Accordingly, acrylamide levels in aerosol can be modulated (*eg.* decreased) by reducing the pool of asparagine generated during curing or drying by modulating (eg. decreasing) the expression or activity of asparagine synthetase that is active during the early phase of curing or drying. Plants are described that have a reduced content of asparagine and exhibit a reduced expression or function of asparagine synthetase. In embodiments, this is achieved using RNA interference or mutation of the asparagine synthetase coding sequences described herein that are expressed during curing or drying. Reducing asparagine levels reduces the amount of acrylamide present in aerosol. When applied to tobacco, this results in lower amounts of acrylamide present in aerosol that is inhaled by a consumer of a smoking article, including a heated or a combusted tobacco product. Further advantages of the present invention are described herein.

ASPECTS AND EMBODIMENTS OF THE INVENTION

Aspects and embodiments of the present invention are set forth in the accompanying claims.

Provided is a mutant, non-naturally occurring or transgenic plant or part thereof having reduced expression or activity of asparagine synthetase, said asparagine synthetase comprising, consisting or consisting essentially of: (i) a polynucleotide sequence comprising, consisting or consisting essentially of a sequence having at least 72% or at least 90% sequence identity to SEQ ID NO:1 or at least 72% sequence identity to SEQ ID NO:3 or SEQ ID NO:5 or SEQ ID NO:7; or (ii) a polypeptide encoded by the polynucleotide set forth in (i); or (iii) a polypeptide having at least 78% sequence identity to SEQ ID NO:2 or SEQ ID NO:4 or SEQ ID NO:6 or SEQ ID NO:8; wherein the expression or activity of the asparagine synthetase set forth in (i), (ii) or (iii) is reduced as compared to a control plant.

In one aspect, there is provided a mutant, non-naturally occurring or transgenic plant or part thereof having reduced expression or activity of asparagine synthetase, said asparagine synthetase comprising, consisting or consisting essentially of : (i) a polynucleotide sequence comprising, consisting or consisting essentially of a sequence having at least 90% sequence identity to SEQ ID NO:1 or at least 72% sequence identity to SEQ ID NO:3 or SEQ ID NO:5 or SEQ ID NO:7; or (ii) a polypeptide encoded by the polynucleotide set forth in (i); or (iii) a polypeptide having at least 78% sequence identity to SEQ ID NO:2 or SEQ ID NO:4 or SEQ ID NO:6 or SEQ ID NO:8; wherein the expression or activity of the asparagine synthetase set forth in (i), (ii) or (iii) is reduced as compared to a control plant.

Suitably, the plant comprises at least one genetic alteration in a regulatory region or in the coding sequence of the polynucleotide encoding the asparagine synthetase.

Suitably, the reduced expression or activity of the asparagine synthetase confers a reduction in the level of asparagine in cured or dried leaf derived from the mutant, non-naturally occurring or transgenic plant or part thereof as compared to the level of asparagine in cured or dried leaf derived from the control plant, suitably wherein the level of asparagine is reduced by at least about 17% as compared to the control plant.

Suitably, the reduced expression or activity of the asparagine synthetase confers a reduction in the level of acrylamide in aerosol produced upon heating or combusting the cured or dried leaf derived from the mutant, non-naturally occurring or transgenic plant or part thereof as compared to cured or dried leaf derived from the control plant, suitably, wherein the level of acrylamide in aerosol is reduced by at least 20% as compared to cured or dried leaf of the control plant.

Suitably, the level of asparagine in cured or dried leaf derived from the mutant, non-naturally occurring or transgenic plant or part thereof is at least about 22% lower and the amount of acrylamide in aerosol produced upon heating or combusting the cured or dried leaf derived from

In another aspect, there is provided plant material or cured or dried plant material from the plant of the present disclosure.

In another aspect, there is provided a plant product comprising at least a part of the plant or the plant material of the present disclosure.

Provided is a method of preparing plant material with reduced levels of asparagine and reduced levels of acrylamide in aerosol derived from said plant material as compared to plant material from a control plant, said method comprising the steps of: (a) providing a plant or part thereof comprising a polynucleotide comprising, consisting or consisting essentially of a sequence encoding an asparagine synthetase and having at least 72% or at least 90 % sequence identity to SEQ ID NO:1 or at least 72 % sequence identity to SEQ ID NO:3 or SEQ ID NO:5 or SEQ ID NO:7; (b) reducing the expression of the polynucleotide or the activity of the protein encoded thereby in the plant or part thereof; (c) harvesting plant material from the plant or part thereof; (d) drying or curing the plant material; (e) optionally, measuring the levels of asparagine in the plant or part thereof and/or measuring the levels of acrylamide in aerosol derived from the plant or part thereof; and (f) obtaining cured or dried plant material with reduced levels of asparagine and reduced levels of acrylamide in aerosol derived from said plant material, suitably, wherein the level of nicotine is substantially the same as the level of nicotine in the control plant; suitably, wherein the formation of glutamine, aspartic acid and glutamic acid is increased as compared to cured or dried leaf of the control plant.

In one aspect, there is provided a method of preparing plant material with reduced levels of asparagine and reduced levels of acrylamide in aerosol derived from said plant material as compared to plant material from a control plant, said method comprising the steps of: (a) providing a plant or part thereof comprising a polynucleotide comprising, consisting or consisting essentially of a sequence encoding an asparagine synthetase and having at least 90 % sequence identity to SEQ ID NO:1 or at least 72 % sequence identity to SEQ ID NO:3 or SEQ ID NO:5 or SEQ ID NO:7; (b) reducing the expression of the polynucleotide or the activity of the protein encoded thereby in the plant or part thereof; (c) harvesting plant material from the plant or part thereof; (d) drying or curing the plant material; (e) optionally, measuring the levels of asparagine in the plant or part thereof and/or measuring the levels of acrylamide in aerosol derived from the plant or part thereof; and (f) obtaining cured or dried plant material with reduced levels of asparagine and reduced levels of acrylamide in aerosol derived from said plant material, suitably, wherein the level of nicotine is substantially the same as the level of nicotine in the control plant; suitably, wherein the formation of glutamine, aspartic acid and glutamic acid is increased as compared to cured or dried leaf of the control plant.

Suitably, the plant material is cured or dried for at least about 3 days after harvesting.

Suitably, the plant material is air cured, suitably, wherein the air cured leaf is sun cured or fire cured.

Suitably, the plant material is air dried or sun dried or fire dried.

In one aspect, there is provided plant material obtained or obtainable by this method.

Provided is a method for identifying one or more genetic alterations in a plant that correlates with reduced levels of asparagine in cured or dried plant material from the plant and reduced levels of acrylamide in aerosol derived from the cured or dried plant or part thereof as compared to a control plant that does not comprise the one or more genetic alterations, said method comprising the steps of: (a) identifying a cured or dried plant with reduced levels of asparagine in plant material from the plant and reduced levels of acrylamide in aerosol derived from the cured or dried plant material as compared to plant material from a control plant; (b) providing a nucleic acid sample from the plant identified in step (a); and (c) identifying in the nucleic acid sample from step (b) one or more genetic alterations in a polynucleotide sequence encoding asparagine synthetase and having at least 72% or at least 90% sequence identity to SEQ ID NO:1 or at least 72% sequence identity to SEQ ID NO:3 or SEQ ID NO:5 or SEQ ID NO:7.

In another aspect, there is provided a method for producing plant material with reduced levels of asparagine and reduced levels of acrylamide in aerosol derived from cured or dried plant material

as compared to a control plant, said method comprising the steps of: (a) providing the plant or the plant material according to the present disclosure; (b) harvesting plant material from the plant; (c) curing or drying the plant material for a period of time; and (d) obtaining cured or dried plant material that has reduced levels of asparagine and reduced levels of acrylamide in aerosol derived from the cured or dried plant material, suitably, wherein the level of nicotine is substantially the same as the level of nicotine in the control plant; suitably, wherein the formation of glutamine, aspartic acid and glutamic acid is increased as compared to the control plant.

Also provided is an isolated polynucleotide sequence comprising, consisting or consisting essentially of a sequence encoding asparagine synthetase and having at least 72% or at least 90% sequence identity to SEQ ID NO:1 or at least 72% sequence identity to SEQ ID NO:3 or SEQ ID NO:5 or SEQ ID NO:7 or comprising, consisting or consisting essentially of a sequence encoding asparagine synthetase and having at least 72% sequence identity to SEQ ID Nos 19, 21 and 23 with the proviso that the sequence with at least 72% sequence identity to SEQ ID Nos 19, 21 and 23 comprises a nucleotide sequence encoding a stop codon at a position equivalent to the location of the stop codon in SEQ ID Nos. 19, 21 and 23, preferably wherein said isolated polynucleotide is a synthetic polynucleotide or cDNA.

Further provided is an isolated polypeptide encoded by the polynucleotide described herein.

Yet further provided is an isolated polypeptide comprising, consisting or consisting essentially of a sequence encoding asparagine synthetase and having at least 78% sequence identity to SEQ ID NO:2 or SEQ ID NO:4 or SEQ ID NO:6 or SEQ ID NO:8 or SEQ ID NO: 20 or SEQ ID NO: 22 or SEQ ID NO: 24 or comprising, consisting or consisting essentially of a sequence encoding asparagine synthetase and having at least 78% sequence identity to SEQ ID NO:2 or SEQ ID NO:4 or SEQ ID NO:6 or SEQ ID NO:8 or SEQ ID NO: 20 or SEQ ID NO: 22 or SEQ ID NO: 24 with the proviso that the sequence with at least 78% sequence identity to SEQ ID NO: 20 or SEQ ID NO: 22 or SEQ ID NO: 24comprises a stop codon at a position equivalent to the location of the stop codon in SEQ ID NO: 20 or SEQ ID NO: 22 or SEQ ID NO: 24, preferably wherein said isolated polynucleotide is a synthetic polynucleotide or cDNA.

Provided a construct, vector or expression vector comprising the isolated polynucleotide described herein.

Provided a mutant, non-naturally occurring or transgenic plant cell from the plant described herein.

Provided cured or dried plant material comprising the cell described herein.

In another aspect, there is provided a tobacco product or a smoking article comprising the plant material described herein.

Combinations of one or more of the embodiments set forth is also disclosed.