid transformation, the board, like the appellants, considers document D3 as the closest prior art document.

Problem to be solved and its solution

7. The appellants consider that the problem to be solved in relation to document D3 is "(i) to provide an alternative to non-lethal (antibiotic) selection markers for plastid transformation (ii) which further allows a higher chloroplast transformation efficiency and a rapid regeneration of chloroplast transgenic plants obtained under antibiotic-free phytotoxic aldehydes selection, and preferably betaine aldehyde (BA) selection, in comparison with spectinomycin/ streptomycin selection" (see section VI above).

8. In view of the disclosure in document D3 highlighting the efficiency of plastid transformation by selection based on the non-lethal marker aminoglycoside 3" adenyltransferase and spectinomycin or streptomycin as selective agents (see point 5 above) and in view of the comparative example in the application disclosing the very same system (see page 10, lines 3 and 4; pages 13 and 14, section "Selection and heightened, rapid regeneration of homoplasmic transgenic plants"), the reference in the problem formulated in point 7 above to

"non-lethal (antibiotic)" selection is taken to refer to selection based on aminoglycoside 3" adenyltransferase.

9. Hence, in other words, the problem to be solved according to the appellants is the provision of selection markers for plastid transformation which are an alternative to the non-lethal aminoglycoside 3" adenyltransferase - spectinomycin/streptomycin selection marker system and by comparison improved in terms of transformation efficiency, regeneration time and the absence of antibiotics.

10. It is established case law (see for example decision T 939/92, EPO OJ 1996, 309; point 2.6 of the reasons) that the objective technical problem, i.e. the problem which is taken into account for the problem-solution approach, is a problem which can be accepted as having been solved by substantially all embodiments of the claims.

11. The solution to the problem formulated above is according to claim 1 the use of a vector for the transformation of any plastid genome from any plant whose growth is inhibited by any of the phytotoxic aldehydes selected from the group of acetaldehyde, formaldehyde, proprionaldehyde, butyraldehyde and betaine aldehyde. Moreover, the vector comprises a DNA sequence encoding any aldehyde dehydrogenase capable of detoxifying the above mentioned aldehydes.

12. Document D1, naming the same inventor and (partly) the same applicant as the present application, discloses on page 5, lines 16 to 22 and in Example 2 - both relating

to the transformation of tobacco - when compared to Examples 3 and 5 to 8 - relating to the transformation of corn, peanut, soybean, sweet potato and grape tissue - that, among these plants, tobacco is the only one for which the selection for resistance to spectinomycin (or streptomycin) on the basis of the enzyme aminoglycoside 3" adenyltransferase is "non-lethal".

13. According to page 18 of the application plants falling under the definition in claim 1 of "a plant where [sic] growth is inhibited by an antibiotic-free phytotoxic aldehyde" are inter alia maize, soybean, peanut, sweet potato and grape.

14. Thus, claim 1 encompasses inter alia the use of the aldehyde dehydrogenase marker system in the transformation of maize, soybean, peanut, sweet potato or grape.

15. However, as far as the use according to claim 1 relates to a use of the betaine aldehyde dehydrogenase marker system in these plants, it cannot be regarded as an alternative to the use of **non-lethal** aminoglycoside 3" adenyltransferase - spectinomycin/streptomycin selection marker system as required by the problem to be solved recited in point 7 above, i.e. to a system which is **not non-lethal** because as follows from the relevant disclosure in document D1 summarized in point 12 above, the aminoglycoside 3" adenyltransferase - spectinomycin/streptomycin selection marker system is a **lethal** system in these plants. Thus, these embodiments of claim 1 are rather to be regarded as alternatives to the **lethal** aminoglycoside 3"

adenyltransferase - spectinomycin/streptomycin selection marker system.

Hence, since only for tobacco the problem to be solved would be defined as the provision of an alternative to the non-lethal aminoglycoside 3" adenyltransferase - spectinomycin/streptomycin selection marker system (see point 12 above), and since the claim also relates to other plants (see point 14 above), the objective technical problem cannot be generally formulated as the provision of alternatives to the non-lethal aminoglycoside3" adenyltransferase - spectinomycin/streptomycin selection marker system.

16. In the board's view, a problem which is derivable from the application as filed and can be considered as being solved by the subject-matter of the claims of the main request may be formulated as the provision of an alternative selection marker system useful in the transformation of plastids which does not require antibiotics as the selective agent.

17. A particular solution to this problem is claimed in claim 6, i.e. the use of betaine aldehyde dehydrogenase which detoxifies the phytotoxic betaine aldehyde to its non-toxic counterpart glycine betaine.

Obviousness of the claimed solution

18. The question to be answered when evaluating the obviousness of the claimed subject-matter is whether or not in view of the problem formulated in point 16 above the skilled person would be motivated to replace the

marker system disclosed in document D3 by, in particular, the one referred to in claim 6.

19. Document D4 discloses in column 1, lines 31 to 37 that "[a]lthough such antibiotic resistance marker gene constructs are useful in generating transformed plants, it is desirable to develop additional selectable marker systems to provide options for plant transformation, for example for a second transformation of a transgenic plant where a second marker is required, and to provide selection systems which do not rely on antibiotic resistance for selection".

Thus, document D4 would not have escaped the skilled person faced with the problem of providing selection systems for plant transformation which do not require antibiotics as the selective agent.

20. Document D4 is concerned with the transformation of the plant nuclear, and not the plant plastid, genome as can be deduced from the absence of any plastid targeting sequences in the transformation constructs disclosed in that document (Example 2).

The marker system disclosed in document D4 relies on the use of an aldehyde dehydrogenase which is capable of detoxifying a phytotoxic aldehyde (see for example the abstract). In particular, document D4 discloses the use of betaine aldehyde dehydrogenase (BADH) to detoxify betaine aldehyde. In view of the experiments, the author of document D4 concludes that "expression of BADH and selection on betaine aldehyde provides a useful selectable marker system for plant transformation" (column 8, lines 49-51).

21. Hence, document D4 discloses the same marker system for plant transformation as the one exemplified in the present application (see for example page 6, lines 21 to 22) and which is also referred to in claim 6 (see section III and point 17 above), with the only difference that it is disclosed in document D4 for the transformation of the nuclear and not the plastid genome of the plant.

22. Since according to the problem formulated in point 16 above the objective of the present application is the provision of a marker system for the transformation of the plant plastid, and in particular the chloroplast, genome, the question arises whether or not the skilled person would contemplate using the system disclosed in document D4, in particular betaine aldehyde dehydrogenase, for selection in the framework of plastid transformation.

23. In contrast to the marker systems dealt with in the closest prior art document D3, betaine aldehyde dehydrogenase provides selection under lethal conditions.

Document D1, published like document D3 in 1999, establishes that selection of chloroplast transformants is possible under lethal conditions (see last paragraphs of Examples 3 and 5 to 8). Thus, in view of the disclosure in document D1 the board is not convinced by the appellants' submission that the skilled person would consider that selection under non-lethal conditions is critical for efficient plant chloroplast transformation and would therefore from the

outset have dismissed the "lethal" marker betaine aldehyde dehydrogenase as an alternative for the non-lethal marker disclosed in document D3.

24. Documents D5 and D6 establish - and this is not contested by the appellants - that (nuclear-genome encoded) betaine aldehyde dehydrogenase (BADH), be it of bacterial (document D5) or plant (document D6) origin, is active in the chloroplast. Document D5 also discloses that the activity of the enzyme is higher in chloroplasts than in the cytoplasm (document D5, page 753, first column, first full paragraph). Both documents disclose that plants are resistant to betaine aldehyde as a consequence of the activity of betaine aldehyde dehydrogenase.

25. Document D6 discloses experiments demonstrating that exogenously supplied betaine aldehyde is efficiently transported into the chloroplast. The results are summarized as follows on page 159, second column:

"[T]he in vivo rates of oxidation of d_3-betaine aldehyde to glycine betaine were significantly correlated (r^2 = 0.77, $P<0.05$) with extractable BADH activity (Fig. 7). Such a correlation would not be expected if transport of betaine aldehyde was rate limiting".

26. In this context the board notes that document D5 discloses that plants in which betaine aldehyde dehydrogenase is present in the cytoplasm are more resistant to betaine aldehyde than plants in which the enzyme is present in chloroplast and that this is so despite the fact that the enzyme is more active in chloroplasts. The authors reason that "[a] possible

explanation for this phenotype is that betaine aldehyde is not effectively transported to the chloroplast where it could be detoxified." (page 753, first column, line 8 from the bottom et seq). However, in the board's view, the skilled person would perceive that this statement does not express a technical reality in view of the way in which it is drafted (see for example the expression "a possible explanation") and since it is not supported by experiments. Moreover, it is contradicted by the experimental results of document D6 (see point 25 above).

27. Thus, in summary, at the priority date of the present application the skilled person knew that exogenously supplied betaine aldehyde is transported into the chloroplast of a plant cell and that it is metabolized there by nuclear-encoded, and chloroplast-located betaine aldehyde dehydrogenase such that the plant cells become resistant to betaine aldehyde.

28. In the board's judgement, this knowledge would give the skilled person good reasons for considering betaine aldehyde dehydrogenase as an alternative to the chloroplast selection system disclosed in document D3 and this is the more so, since it does not rely on the use of antibiotics as the selective agent - which was a desired property at the priority date of the patent (see point 19 above).

29. The appellants argue that that the skilled person would not have considered betaine aldehyde dehydrogenase as an alternative to the chloroplast selection system disclosed in document D3 and this is so not only for the reason that betaine aldehyde dehydrogenase is a

lethal marker (see point 23 above), but also for the following reasons.

30. The appellants submit that the skilled person would derive from the teaching in documents D4 and D5 that the cytoplasmic location of betaine aldehyde dehydrogenase was critical for the proper functioning of the selection with betaine aldehyde.

31. As regards document D4 the appellants point to the sentence highlighted below. The whole passage of which the sentence is part reads (column 5, lines 24 to 42):

"**For detoxification of betaine aldehyde provided in plant cell culture media, production of BADH in the cytoplasm of transgenic plant cells may provide for higher selection efficiency**. The cytoplasm of cells in direct contact with media containing betaine aldehyde likely accumulate [sic] high levels of betaine aldehyde in the cytoplasm, and hence, metabolism of the betaine aldehyde into glycine betaine via the action of BADH, would effectively remove the betaine aldehyde from the cell. There are several approaches that could be used to provide for localization of the BADH protein in the cytoplasm. For example, a BADH gene from an organism which does not require plastid targeting mechanisms could be used, such as BADH from E. coli or yeast. Where plant BADH genes are used, a construct in which the BADH transit peptide region is remove [sic] can be prepared. Alternatively, one could use a translational fusion construct between the BADH cDNA and a protein or peptide fragment that destroys chloroplast targeting and encodes a functional BADH protein".

32. However, in the board's opinion, although the skilled person may find it comprehensible from a common sense point of view - that in order to reach the cytoplasm betaine aldehyde only needs to cross the wall of the plant cell and not the wall of the plant cell and that of the plastid - the skilled person would, in view of the terms "may" and "could", in the first place qualify the information conveyed by the cited passage as an assumption and not as a definite teaching of an exclusive way of proceeding when selecting for chloroplast transformants. The skilled person would find confirmation for this view in the actual experiments disclosed in document D4 where chloroplast targeting was not disrupted.

33. As regards document D5, the appellants refer to the following passage on page 753, first column, second full paragraph, line 13 et seq.:

"Interestingly, transgenic plants where BADH was directed to the chloroplasts were not as resistant to betaine aldehyde as those where BADH was cytoplasmic though the former showed higher BADH activity".

34. However, even if betaine aldehyde dehydrogenase when targeted to chloroplasts shows reduced resistance to betaine aldehyde, it is still efficient in conferring resistance. It is reported on page 753, first column, second full paragraph, lines 18 to 21 that "plants with BADH localized to chloroplast exhibited clearly reduced root growth while the transformants with the enzyme in the cytoplasm showed normal root growth".

Moreover, the results reported and summarized in the passages cited above in points 33 and 34 do not even lead the authors of document D5 to the conclusion that for efficient resistance to betaine aldehyde the location of betaine aldehyde dehydrogenase in the chloroplast should be avoided. It is stated in the last paragraph on page 753:

"In conclusion, we have demonstrated that the bacterial betB gene encoding betaine-aldehydedehydrogenase placed under the control of plant promoters can be expressed in tobacco where it directs the synthesis of the corresponding protein. If the gene is provided with a sequence for chloroplast targeting the protein is imported into the chloroplasts and processed. We have also shown that the enzyme produced is active and able to carry out its normal biological function, biosynthesis of betaine in the cytoplasm as well as in the chloroplast."

35. The skilled person would also not adopt the appellants' interpretation of documents D4 and D5 that the cytoplasmic location of betaine aldehyde dehydrogenase is critical (see point 30 above) for the reason that it would directly contradict the teachings in document D6 (see points 24 and 25 above).

36. Thus, in the board's view, the skilled person would not derive from documents D4 and D5 that the cytoplasmic location of betaine aldehyde dehydrogenase is critical for the proper functioning of the selection with betaine aldehyde.

37. Hence, the appellants' arguments do not convince the board to change its position arrived at in point 28 above.

38. Consequently, the board concludes that in the light of the problem formulated in point 16 above the skilled person would have been motivated in the light of the teachings in documents D4 and D6 to use the selection with betaine aldehyde dehydrogenase as an alternative to the selection system disclosed in document D3. The skilled person would thus have arrived at the subject-matter of dependent claim 6 of the main request in an obvious manner. Consequently, the subject-matter of this claim does not fulfil the requirements of Article 56 EPC. Therefore the main request is rejected.

Auxiliary Request

39. Although claim 1 of this request is restricted to the detoxification of betaine aldehyde, the subject-matter of claim 6 of the auxiliary request is identical with that of claim 6 of the main request (see section III above). Thus, the subject-matter of claim 6 of the auxiliary request does not fulfil the requirements of Article 56 EPC for the reasons given in relation to the main request. Consequently, the auxiliary request is also rejected.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

P. Cremona C. Rennie-Smith