agronomic phenotypes such as poor biomass yield and delay in plant maturation. Limiting nitrogen is therefore not a commercially viable approach to reduce TSNA levels. Trying to manipulate nitrate accumulation in tobacco leaf is thus a major challenge.

WO98/58555 describes the treatment of tobacco leaves before or during flue-curing by microwaving for reducing TSNAs. US 5,810,020 describes a process for removing TSNAs from tobacco by contacting the tobacco material with a trapping sink, wherein the trapping sink comprises a select transition metal complex which is readily nitrosated to form a nitrosyl complex with little kinetic or thermodynamic hindrance. US 6,202,649 describes a method of substantially preventing formation of TSNAs by, among other things, curing tobacco in a controlled environment having a sufficient airflow to substantially prevent an anaerobic condition around the vicinity of the tobacco leaf. The controlled environment is provided by controlling one or more curing parameters, such as airflow, humidity, and temperature. However, methods such as these can add considerable cost and time to the production of tobacco and therefore are less likely to be accepted by the tobacco industry. Thus, a need remains for an effective and relatively inexpensive method for reducing TSNAs.

Molecular based methods for reducing the levels of TSNAs in plants are highly desirable since they do not require expensive, and often complex, methods to achieve the reduced levels of TSNAs. One such molecular based approach is disclosed in WO2011/088180. Compositions and methods are disclosed for inhibiting the expression or function of root-specific nicotine demethylase polypeptides that are involved in the metabolic conversion of nicotine to nornicotine in the roots of tobacco plants. The gene sequence of the CYP82E10 nicotine demethylase gene is disclosed. Reducing the expression of this gene was found to reduce the levels of NNN in cured tobacco leaves. Whilst reduced levels of NNN may be obtained, there is more than one TSNA that has been reported to be carcinogenic which will still remain in the modified plants. Other nicotine demethylase genes include CYP82E4 and CYP82E5 which participate in the conversion of nicotine to nornicotine and are described in WO2006091194, WO2008070274 and WO2009064771.

We have now found that modifying certain CLC family genes can also provide an increase in the biomass yield of the plant.

SUMMARY

In one aspect, the present disclosure provides a mutant, non-naturally occurring or transgenic plant cell comprising: (i) a polynucleotide comprising, consisting or consisting essentially of a sequence encoding a member of the CLC family of chloride channels and having at least 60% sequence identity to SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3 or SEQ ID NO:4 or SEQ ID NO:10 or SEQ ID NO:11; (ii) a polypeptide encoded by the polynucleotide set forth in (i); (iii) a polypeptide comprising, consisting or consisting essentially of a sequence encoding a member of the CLC family of chloride channels and having at least 60% sequence identity to SEQ ID NO:5 or SEQ ID

NO:6 or SEQ ID NO:7 or SEQ ID NO:12 or SEQ ID NO:13 or SEQ ID NO:14; or (iv) a construct, vector or expression vector comprising the isolated polynucleotide set forth in (i), and wherein the expression or activity of the polynucleotide or the polypeptide is modulated as compared to a control plant containing a control plant cell and wherein the biomass levels in the mutant, non-naturally occurring or transgenic plant containing the mutant, non-naturally occurring or transgenic plant cell are modulated as compared to the control plant containing the control plant cell.

In another aspect, the present disclosure provides a mutant, non-naturally occurring or transgenic plant or component thereof comprising the plant cell as disclosed herein.

In a further aspect, the present disclosure provides a method for modulating at least the biomass content of a plant or a component thereof, comprising the steps of:

(a) modulating the expression or activity of:

(i) a polynucleotide comprising, consisting or consisting essentially of a sequence encoding a member of the CLC family of chloride channels and having at least 60% sequence identity to SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3 or SEQ ID NO:4 or SEQ ID NO:10 or SEQ ID NO:11;

(ii) a polypeptide encoded by the polynucleotide set forth in (i); or

(iii) a polypeptide comprising, consisting or consisting essentially of a sequence encoding a member of the CLC family of chloride channels and having at least 60% sequence identity to SEQ ID NO:5 or SEQ ID NO:6 or SEQ ID NO:7 or SEQ ID NO:12 or SEQ ID NO:13 or SEQ ID NO:14; and measuring the biomass yield of the mutant;

(b) optionally measuring at least the nitrate content in at least a part of the mutant, non-naturally occurring or transgenic plant obtained in step (a); and

(c) identifying a mutant, non-naturally occurring or transgenic plant in which at least the biomass has changed in comparison to a control plant in which the expression or activity of the polynucleotide or the polypeptide set forth in (a) has not been modulated.

In yet another aspect, the present disclosure provides a plant or a component thereof that is obtained or obtainable by the method as disclosed herein.

In another aspect, the present disclosure provides a plant material including biomass, seed, stem or leaves from the plant as disclosed herein.

In another aspect, the present disclosure provides a tobacco product comprising the plant cell, at least a part of the plant, plant material, or suitably processed plant material as disclosed herein.

In a further aspect, the present disclosure provides an isolated polynucleotide comprising, consisting, or consisting essentially of a sequence encoding a member of the CLC family of chloride channels and having at least 60% sequence identity to SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3 or SEQ ID NO:4 or SEQ ID NO:10 or SEQ ID NO:11.

In yet another aspect, the present disclosure provides a polynucleotide construct, vector, or expression vector comprising a polynucleotide as disclosed herein.

In another aspect, the present disclosure provides an isolated polypeptide encoded by a polynucleotide as disclosed herein.

In another aspect, the present disclosure provides a plant or a part thereof comprising a suitably homozygous mutation in a CLC gene as set forth in SEQ ID No. 15.

The inventors have cloned novel genes encoding various members of the CLC family of chloride channels from plants belonging to the genus *Nicotiana* and denoted as *CLC-Nt2* and *NtCLCe*. Two copies of the orthologous gene originating from two ancestors, *N. tomentosiformis* and *N. sylvestris* exist in *Nicotiana tabacum*, and are denoted herein as *CLC-Nt2-t* and *CLC-Nt2-s* or *NtCLCe-t* and *NtCLCe-s*, respectively. The polynucleotide sequences of these genes are set forth in SEQ ID NOs: 1-4, 10 and 11 and the polypeptide sequences of these genes are set forth in SEQ ID NOs: 5-7 and 12-14. By reducing the expression of these genes in tobacco plants a reduction

in nitrate levels in plants is seen. In particular, a reduction in nitrate levels in green leaves is seen. Total TSNA content after curing of leaves is reduced in these plants. This suggests that reduced levels of nitrate can cause the formation of lower levels of TSNAs in cured plant material – such as cured leaves. The inventors unexpectedly found that a reduction in at least NNK is seen in cured plant material from both NtCLCe-RNAi and CLC-Nt2-RNAi plants. A reduction in total TSNA content was also observed. Reducing the expression of *NtCLCe* and/or *CLC-Nt2* therefore contributes to reducing nitrate levels in tobacco leaves. After curing, at least NNK and optionally other TSNAs, which may include NNN or NAB or NAT or a combination of two or more thereof, can be reduced. In addition, the visual appearance of the plants is not substantially altered which is an important criterion for acceptance by the industry and for maximising plant yields and the like. The inventors have moreover unexpectedly found that certain CLC mutations described herein can result in an increased biomass yield in the plant. Furthermore, the inventors have unexpectedly found that certain CLC mutations described herein can result in modulation of more than one property, for example, modulation of nitrate/TSNA levels as well as modulation of biomass production in plants. The present disclosure may therefore be particularly useful to modulate (*eg.* increase or decrease) levels of nitrate, total TSNAs and/or biomass production in plants, including at least NNK. In particular, the present disclosure may be particularly useful when combined with other methods that are able to reduce the levels of TSNAs. Thus, it may be desirable in certain embodiments to reduce the expression of the one or more polynucleotides described herein together with reducing the expression of one or more nicotine demethylase genes in a tobacco plant. This combination would be expected to reduce at least NNK and NNN levels in a cured plant material which would be highly desirable since NNK and NNN have both been reported to be carcinogenic when applied to animals in laboratory studies. The tobacco products derived from the tobacco plants described herein may find use in methods for reducing the carcinogenic potential of these tobacco products, and reducing the exposure of humans to carcinogenic nitrosamines. Mutants of the polypeptide sequences described herein that can modulate nitrate content and/or biomass production in plants are also described. Therefore some mutants described herein may result in modulation of nitrate/TSNA levels only, whereas some mutants described herein may result in modulation of nitrate/TSNA levels and of biomass production.

ASPECTS AND EMBODIMENTS OF THE INVENTION

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

In a first aspect, there is described a mutant, non-naturally occurring or transgenic plant cell comprising: (i) a polynucleotide comprising, consisting or consisting essentially of a sequence encoding a member of the CLC family of chloride channels and having at least 60% sequence identity to *SEQ ID NO:1* or *SEQ ID NO:2* or *SEQ ID NO:3* or *SEQ ID NO:4* or *SEQ ID NO:10* or SEQ ID NO:11; (ii) a polypeptide encoded by the polynucleotide set forth in (i); (iii) a polypeptide