and anti-platelet aggregating properties. A large number of beneficial health effects have been shown for DHA or mixtures of EPA and DHA.

Vitamin E (tocopherol) is a lipid soluble antioxidant that is important for preventing oxidative damage in both plants and animals and is known to have a beneficial effect in the prevention of cardiovascular disease. Vitamin E naturally occurs in vegetable oils, where it functions to prevent oxidative damage. Vegetable oils therefore represent a useful source of vitamin E in the human diet. Additionally, vitamin E extracted from vegetable oils is used as an additive in other food, health supplement, and cosmetic products.

Up to now it has not been possible to correlate vitamin E concentration with any n-3 VLC-PUFA (i.e., EPA or DHA) component of oil. Vitamin E occurs in plants as various forms of tocopherol, including alpha-, beta-, gamma-, and delta. A study containing 52 landraces and 15 breeding lines of Brassica napus revealed a significant positive correlation between alpha-tocopherol and 18:1+18:2, but no correlation between gamma-tocopherol and any fatty acid component (Li et al. (2013) J Agric Food Chem 61:34-40). Tocopherol concentrations have not been correlated with the degree of unsaturation in various Brassica napus seeds with genetically altered fatty acid composition (Abidi et al (1999) J Am Oil Chem Soc 76, 463-467, and Dolde et al (1999) J Am Oil Chem Soc 76, 349-355).

There is thus the need to provide a reliable source for plants, in particular seeds, comprising tocopherol in preferably high concentrations.

SUMMARY

The disclosure is thus concerned with a method for increasing the tocopherol content of a plant relative to a control plant, comprising expressing in a plant at least one polynucleotide encoding a delta-12-desaturase, at least one polynucleotide encoding a delta-6-desaturase, at least one polynucleotide encoding a delta-6-elongase, and at least one polynucleotide encoding a delta-5-desaturase.

In an aspect, there is provided a method for increasing the tocopherol content of a plant of genus Brassica relative to a control plant, comprising expressing in a plant of genus Brassica at least one polynucleotide encoding a delta-12-desaturase, at least one polynucleotide encoding a delta-6-desaturase, at least one polynucleotide encoding a delta-6-elongase, and at least one polynucleotide encoding a delta-5-desaturase.

In another aspect, there is provided a method for increasing the tocopherol content of a plant of genus Brassica relative to a control plant, comprising expressing in a plant of genus Brassica at least one polynucleotide encoding a delta-12-desaturase, at least one polynucleotide encoding a delta-6-desaturase, at least one polynucleotide encoding a delta-6-elongase, and at least one polynucleotide encoding a delta-5-desaturase and further comprising the step of selecting for a plant having an increased tocopherol content relative to a control plant.

In an example, the method further comprises expressing in the plant at least one polynucleotide encoding an omega-3-desaturase.

In an example, the method further comprises expressing in the plant at least one polynucleotide encoding a delta-5-elongase.

In an example, the method further comprises expressing in the plant at least one polynucleotide encoding a delta-4-desaturase. Preferably, two or more polynucleotides encoding a delta-4-desaturase are expressed. More preferably, at least one polynucleotide encoding a Coenzyme A dependent delta-4-desaturase and at least one polynucleotide encoding a phospholipid dependent delta-4-desaturase.

Preferably, at least two of the further polynucleotides are expressed. Further, the present disclosure contemplates the expression of all three further polynucleotides. Thus, the method may further comprise expressing at least one polynucleotide encoding a delta-5-elongase, at least one polynucleotide encoding a delta-4-desaturase (preferably at least one polynucleotide for a Coenzyme A dependent delta-4 desaturase and at least one for a phospholipid dependent delta-4 desaturase), and at least one polynucleotide encoding an omega-3 desaturase.

Moreover, the method of the present disclosure may further comprise expressing in the plant at least one polynucleotide encoding a delta-15-desaturase.

In an example, at least one polynucleotide encoding a delta-6 elongase from Physcomitrella patens, at least one polynucleotide encoding a delta-12 desaturase from Phythophthora sojae, at least one polynucleotide encoding a delta-6 desaturase from Ostreococcus tauri, at least one polynucleotide encoding a delta-6 elongase from Thalassiosira pseudonana, at least one polynucleotide (preferably at least two polynucleotides) encoding a delta-5 desaturase from Thraustochytrium sp. (preferably from Thraustochytrium sp. ATCC21685), and optionally at least one polynucleotide (preferably, at least two polynucleotides) encoding a omega-3 desaturase from Pythium irregulare, at least one polynucleotide encoding a omega-3-desaturase from Phythophthora infestans, at least one polynucleotide encoding a delta-5 elongase from Ostreococcus tauri, and at least one polynucleotide encoding a delta-4 desaturase from Thraustochytrium sp., and at least one polynucleotide encoding a delta-4 desaturase from Pavlova lutheri are expressed. Preferably, at least two polynucleotides encoding a delta-5 desaturase from Thraustochytrium sp. (preferably from Thraustochytrium sp. ATCC21685) are expressed. Moreover, it is envisaged to express at least two polynucleotides encoding a omega-3 desaturase from Pythium irregulare. As set forth elsewhere herein, also variants of the aforementioned polynucleotides can be expressed.

In accorandance with the method of the present disclosure, it is envisaged that at least one polynucleotide encoding a delta-12-desaturase, at least one polynucleotide encoding a delta-6-desaturase, at least two polynucleotides encoding a delta-6-elongase, at least two polynucleotides encoding a delta-5-desaturase, and optionally at least three polynucleotides encoding an omega-3-desaturase, and at least one polynucleotide encoding a delta-5-elongase, and at least two polynucleotides encoding a delta-4-desaturase are expressed. Preferably, at least one polynucleotide encoding a Coenzyme A dependent delta-4-desaturase and at least one polynucleotide encoding a phospholipid dependent delta-4-desaturase are expressed.

In an example, the polynucleotides are expressed in the seeds of the plant.

In accordance with the present dislcosure, the tocopherol content shall be preferably increased in the seeds of the plant as compared to the tocopherol content in seeds of a control plant, in particular the tocopherol content is increased in the seed oil of the plant as compared to the seed oil of a control plant.

Preferably, the polynucleotides encoding the elongases and desaturases referred to above are recombinant polynucleotides. They may be expressed in a plant by introducing them into the plant by recombinant means such as Agrobacterium-mediated transformation. Thus, the method may comprise the steps of introducing and expressing the above-referenced polynucleotides.

In one example, the method may further comprise the step of selecting for plants having an increased tocopherol content (as compared to a control plant).

In accordance with the present disclosure, polynucleotides are referred to herein above present on one T-DNA or construct (and thus on the same T-DNA or construct). Said construct or T-DNA shall be is stably integrated in the genome of the plant. In an example, the plant is homozygous for the T-DNA. In another example, the plant is hemizygous for the T-DNA. If the plant is homozygous for one T-DNA at one locus, this is nevertheless considered as a single copy herein, i.e. as one copy. Double copy, as used herein, refers to a plant in which two T-DNAs have been inserted, at one or two loci, and in the hemizygous or homozygous state.

The present disclosure also relates to a construct or T-DNA comprising expression cassettes for the polynucleotides as set forth in the context of method of the present dislcosure for increasing the tocopherol content.

Preferably, the construct or T-DNA shall comprise expression cassettes for at least one polynucleotide encoding a delta-12-desaturase, at least one polynucleotide encoding a delta-6-desaturase, at least one polynucleotide encoding a delta-6-elongase, and at least one polynucleotide encoding a delta-5-desaturase, and optionally for at least one of the further polynucleotides encoding the desaturases or elongases referred to above.

The present disclosure further concerns the use of the polynucleotides as set forth in the context of the present disclosure, or of a construct or T-DNA comprising expression cassettes for said polynucleotides for increasing the tocopherol content of a plant relative to control plants.

The present disclosure also relates to a plant comprising expression cassettes for the polynucleotides as referred to in the context of the method of the present disclosure for increasing the tocopherol content, or comprising the T-DNA or construct of the present disclosure.

The present disclosure also relates to a seed of the plant of the present disclosure. Said seed shall comprise expression cassettes for the polynucleotides as referred to in the context of the method of the present disclosure for increasing the tocopherol content, or comprising the T-DNA or construct of the present disclosure. In an example, the seed shall comprise an of oil the present disclosure. The oil is described herein below.

Preferably, the method for increasing the tocopherol content comprises the further step of obtaining an oil from the plant, in particular from the seeds of the plant. Said oil shall have an increased tocopherol content as specified elsewhere herein. In addition, the oil shall have an

increased content of VLC-PUFAs. In accordance with the present disclosure, the oil shall be obtained from the plant under conditions which maintain the tocopherol content. Such methods are well known in the art.

The disclosure also provides methods of producing an oil, wherein the oil has a high content of tocopherol. In addition, the oil may have a high VLC-PUFA content, in particular a high content of EPA and/or DHA. In particularly preferred examples these methods are for producing a corresponding plant oil. Thus, the disclosure also provides methods of producing an oil.

The disclosure also provides methods for creating a plant, such that the plant or progeny thereof can be used as a source of an oil having a high content of tocopherol. Preferably, the oil further has a high VLC-PUFA content, in particular a high content of EPA and/or DHA. Thus, the disclosure beneficially also provides methods for the production of plants having a heritable phenotype of high tocopherol content in seed oil. Further, the plants may have a hight VLC-PUFA content in one or more of their tissues or components, preferably a high content of EPA and/or DHA in seed oil.

DETAILED DESCRIPTION OF THE INVENTION

Various aspects of the invention are hereinafter described in more detail. The definitions and explantions given in the previous section apply accordingly. It is to be understood that the detailed description is not intended to limit the scope of the claims.

Tocopherols are well known in the art. The term “content of tocopherol” preferably refers to the total tocopherol content, i.e. to the sum of the amounts of the tocopherols present in the plant, plant part (preferably in the seed) or oil (in particular in seed oil) thereof. In particular, the term refers to the sum of the amounts of alpha-tocopherol, beta-tocopherol, gamma-tocopherol, and delta-tocopherol. However, it is also envisaged that term refers to the amount of alpha-tocopherol, the amount of beta-tocopherol, andr to the amount of gamma-ocopherol, or the amount of delta-tocopherol. In a preferred embodiment, the term refers to the amount of gamma-tocopherol. In another preferred embodiment, the term term to the amount of delta-tocopherol. Also preferably, the term refers to the amount of total tocopherol, gamma-tocopherol and/or delta-tocopherol.

Thus, “tocopherol” in the context of the present invention, preferably, refers to total tocopherol, alpha-tocopherol, beta-tocopherol, gamma-tocopherol, and/or delta-tocopherol. In particular, the term refers to total tocopherol, gamma-tocopherol, or delta-tocopherol
The term “amount” or “content” preferably refers to the absolute amount or the concentration (preferably, in the plant, more preferably in the seed and most preferably in the seed oil). In an embomdiment, the content of tocopherol is increased in the seed oil of a plant as compared to the seed oil of a control plant.

CLAIMS

1. A method for increasing the tocopherol content of a plant of genus Brassica relative to a control plant, comprising expressing in a plant of genus Brassica at least one polynucleotide encoding a delta-12-desaturase, at least one polynucleotide encoding a delta-6-desaturase, at least one polynucleotide encoding a delta-6-elongase, and at least one polynucleotide encoding a delta-5-desaturase.

2. A method for increasing the tocopherol content of a plant of genus Brassica relative to a control plant, comprising expressing in a plant of genus Brassica at least one polynucleotide encoding a delta-12-desaturase, at least one polynucleotide encoding a delta-6-desaturase, at least one polynucleotide encoding a delta-6-elongase, and at least one polynucleotide encoding a delta-5-desaturase and further comprising the step of selecting for a plant having an increased tocopherol content relative to a control plant.

3. The method according to claim 1 or 2, further comprising expressing in the plant at least one polynucleotide encoding an omega-3-desaturase.

4. The method according to any one of claims 1 to 3, further comprising expressing in the plant at least one polynucleotide encoding a delta-5-elongase.

5. The method according to any one of claims 1 to 4, further comprising expressing in the plant at least one polynucleotide encoding a delta-4-desaturase.

6. The method according to any one of claims 1 to 5, wherein at least one polynucleotide encoding a delta-12-desaturase, at least one polynucleotide encoding a delta-6-desaturase, at least two polynucleotides encoding a delta-6-elongase, at least two polynucleotides encoding a delta-5-desaturase, and optionally at least three polynucleotides encoding an omega-3-desaturase, and at least one polynucleotide encoding a delta-5-elongase, and at least two polynucleotides encoding a delta-4-desaturase are expressed.

7. The method of any one of claims 1 to 6, wherein at least one polynucleotide encoding a delta-6 elongase from Physcomitrella patens, at least one polynucleotide encoding a delta-6 elongase from Thalassiosira pseudonana, at least one polynucleotide encoding a delta-12 desaturase from Phythophthora sojae, at least one polynucleotide encoding a delta-6 desaturase from Ostreococcus tauri, and at least one polynucleotide encoding a delta-5 desaturase from Thraustochytrium sp. (in particular at least two polynucleotides encoding a delta-5 desaturase from Thraustochytrium sp.), and optionally at least one polynucleotides encoding a omega-3 desaturase from Pythium irregulare (in particular at least two polynucleotides encoding a omega-3 desaturase from Pythium irregulare), at least one polynucleotide encoding a omega-3-desaturase from Phythophthora infestans, and at least one polynucleotide encoding a delta-5 elongase from Ostreococcus tauri, and at least one polynucleotide encoding a delta-4 desaturase from Thraustochytrium sp., and at least one polynucleotide encoding a delta-4 desaturase from Pavlova lutheri are expressed.

8. The method of any one of claims 1 to 7, wherein the polynucleotides are recombinant polynucleotides.

9. The method of any one of claims 1 to 8, wherein the polynucleotides are present on one T-DNA or construct which is stably integrated in the genome of the plant.

10. The method of any one of claims 1 to 9, wherein the polynucleotides are expressed in the seeds of the plant.

11. The method of any one of claims 1 to 10, wherein the tocopherol content in the seeds is increased as compared to the tocopherol content in seeds of a control plant.

12. The method of any one of claims 1 to 11, wherein the tocopherol content is the total tocopherol content, the content of alpha-tocopherol, the content of beta-tocopherol, the content of gamma tocopherol, and/or the content of delta-tocopherol.

13. The method of claim 12, wherein the tocopherol content is the total tocopherol content, the content of gamma tocopherol, and/or the content of delta-tocopherol.

14. The method of any one of claims 1 to 13, wherein the plant is of a species comprising a genome of one or two members of the species Brassica oleracea, Brassica nigra and Brassica rapa.

15. The method of claim 14, wherein the plant is of the species Brassica napus, Brassica carinata, Brassica juncea, Brassica oleracea, Brassica nigra or Brassica rapa.

16. The method of any one of claims 1 and 3 to 15, further comprising the step of a selecting for a plant having an increased tocopherol content relative to a control plant.

17. The method of any one claims 1 to 16, further comprising the step of obtaining an oil from the plant, said oil having an increased tocopherol content relative to a control plant.

18. The method of claim 17, wherein said oil is obtained under conditions which maintain the tocopherol content of the oil.