CLAIMS

1. A mutant, non-naturally occurring or transgenic tobacco plant, wherein the plant comprises a plant cell comprising:

(i) at least one polynucleotide comprising, consisting or consisting essentially of a sequence encoding an isopropylmalate synthase and having at least 80% sequence identity to SEQ ID NO:1 or SEQ ID NO:10 or SEQ ID NO: 12 or SEQ ID NO:14; or

(ii) a polypeptide encoded by the polynucleotide(s); or

(iii) a polypeptide having at least 80% sequence identity to SEQ ID NO:2 or SEQ ID NO:11 or SEQ ID NO:13 or SEQ ID NO:15; or

(iv) a construct, vector or expression vector comprising the polynucleotide sequence(s), optionally in which the construct, vector or expression vector additionally comprises a promoter comprising, consisting or consisting essentially of the sequence set forth in SEQ ID NO:8 or a variant thereof with at least ~~about~~ 60% identity thereto or a trichome promoter or a naturally occurring isopropylmalate synthase promoter; and wherein the visual appearance of said tobacco plant is substantially the same as the control tobacco plant; and wherein the chlorophyll content and/or the colouration of the leaves of the mutant, non-naturally occurring or transgenic plant is substantially the same as the control plant at three months after transplant or 36 days after topping.

~~2. A mutant, non-naturally occurring or transgenic plant comprising a plant cell according to Claim 1.~~

~~3~~2. A method for modulating the quantity or type of sucrose esters in at least a part of a tobacco plant, comprising the steps of:

(i) modulating the expression or activity of isopropylmalate synthase in the plant, in which the isopropylmalate synthase comprises a polynucleotide comprising, consisting or consisting essentially of a sequence encoding isopropylmalate synthase and having at least 80% sequence identity to SEQ ID NO:1 or SEQ ID NO:10 or SEQ ID NO: 12 or SEQ ID NO:14; or is encoded by a polypeptide encoded by the polynucleotide(s); or in which the isopropylmalate synthase is encoded by a polypeptide having at least 80% sequence identity to SEQ ID N0:2 or SEQ ID N0:11 or SEQ ID N0:13 or SEQ ID N0:15;

(ii) optionally measuring the quantity or determining the type of one or more sucrose esters in at least a part of the mutant, non-naturally occurring or transgenic plant obtained in step (i); and

(iii) identifying a mutant, non-naturally occurring or transgenic plant in which the quantity or type of the one or more sucrose esters therein has changed in comparison to

a control plant in which the expression or activity of isopropyl malate synthase has not been modulated and, in which the visual appearance of the mutant, non-naturally occurring or transgenic plant is substantially the same as the control plant, and wherein the chlorophyll content and/or the colouration of the leaves of the mutant, non-naturally occurring or transgenic plant is substantially the same as the control plant at three months after transplant or 36 days after topping.

~~4. A mutant, non-naturally occurring or transgenic plant that is obtained or obtainable by a method according to Claim 3.~~

~~5~~3. A mutant, non-naturally occurring or transgenic tobacco plant according to Claim 1, in which expression of isopropylmalate synthase or the activity of the protein encoded thereby is modulated and at least a part of the tobacco plant has a change in the composition of one or more sucrose esters as compared to a control tobacco plant in which the expression or the activity of isopropylmalate synthase has not been modulated ~~and in which the visual appearance of the tobacco plant is substantially the same as the control tobacco plant~~.

~~6~~4. Plant material including biomass, seed or leaves comprising cells or tissue from a plant according to Claim 1 or Claim 3~~4 or Claim 5~~.

~~7~~5. A tobacco product comprising a part of a plant according to Claim 1 or Claim 3~~4 or Claim 5~~ or plant material according to Claim 4~~6~~.

~~8~~6. A method for producing a composition comprising one or more sucrose esters comprising the steps of:

(i) providing at least part of a mutant, non-naturally occurring or transgenic tobacco plant according to Claim 1 or Claim 3~~4 or Claim 5~~, plant material according to Claim 4~~6~~ or a tobacco product according to Claim ~~7~~5;

(ii) extracting the sucrose ester(s) therefrom; and

(iii) optionally, isolating or purifying the extracted sucrose ester(s).

~~97~~. A method according to Claim ~~32~~ or ~~86~~ or the mutant, non-naturally occurring or transgenic plant according to Claims 1 or ~~34 or 5~~, in which one or more of the sucrose esters has the structure as shown in Figure 5 and in which R3 is acetyl or hydrogen, preferably acetyl; one or more of R1, R2 and R4 comprise an acyl chain with 6 carbons, preferably beta-methylvaleryl; and R5 is an acetyl or hydrogen, preferably, hydrogen.

~~108~~. A method for modulating the flavour of tobacco or a tobacco product comprising: (i) adding to tobacco or a tobacco product, a part of a plant, preferably leaves, from a mutant,

non-naturally occurring or transgenic plant according to any of Claims ~~2,~~ 41 or ~~53~~ or plant material according to Claim ~~64~~; or (ii) adding to tobacco or a tobacco product a composition comprising sucrose esters obtained or obtainable by a method according to Claims ~~86~~ or ~~97~~.

~~119~~. A method for producing beta-methylvaleric acid comprising the steps of:
(i) providing at least part of a mutant, non-naturally occurring or transgenic plant according to any of Claims ~~2,~~ 41 or ~~53~~, plant material according to Claim ~~64~~ or a tobacco product according to Claim ~~75~~;
(ii) hydrolysing the material or an extract thereof provided in step (i); and
(iii) optionally isolating or purifying the beta-methylvaleric acid.

~~12. An isolated polynucleotide comprising, consisting or consisting essentially of a sequence encoding an isopropylmalate synthase and having at least 80% sequence identity to SEQ ID NO:1 or SEQ ID NO:10 or SEQ ID NO: 12 or SEQ ID NO:14.~~

~~13. An isolated polypeptide encoded by a polynucleotide set forth in Claim12.~~

~~14. An isolated polypeptide having at least 80% sequence identity to SEQ ID NO:2 or SEQ ID NO:11 or SEQ ID NO:13 or SEQ ID NO:15.~~

~~15. A construct, vector or expression vector comprising an isolated polynucleotide sequence according to Claim 12, optionally in which the construct, vector or expression vector additionally comprises a promoter comprising, consisting or consisting essentially of the sequence set forth in SEQ ID NO:9 or a variant thereof with at least about 60% identity thereto or a trichome promoter or a natural isopropylmalate synthase promoter.~~

transformed via *Agrobacterium* into *Nicotiana tabacum* T.I. 1068, *Nicotiana glutinosa* cv. 24a and *S. pennellii.* The results obtained using *N. tabacum* plants showed some changes in the acyl group abundance of sugar esters through reduced beta-methlyvaleryl and increased 2-methylbutyryl-acylation compared to non-transformed controls. However, the *N. tabacum* plants that were obtained were phenotypically impaired since they were chlorotic and some had curly leaves. Likewise, the transformed *N. glutinosa* and S. *pennellii* plants were phenotypically impaired. The authors conclude that isopropylmalate synthase is a critical enzyme and that its impairment probably would lead to an unhealthy plant.

There is a need in the art for plants in which the sucrose ester composition is modulated and whilst minimising undesirable effects on the plant. It is an object of the present invention to satisfy this need.

SUMMARY ~~OF THE INVENTION~~

The present ~~invention~~ disclsoure is based, at least in part, on the surprising finding that modulating the activity or expression of the isopropylmalate synthase gene from *Nicotiana tabacum* results in a change in sucrose ester composition with fewer undesirable effects on the overall metabolism of the plant. Most of the effects are concentrated in the trichomes of the plant and to secondary metabolism. Thus, modulating the activity or expression of the isopropylmalate synthase gene from *Nicotiana tabacum* does not substantially result in alterations in the visual appearance of the plant as compared to a control plant. This is advantageous because plants can be used for the commercial production of various products where alterations in visual appearance would either not be acceptable to the industry or could result in unacceptably reduced production yields. Advantageously the flavour profile of the aerosol generated from tobacco can be modified such that new flavour profiles can be created. Moreover, the pest resistance of the plants in which the sucrose ester profile has been modified can be changed. Compositions of sucrose esters can be extracted from the plants for various uses - such as in drugs, food additives smoking flavourants and as components of organic pesticides and the like.

In an aspect, there is provided a mutant, non-naturally occurring or transgenic tobacco plant, wherein the plant comprises a plant cell comprising:

(i) at least one polynucleotide comprising, consisting or consisting essentially of a sequence encoding an isopropylmalate synthase and having at least 80% sequence identity to SEQ ID NO:1 or SEQ ID NO:10 or SEQ ID NO: 12 or SEQ ID NO:14; or

(ii) a polypeptide encoded by the polynucleotide(s); or

(iii) a polypeptide having at least 80% sequence identity to SEQ ID NO:2 or SEQ ID NO:11 or SEQ ID NO:13 or SEQ ID NO:15; or

(iv) a construct, vector or expression vector comprising the polynucleotide sequence(s), optionally in which the construct, vector or expression vector additionally comprises a promoter comprising, consisting or consisting essentially of the sequence set forth in SEQ ID NO:8 or a variant thereof with at least 60% identity thereto or a trichome promoter or a naturally occurring isopropylmalate synthase promoter; and wherein the visual appearance of said tobacco plant is substantially the same as the control tobacco plant; and wherein the chlorophyll content and/or the colouration of the leaves of the mutant, non-naturally occurring or transgenic plant is substantially the same as the control plant at three months after transplant or 36 days after topping.

In another aspect, there is provided a method for modulating the quantity or type of sucrose esters in at least a part of a tobacco plant, comprising the steps of:

(i) modulating the expression or activity of isopropylmalate synthase in the plant, in which the isopropylmalate synthase comprises a polynucleotide comprising, consisting or consisting essentially of a sequence encoding isopropylmalate synthase and having at least 80% sequence identity to SEQ ID NO:1 or SEQ ID NO:10 or SEQ ID NO: 12 or SEQ ID NO:14; or is encoded by a polypeptide encoded by the polynucleotide(s); or in which the isopropylmalate synthase is encoded by a polypeptide having at least 80% sequence identity to SEQ ID NO:2 or SEQ ID NO:11 or SEQ ID NO:13 or SEQ ID NO:15;

(ii) optionally measuring the quantity or determining the type of one or more sucrose esters in at least a part of the mutant, non-naturally occurring or transgenic plant obtained in step (i); and

(iii) identifying a mutant, non-naturally occurring or transgenic plant in which the quantity or type of the one or more sucrose esters therein has changed in comparison to a control plant in which the expression or activity of isopropyl malate synthase has not been modulated and, in which the visual appearance of the mutant, non-naturally occurring or transgenic plant is substantially the same as the control plant, and wherein the chlorophyll content and/or the colouration of the leaves of the mutant, non-naturally occurring or transgenic plant is substantially the same as the control plant at three months after transplant or 36 days after topping.

In another aspect, there is provided plant material including biomass, seed or leaves comprising cells or tissue from a plant which is disclosed herein.

In another aspect, there is provided a tobacco product comprising a part of a plant which is disclosed herein.

In another aspect, there is provided a method for producing a composition comprising one or more sucrose esters comprising the steps of:
(i) providing at least part of a mutant, non-naturally occurring or transgenic tobacco plant which is disclosed herein;
(ii) extracting the sucrose ester(s) therefrom; and
(iii) optionally, isolating or purifying the extracted sucrose ester(s).

In another aspect, there is provided a method for modulating the flavour of tobacco or a tobacco product comprising: (i) adding to tobacco or a tobacco product, a part of a plant, preferably leaves, from a mutant, non-naturally occurring or transgenic plant which is disclosed herein; or (ii) adding to tobacco or a tobacco product a composition comprising sucrose esters obtained or obtainable by a method which is disclosed herein.

In another aspect, there is provided a method for producing beta-methylvaleric acid comprising the steps of:
(i) providing at least part of a mutant, non-naturally occurring or transgenic plant which is disclosed herein;
(ii) hydrolysing the material or an extract thereof provided in step (i); and
(iii) optionally isolating or purifying the beta-methylvaleric acid.

ASPECTS AND EMBODIMENTS OF THE INVENTION

Aspect and embodiments of the present invention are set forth in the accompanying claims. In a first aspect there is provided an isolated polynucleotide comprising consisting or consisting essentially of a sequence encoding an isopropylmalate synthase and having at least 60% sequence identity to SEQ ID NO: 1 or SEQ ID NO: 10 or SEQ ID NO: 12 or SEQ ID NO: 14. In another aspect, there is provided an isolated polypeptide encoded by the polynucleotide.

In another aspect, there is provided an isolated polypeptide having at least 60% sequence identity to SEQ ID NO: 2 or SEQ ID NO: 11 or SEQ ID NO: 13 or SEQ ID NO: 15.