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P.O. BOX 18558, NL-2502 EN THE HAGUE, 29 January 2007

Our ref.: L/DU62/ES/4
Your ref.:

Re.: European Patent application No. 00943766.6
European Patent No. 1 185 161
"Asclepic acid"
In the name of Consejo Superior de Investigaciones Cientificas

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In preparation to the Oral Proceedings of 28 March 2007 the following is submitted.

Article 83: Sufficiency

The present invention relates to a new trait in sunflowers. The trait is a certain fatty acid profile of the oil that is present in the seeds. The trait can be defined as "high palmitic and low palmitoleic and asclepic".

The DNA sequences of the genes or genetic elements that cause this trait were not known at the priority date. It was, however, known that only a limited number of enzymes, i.e. a limited number of genes, is involved in the fatty acid biosynthetic pathway (see my submission of 16 January 2006). The mutagenesis treatment of the

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parents has led to certain mutations in one or more of these genes. These mutations were combined by crossing the parents and the resulting progeny plant has the claimed fatty acid profile.

The set of mutated genes that leads to this profile is in a sense a "black box" because there is no sequence information.

The elements of the black box, although anonymous, do however exist and cause the trait. The black box is inheritable and is thus analogous to a transgene or recombinant DNA sequence, the only difference being that the latter are usually not anonymous.

Article 83 requires that the application discloses the invention in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art.

The invention relates to plants and seeds with a certain trait. The application provides the information to make the black box that causes this trait. The black box is the tool to bestow the desired trait on other plants. This follows from the application which informs the skilled person that the trait, i.e. the black box, is inheritable (cf. paragraphs 19 and 34) and can be bestowed on other plants by crossing (cf. paragraphs 32 and 34).

Example 4 explains how the parents CAS-3 and IG-1297M can be crossed, how to select F2 parent seeds on the basis of defined criteria and that inbred lines of these F2 lines are to be selected for having a high oleic background, more than 20% palmitic acid and less than 4% of both palmitoleic and asclepic acid. This is all the information needed to produce the black box.

It furthermore explains how inbred lines are produced that can be crossed to form hybrid seed which will produce F2 seed

evidencing the desired oil traits of the invention (paragraph 32).

G1/98 in point 3.8 makes a distinction between the development of a plant variety and a genetic engineering invention. The first case is concerned with requirements like homogeneity and stability, whereas the latter case provides tools whereby a desired property can be bestowed on plants by inserting a gene into the genome of those plants.

The next step after having provided this tool is introduction of the gene into a specific plant. According to G1/98 this is a matter of routine breeding. G1/98 realised that the contribution of the inventor in the genetic field made it possible to take the second step and come to a marketable product and therefore wanted to provide the inventor in the genetic field with appropriate protection.

The present invention is no different than the genetic engineering invention of G1/98. Here too, the inventors have provided the genome of a plant with the genetic elements for a desired property. They did not do it by means of genetic engineering but by mutagenesis, crossing and selection but nevertheless the result is the same. They have provided a new trait and described and deposited the means to use this trait in each and every sunflower desired, be it a variety or not.

Thus, the skilled person is provided with all the means to carry out the invention. The OD has stated that the claimed subject matter "can only be carried out by using the deposited sunflower lines" and that "all remaining claimed subject matter cannot be carried out". It is not clear to the proprietor what subject matter that would be. The subject matter of the claims is seed with a certain fatty acid profile. There is no other subject matter than that. And this seed is provided with this trait by the presence in its genome

of a set of mutations in a set of genes that is involved in the fatty acid biosynthesis (the so-called "black box").

Clearly, the deposits are made to enable the skilled person to make the black box that causes the trait, they are intended to be used exactly for that purpose. There is no need to repeat the mutations because they are all present in the deposited material. There is no need to identify them because they are there and can be crossed into other plants by routine breeding techniques.

Rule 28 EPC provides that biological material which is not available to the public and cannot be described may be deposited for the purpose of Article 83. This is exactly what was done here. With the seeds of these deposits and the instructions in Example 4 the skilled person can produce a plant with the desired trait that can be used to introduce the trait in other plants.

Thus, it is totally irrelevant that the mutagenesis process is unrepeatable. There is no need to repeat it for carrying out the invention. There is no need to have information on the specific mutations because the skilled person does not need to go back to the mutagenesis step since he has the deposited parents that have all the relevant mutations.

In view of the above it follows that the invention is sufficiently disclosed and that the claims thus meet the requirements of Article 83 EPC.

Article 53b: Plant varieties

Whether or not the subject matter of a claim relates solely to plant varieties is assessed on the basis of the definition of Rule 23b(4) EPC. This definition is however rather unclear and at least some of its requirements are not capable of distinguishing plant varieties from other plant groupings that are not varieties as I will illustrate hereinbelow.

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In order to assess whether a plant grouping defined in a claim does in fact relate to a plant variety, the following four questions are asked.

1. *Does the plant grouping belong to the lowest taxonomical rank?*
   Article 4.1 of the International Code of Botanical Nomenclature (Saint Louis Code) defines variety as a taxonomical rank below species (cf. Exhibit 1). Thus when a species is claimed the claim does not automatically relate to a plant grouping belonging to a taxon of the lowest known rank.

   For example, when a patent proprietor chooses to claim "watermelons" instead of "plants" in general for the simple reason that the new trait he has invented is only relevant for watermelons he should not be deprived of patent protection because he appears to claim a species.

   The same applies to the present situation. The OD have implied that the claimed subject matter belongs to "any plant grouping within a single botanical taxon of the lowest known rank". This is not correct. "Sunflower" is the species *Helianthus annuus* to which belong multiple subspecies and varieties. Thus, the term "sunflower" does not denominate a taxon of the lowest known rank.

2. *Can the plant grouping be defined by the expression of the characteristics that result from a given genotype or combination of genotypes?*
   This is a very general requirement. The genotype inherently defines the characteristics of a plant. The phenotype is caused by the genotype. Thus, a group of plants, either transgenic or not, that all express a certain pathogen resistance that results from a gene or set of genes or a mutation or a set of mutations, meets this requirement.

However, they are not excluded from patent protection because the pathogen resistance is a general concept that can be applied to all members of the genus or species to which the plants belong, even if varieties can also have the resistance.

This is confirmed by G1/98, which clearly states that reference to "the expression of the characteristics that result from a given genotype or combination of genotypes" is to be considered as a reference to the entire genetic constitution of a plant.

Moreover, according to G1/98 "a plant defined by single recombinant DNA sequences is not a plant grouping to which an entire constitution can be attributed". It is rather an abstract definition defined by "part of its genome or by a property bestowed on it by that part" and embracing an indefinite number of individual entities.

The OD have stated that in the present case the genotype does indeed define the characteristics. However, here the black box of mutations that lead to the altered fatty acid profile is analogous to the "single recombinant DNA sequence". It is abstract and does not define the entire plant. This set can be present in any sunflower plant and confers the desired fatty acid profile. It is not limited to particular varieties. The black box is a constant factor whereas the remainder of the genome is undefined and can be anything.

3. *Is the plant grouping distinguished from any other plant grouping by the expression of at least one of the said characteristics?*
G1/98 has allowed patent protection for plants that incorporate a new feature, wherein the feature is a concept that can be used in different plants. Strictly speaking a

plant grouping in which the individuals all comprise the same transgene that leads for example to pathogen resistance are distinguished from any other plant grouping (namely the groupings that are not resistant to that pathogen) by the expression of that characteristic (resistance gene). Still, such plants would be patentable and would not fall within the exception of Art. 53(b) EPC.

The OD is of the opinion that the condition is met by the distinguishing oil profile. This may be the case when plants having the fatty acid profile of the invention are compared with plant groupings that do not have this trait. But this does not automatically mean that therefore the present sunflowers are a variety.

4. *Is the plant grouping considered as a unity with regard to its suitability for being propagated unchanged?*
For varieties this is a very important requirement. Varieties usually demonstrate multiple desirable traits in an agronomically desirable background. Next generations need to have the same combination of desirable traits in the same background. It cannot be the case that within a new tomato variety that has a particularly sweet taste (which is the new feature), and in addition a bright red colour and is resistant to three different pathogens the next generations are no longer resistant to one of the pathogens and the colour is dark red instead of bright. If this happens still all progeny members have the sweet taste but the plant grouping is not unchanged and is not classified as a variety.

In the framework of G1/98 losing certain desirable properties is not relevant. The only relevant property is the one that is claimed and that is independent from other properties that need to go together in a variety but do not need to be present in plants of the invention.

In the present case the OD has implied that this condition is met when the original sunflower seeds that were mutated were those of a variety. This is not a relevant question since the subject matter of the claims is not directed to the original sunflower seeds. The claims are directed to all sunflower seeds that have the defined fatty acid profile. Since this fatty acid profile can be introduced into each and every sunflower the claims are totally independent from the original parents.

Moreover, since the mission of the EPC is to grant patents and because the EPO has no means to determine whether the claimed plants are variety it appears that when a claim is clearly not directed to plant varieties the EPO should accept such claim as being of a higher order than a claim to a variety *per se* unless and until an opponent proves otherwise.

This being said, it should be noted that the original used seed material RDF-1-532 and the seeds used in Example 3 are not varieties. Rather, they are sunflower populations that are not homogeneous, which is one of the requirements of a variety.

Conclusion

From the above it follows that for claims to seeds and plants some of the questions above may be answered in the affirmative even though such claims are in substance clearly patentable. These questions are therefore not the only tool to decide whether the subject matter of a claim is directed to plant varieties or not. G1/98 also indicated that the substance of a claim is decisive in assessing the subject matter to which the claim is directed.

When properly interpreted it follows that the present claims do not relate to plant varieties. Their substance relates to the concept of introducing a particular set of mutations into

any sunflower plant to bestow upon the oil in the seeds of this sunflower plant a particular fatty acid profile. This concept is broadly applicable.

It should also be kept in mind that the legislator choose to exclude plant varieties in general for two reasons. First, because it was not appropriate to allow patent protection for varieties that fell under the plant variety protection laws of some of the contracting states and the then existing ban on dual protection. Second, he could not exclude only those varieties for which plant variety protection was available because this differed in the various contracting states. The wording of Art. 53b was nothing more than a pragmatic solution to this problem. Everyone agrees that the legislator did not wish to exclude plant patents entirely.

If necessary we can provide a Declaration by Advanta Seeds stating that the trait of the invention was transferred to several of their varieties. This clearly demonstrates that the concept of this invention is of a higher order and is not linked to a particular variety.

Article 53b: Essentially biological process

The OD has indicated that processes for producing new plants by irradiation of the plants or seeds with isotopes would not be excepted from patentability. It is submitted that isotopes are not essentially different from chemicals. Thus, claim 17 of the auxiliary request should also fall outside the exclusion of Article 53b.

Article 56 EPC: Inventive step

The OD has argued that claims 12, 13 and 20-25 lack an inventive step.

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I herewith file a new 1st auxiliary request in which claim 12 has been amended to indicate that the claimed oil is directly obtained from the claimed seeds. This excludes oil mixtures having the same fatty acid profile. Claim 13 is deleted and claims 14-25 have been renumbered.

The OD has defined the problem to be solved as provision of sunflower oil that meets the requirements of the food industry in terms of high thermostability and plasticity to be spread.

It was argued that the solution is well known, i.e. increasing the levels of saturated fatty acids, i.e. palmitic and stearic.

The invention is however not that simple. According to the invention the saturated fatty acids contents are increased while keeping the undesirable fatty acids palmitoleic and asclepic at an acceptably low level.

Paragraphs 5 and 9 describe prior art oils that have increased palmitic levels but then also palmitoleic acid contents above 4% and higher asclepic acid contents.

The inventors have now constructed a plant in which the desaturation of palmitic into palmitoleic in high palmitic mutants could be reduced by incorporating the stearoyl desaturase enzymatic activity of the high stearic mutant lines. This combination leads to oils that maintain reduced levels of palmitoleic and asclepic acid.

There is no indication in the prior art that this combination would lead to such fatty acid profile. The invention is thus not obvious.

Since claim 12 is now limited to oil from seeds that have this particular combination of mutated enzymatic activities and the use claim refer back to claim 12 it appears that also claims

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20-25 (now claims 19-24) meet the requirements of Article 56 EPC.

Additional requests

Further to the requests that are already on file I herewith file a second auxiliary request. In this request claim 1 is a product-by-process claim.

I also reserve the right to file further request if this is deemed necessary in the light of the discussions during the Oral Proceedings.

The Representative,

VAN SOMEREN, Petronella Francisca Hendrika Maria

Encl.

1st AUXILIARY REQUEST

1. Sunflower seeds that contain an oil having an oleic acid content of more than 5% and less than 65% by weight based upon the total fatty acid content, a linoleic acid content of more than 1% and less than 65% by weight based upon the total fatty acid content, a palmitic acid content of more than 20% and less than 40% by weight based upon the total fatty acid content, a stearic acid content of more than 3% and less than 15% based upon the total fatty acid content, characterized in that

- the palmitoleic acid content is less than 4% based upon the total fatty acid content; and
- the asclepic acid content is less than 4% based upon the total fatty acid content.

2. Seeds according to claim 1, characterized in that the palmitoleic acid content in the oil is less than 3% based upon the total fatty acid content.

3. Seeds according to claim 1 or 2, characterized in that the asclepic acid content in the oil is less than 2% based upon the total fatty acid content.

4. Seeds according to claims 1-3, characterized in that the oleic acid content in the oil is at least 40% by weight based upon the total fatty acid content.

5. Seeds according to claims 1-4, characterized in that the total level of saturated fatty acids in the oil is at least 24% by weight based upon the total fatty acid content.

6. Seeds according to claim 5, characterized in that the total level of saturated fatty acids in the oil is at least 35% by weight based upon the total fatty acids content.

7. Seeds according to claim 6, characterized in that the total level of saturated fatty acids in the oil is at least 45% by weight based upon the total fatty acids content.

8. Seeds according to claims 1-7, characterized in that the linoleic acid content in the oil is less than 18% by weight based upon the total fatty acids content.

9. Seeds according to claims 1-8, characterized in that the oil has less than 10% by weight of the saturated fatty acid groups in the 2 position of the triacylglycerol molecules of the oil.

10. Seeds according to claim 9, characterized in that the oil has a maximum of 5% of the saturated fatty acid groups in the 2 position of the triacylglycerol molecules of the oil.

11. Seeds according to any one of the claims 1-10, obtainable by crossing sunflower seeds of the mutant sunflower line IG-1297M deposited on 20 January 1998 with ATCC under deposit accession number ATCC-209591 with the mutant sunflower line CAS-3, deposited on 14 December 1994 with the ATCC under deposit accession number ATCC-75968.

12. Sunflower oil having an oleic acid content of more than 5% and less than 65% by weight based upon the total fatty acid content, a linoleic acid content of more than 1% and less than 65% by weight based upon the total fatty acid content, a palmitic acid content of more than 20% and less than 40% by weight based upon the total fatty acid content, a stearic acid content of more than 3% and less than 15% based upon the total fatty acid content, characterized in that the palmitoleic acid content is less than 4% based upon the total fatty acid content, and the asclepic acid content is less than 4% based upon the total fatty acid content and which oil is directly obtained from seeds as claimed in any one of the claims 1-11.

13. Sunflower plants producing the seeds as claimed in claims 1-11.

14. Plants, obtainable by growing seeds as claimed in claims 1-11.

15. Progeny of the plants according to claims 13-14.

16. Method for preparing sunflower seeds as claimed in 11, comprising the steps of:

a) crossing sunflower seeds of the mutant sunflower line IG-1297M deposited on 20 January 1998 with ATCC under deposit accession number ATCC-209591 with the mutant sunflower line CAS-3, deposited on 14 December 1994 with the ATCC under deposit accession number ATCC-75968;

b) self-pollinating F1 progeny plants of step a) for at least two generations to produce inbred plants.

c) selecting from the progeny of step b) plants with seeds containing an oil having a palmitic acid content of more than 20%, a palmitoleic acid content of less than 4% and an asclepic acid content of less than 3%.

d) collecting progeny seeds from step c) and optionally

e) repeating the cycle of self-pollination, selection and collection of seeds.

17. Method for preparing an oil according to claim 12, by extracting seeds as claimed in claims 1-11.

18. Use of oil according to claims 12 at high temperature conditions.

19. Use of oil as claimed in claim 18, wherein the high temperature conditions constitute baking.

20. Use of oil as claimed in claim 18, wherein the high temperature conditions constitute cooking.

21. Use of oil as claimed in claim 19, wherein the high temperature conditions constitute roasting.

22. Use of oil as claimed in claim 19, wherein the high temperature conditions constitute heating by any means at temperatures of at least 70°C.

23. Use of the oil according to claims 12 in the production of edible fats or fat mixtures, such as margarine or vegetable-dairy.

24. Use of the oil according to claims 12 in confectionery or bakery.

2nd AUXILIARY REQUEST

1. Sunflower seeds that contain an oil having an oleic acid content of more than 5% and less than 65% by weight based upon the total fatty acid content, a linoleic acid content of more than 1% and less than 65% by weight based upon the total fatty acid content, a palmitic acid content of more than 20% and less than 40% by weight based upon the total fatty acid content, a stearic acid content of more than 3% and less than 15% based upon the total fatty acid content, characterized in that

- the palmitoleic acid content is less than 4% based upon the total fatty acid content; and
- the asclepic acid content is less than 4% based upon the total fatty acid content

which seeds are obtainable by a method, comprising the steps of:

a) crossing sunflower seeds of the mutant sunflower line IG-1297M deposited on 20 January 1998 with ATCC under deposit accession number ATCC-209591 with the mutant sunflower line CAS-3, deposited on 14 December 1994 with the ATCC under deposit accession number ATCC-75968;

b) self-pollinating F1 progeny plants of step a) for at least two generations to produce inbred plants.

c) selecting from the progeny of step b) plants with seeds containing an oil having a palmitic acid content of more than 20%, a palmitoleic acid content of less than 4% and an asclepic acid content of less than 3%.

d) collecting progeny seeds from step c) and optionally

e) repeating the cycle of self-pollination, selection and collection of seeds.

2. Hybrid sunflower seeds that contain an oil having an oleic acid content of more than 5% and less than 65% by weight based upon the total fatty acid content, a linoleic acid content of more than 1% and less than 65% by weight based upon the total fatty acid content, a palmitic acid content of more than 20% and less than 40% by weight

based upon the total fatty acid content, a stearic acid content of more than 3% and less than 15% based upon the total fatty acid content,
obtainable by crossing a plant grown from a seed as claimed in claim 1 with another sunflower plant.

3. Seeds according to claim 1 or 2, characterized in that the palmitoleic acid content in the oil is less than 3% based upon the total fatty acid content.

4. Seeds according to claim 1, 2 or 3, characterized in that the asclepic acid content in the oil is less than 2% based upon the total fatty acid content.

5. Seeds according to claims 1-3, characterized in that the oleic acid content in the oil is at least 40% by weight based upon the total fatty acid content.

5. Seeds according to claims 1-4, characterized in that the total level of saturated fatty acids in the oil is at least 24% by weight based upon the total fatty acid content.

6. Seeds according to claim 5, characterized in that the total level of saturated fatty acids in the oil is at least 35% by weight based upon the total fatty acids content.

7. Seeds according to claim 6, characterized in that the total level of saturated fatty acids in the oil is at least 45% by weight based upon the total fatty acids content.

8. Seeds according to claims 1-7, characterized in that the linoleic acid content in the oil is less than 18% by weight based upon the total fatty acids content.

9. Seeds according to claims 1-8, characterized in that the oil has less than 10% by weight of the saturated fatty acid groups in the 2 position of the triacylglycerol molecules of the oil.

10. Seeds according to claim 9, characterized in that the oil has a maximum of 5% of the saturated fatty acid groups in the 2 position of the triacylglycerol molecules of the oil.

11. Seeds according to any one of the claims 1-10, obtainable by crossing sunflower seeds of the mutant sunflower line IG-1297M deposited on 20 January 1998 with ATCC under deposit accession number ATCC-209591 with the mutant sunflower line CAS-3, deposited on 14 December 1994 with the ATCC under deposit accession number ATCC-75968.

12. Sunflower oil having an oleic acid content of more than 5% and less than 65% by weight based upon the total fatty acid content, a linoleic acid content of more than 1% and less than 65% by weight based upon the total fatty acid content, a palmitic acid content of more than 20% and less than 40% by weight based upon the total fatty acid content, a stearic acid content of more than 3% and less than 15% based upon the total fatty acid content, characterized in that the palmitoleic acid content is less than 4% based upon the total fatty acid content, and the asclepic acid content is less than 4% based upon the total fatty acid content and which oil is directly obtained from seeds as claimed in any one of the claims 1-11.

14. Sunflower plants producing the seeds as claimed in claims 1-11.

15. Plants, obtainable by growing seeds as claimed in claims 1-12.

16. Progeny of the plants according to claims 14-15.

17. Method for preparing sunflower seeds as claimed in 11, comprising the steps of:

a) crossing sunflower seeds of the mutant sunflower line IG-1297M deposited on 20 January 1998 with ATCC under deposit accession number ATCC-209591 with the mutant sunflower line CAS-3, deposited on 14 December 1994 with the ATCC under deposit accession number ATCC-75968;

b) self-pollinating F1 progeny plants of step a) for at least two generations to produce inbred plants.

c) selecting from the progeny of step b) plants with seeds containing an oil having a palmitic acid content of more than 20%, a palmitoleic acid content of less than 4% and an asclepic acid content of less than 3%.

d) collecting progeny seeds from step c) and optionally

e) repeating the cycle of self-pollination, selection and collection of seeds.

18. Method for preparing an oil according to claim 12-13, by extracting seeds as claimed in claims 1-11.

19. Use of oil according to claims 12-13 at high temperature conditions.

20. Use of oil as claimed in claim 19, wherein the high temperature conditions constitute baking.

21. Use of oil as claimed in claim 19, wherein the high temperature conditions constitute cooking.

22. Use of oil as claimed in claim 19, wherein the high temperature conditions constitute roasting.

23. Use of oil as claimed in claim 19, wherein the high temperature conditions constitute heating by any means at temperatures of at least 70°C.

24. Use of the oil according to claims 12-13 in the production of edible fats or fat mixtures, such as margarine or vegetable-dairy.

25. Use of the oil according to claims 12-13 in confectionery or bakery.