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(71) Applicant: ALTRIA CLIENT SERVICES LLC
[US/US]; 6601 West Broad Street, Richmond, Virginia
23230 (US).

(72) Inventors: DE GODOY LUSO, Marcos Fernando;
6601 West Broad Street, Richmond, Virginia 23230 (US).
ADAMS, Andrew Carl; 6601 West Broad Street, Rich-
mond, Virginia 23230 (US).

(74) Agent: MARSH, David et al.; 601 Massachusetts Ave.,
NW, Washington, District of Columbia 20001-3743 (US).

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(54) Title: DARK TOBACCO INBREDS AND HYBRIDS COMPRISING REDUCED NICOTINE DEMETHYLATION AND NORNICOTINE

(57) Abstract: The present disclosure provides tobacco inbred plants TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, and CMS KY171 SRC, and hybrids PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, and PD7319 SRC. The present disclosure also provides parts of such plants and products made from those parts. The present disclosure also includes progeny of the provided plants including hybrids.



Dark Tobacco Inbreds and Hybrids Comprising Reduced Nicotine Demethylation and Nornicotine

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application claims priority to U.S. Provisional Application No. 62/420,527, filed November 10, 2016, U.S. Provisional Application No. 62/441,855, filed January 3, 2017 and U.S. Provisional Application No. 62/558,072, filed September 13, 2017. Each of these U.S. Provisional Applications are incorporated by reference herein in their entireties.

Incorporation of Sequence Listing

10 A sequence listing contained in the file name "P34475WO00_SEQ.txt" which is 38,136 bytes (measured in MS-Windows[®]) and created on November 7, 2017, is filed electronically herewith and incorporated by reference in its entirety.

Field

15 The present disclosure provides tobacco inbred plants TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, CMS KY171 SRC and hybrid cultivars PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, and PD7319 SRC. The present disclosure also provides parts of such plants and products made from those parts. The present disclosure also includes progeny of the provided plants including hybrids.

Background

20 Tobacco (*Nicotiana tabacum* L.) is an important commercial crop in the United States as well as in other countries. In tobacco plants, N-demethylation of nicotine results in nornicotine, a secondary alkaloid known to be a precursor for formation of N-Nitrosonornicotine ("NNN") in cured leaves. At high levels, NNN is an undesired
25 component of cured leaves.

30 The predominant alkaloid found in commercial tobacco varieties is nicotine, typically accounting for 90 - 95% of the total alkaloid pool. The remaining alkaloid fraction is comprised primarily of three additional pyridine alkaloids: nornicotine, anabasine, and anatabine. Nornicotine is generated directly from nicotine through the activity of the enzyme nicotine N-demethylase. Nornicotine usually represents less than 5% of the total pyridine alkaloid pool, but through a process termed "conversion," tobacco plants that initially

produce very low amounts of nornicotine give rise to progeny that metabolically “convert” a large percentage of leaf nicotine to nornicotine. In tobacco plants that have genetically converted (termed “converters”), the great majority of nornicotine production occurs during the senescence and curing of the mature leaf (Wernsman and Matzinger (1968), *Tob. Sci.*, 12:226-228). Burley tobaccos are particularly prone to genetic conversion, with rates as high as 20% per generation observed in some cultivars.

During the curing and processing of the tobacco leaf, a portion of the nornicotine is metabolized to the compound NNN, a tobacco-specific nitrosamine (TSNA) that has been reported to be carcinogenic in laboratory animals (Hecht and Hoffmann (1990), *Cancer Surveys*, 8:273-294; Hoffmann *et al.* (1994), *J. Toxicol. Environ. Health*, 41:1-52; Hecht (1998), *Chem. Res. Toxicol.*, 11:559-603). In flue-cured tobaccos, TSNA are found to be predominantly formed through the reaction of alkaloids with the minute amounts of nitrogen oxides present in combustion gases formed by the direct-fired heating systems found in traditional curing barns (Peele and Gentry (1999), “Formation of Tobacco-specific Nitrosamines in Flue-cured Tobacco,” CORESTA Meeting, Agro-Phyto Groups, Suzhou, China). Retrofitting these curing barns with heat-exchangers virtually eliminated the mixing of combustion gases with the curing air and dramatically reduced the formation of TSNA in tobaccos cured in this manner (Boyette and Hamm (2001), *Rec. Adv. Tob. Sci.*, 27:17-22.). In contrast, in the air-cured Burley tobaccos, TSNA formation proceeds primarily through reaction of tobacco alkaloids with nitrite, a process catalyzed by leaf-borne microbes (Bush *et al.* (2001), *Rec. Adv. Tob. Sci.*, 27:23-46). Thus far, attempts to reduce TSNA through modification of curing conditions while maintaining acceptable quality standards continue to be challenging for the air-cured tobaccos.

SUMMARY

In an aspect, the present disclosure includes a seed of tobacco cultivar TND950 (phph) SRC, a representative sample seed of the cultivar having been deposited with the American Type Culture Collection (ATCC) under ATCC Accession No. PTA-123665.

In another aspect, the present disclosure includes a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar TND950 (phph) SRC.

In a further aspect, the present disclosure includes a harvested leaf, or part thereof, of a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar TND950 (phph) SRC.

In an aspect, the present disclosure includes a harvested leaf, or part thereof, of a tobacco plant, produced by growing the seed of tobacco cultivar TND950 (phph) SRC, where the leaf has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN).

5 In an aspect, the present disclosure includes a harvested leaf, or part thereof, produced by growing the seed of tobacco cultivar TND950 (phph) SRC, where the leaf has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN), where the reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) is reduced in a smoke stream produced from the leaf.

10 In a further aspect, the present disclosure includes a tobacco product, prepared from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar TND950 (phph) SRC, where the product is selected from the group consisting of pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, shredded tobacco, cut tobacco, and any combination thereof.

15 In an aspect, the present disclosure includes a tobacco product, prepared from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar TND950 (phph) SRC, where the product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, plug chewing tobacco, moist snuff, and
20 nasal snuff.

In another aspect, the present disclosure includes a tobacco product, prepared from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar TND950 (phph) SRC, where the product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco,
25 cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, and further where the product has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN).

In a further aspect, the present disclosure includes a second tobacco product prepared or produced from a first tobacco product prepared from a tobacco plant, or part thereof,
30 produced by growing the seed of tobacco cultivar TND950 (phph) SRC, where first product is selected from the group consisting of pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, shredded tobacco, cut tobacco and any combination thereof, and the second tobacco product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco,

cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, plug chewing tobacco, moist snuff, and nasal snuff. In some aspects, the first and/or second product comprises a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN).

5 In an aspect, the present disclosure includes a part of a tobacco plant, produced by growing the seed of tobacco cultivar TND950 (phph) SRC, where the part is selected from the group consisting of leaf, pollen, ovule, embryo, cotyledon, hypocotyl, meristematic cell, protoplast, root, root tip, pistil, anther, flower, shoot, stem, pod and petiole.

10 In another aspect, the present disclosure includes a tissue culture produced from a protoplast or cell from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar TND950 (phph) SRC, where the cell or protoplast of the tissue culture is produced from a plant part selected from the group consisting of a leaf, pollen, embryo, cotyledon, hypocotyl, meristematic cell, root, root tip, pistil, anther, flower, shoot, stem, pod, and petiole.

15 In an aspect, the present disclosure includes a tobacco plant regenerated from a tissue culture produced from a protoplast or cell from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar TND950 (phph) SRC, where the cell or protoplast of the tissue culture is produced from a plant part selected from the group consisting of a leaf, pollen, embryo, cotyledon, hypocotyl, meristematic cell, root, root tip, pistil, anther, flower, shoot, stem, pod, and petiole where the regenerated plant has all, or essentially all of the morphological and physiological characteristics of cultivar TND950 (phph) SRC.

20 In an aspect, the present disclosure includes an F₁ progeny plant of tobacco cultivar TND950 (phph) SRC.

25 In another aspect, the present disclosure includes an F₁ progeny plant of tobacco cultivar TND950 (phph) SRC, where the F₁ plant is cytoplasmic male sterile (CMS).

 In another aspect, the present disclosure includes an F₁ progeny seed produced crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar TND950 (phph) SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

30 In another aspect, the present disclosure includes a method for producing a tobacco seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar TND950 (phph) SRC.

In another aspect, the present disclosure includes a method for producing a tobacco seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar TND950 (phph) SRC, where the plant of tobacco cultivar TND950 (phph) SRC is the male parent.

In another aspect, the present disclosure includes a method for producing a tobacco seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar TND950 (phph) SRC, where the plant of tobacco cultivar TND950 (phph) SRC is the female parent.

In another aspect, the present disclosure includes a method for producing a tobacco seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar TND950 (phph) SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present disclosure also includes a container of F₁ progeny seeds produced by crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar TND950 (phph) SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present disclosure includes an F₁ progeny plant produced by growing a seed produced by crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar TND950 (phph) SRC, and where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present disclosure also includes a harvested leaf of an F₁ progeny plant produced by growing a seed produced by a method of comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar TND950 (phph) SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In another aspect, the present disclosure further includes a harvested leaf of an F₁ progeny plant having a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) produced by growing a seed produced by a method of comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar TND950 (phph) SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present disclosure also includes a harvested leaf of an F₁ progeny plant having a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) in a smoke stream produced from the leaf, where the plant is produced by growing a seed produced by crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar TND950 (phph) SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present disclosure includes a tobacco product prepared from an F₁ progeny tobacco plant, or part thereof, where the plant or part thereof is produced by growing a F₁ progeny seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is produced by growing a seed of tobacco cultivar TND950 (phph) SRC, where at least one tobacco plant is cytoplasmic male sterile, and where the tobacco product is selected from the group consisting of pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, shredded tobacco, cut tobacco and any combination thereof..

In an aspect, the present disclosure further includes a tobacco product prepared from an F₁ progeny tobacco plant, or part thereof, where the plant or part thereof is produced by growing a F₁ progeny seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is produced by growing a seed of tobacco cultivar TND950 (phph) SRC, where at least one tobacco plant is cytoplasmic male sterile, and where the tobacco product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, plug chewing tobacco, moist snuff, and nasal snuff.

In an aspect, the present disclosure further includes a tobacco product prepared from an F₁ progeny tobacco plant, or part thereof, where the plant or part thereof is produced by growing a F₁ progeny seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is produced by growing a seed of tobacco cultivar TND950 (phph) SRC, where at least one tobacco plant is cytoplasmic male sterile, and where the tobacco product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, and further where the product has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN).

In an aspect, the present disclosure includes a seed of tobacco cultivar Narrow Leaf Madole (phph) SRC, a representative sample seed of the cultivar having been deposited with the American Type Culture Collection (ATCC) under ATCC Accession No. PTA-123664.

5 In another aspect, the present disclosure includes a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar Narrow Leaf Madole (phph) SRC.

In a further aspect, the present disclosure includes a harvested leaf, or part thereof, of a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar Narrow Leaf Madole (phph) SRC.

10 In an aspect, the present disclosure includes a harvested leaf, or part thereof, of a tobacco plant, produced by growing the seed of tobacco cultivar Narrow Leaf Madole (phph) SRC, where the leaf has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN).

15 In an aspect, the present disclosure includes a harvested leaf, or part thereof, produced by growing the seed of tobacco cultivar Narrow Leaf Madole (phph) SRC, where the leaf has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN), where the reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) is reduced in a smoke stream produced from the leaf.

20 In a further aspect, the present disclosure includes a tobacco product, prepared from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar Narrow Leaf Madole (phph) SRC, where the product is selected from the group consisting of pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, shredded tobacco, cut tobacco, and any combination thereof.

25 In an aspect, the present disclosure includes a tobacco product, prepared from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar Narrow Leaf Madole (phph) SRC, where the product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, plug chewing tobacco, moist snuff, and nasal snuff.

30 In another aspect, the present disclosure includes a tobacco product, prepared from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar Narrow Leaf Madole (phph) SRC, where the product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco,

shredded tobacco, cut tobacco, loose leaf chewing tobacco, and further where the product has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN).

In a further aspect, the present disclosure includes a second tobacco product prepared or produced from a first tobacco product prepared from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar Narrow Leaf Madole (phph) SRC, where first product is selected from the group consisting of pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, shredded tobacco, cut tobacco and any combination thereof, and the second tobacco product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, plug chewing tobacco, moist snuff, and nasal snuff. In some aspects, the first and/or second product comprises a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN).

In an aspect, the present disclosure includes a part of a tobacco plant, produced by growing the seed of tobacco cultivar Narrow Leaf Madole (phph) SRC, where the part is selected from the group consisting of leaf, pollen, ovule, embryo, cotyledon, hypocotyl, meristematic cell, protoplast, root, root tip, pistil, anther, flower, shoot, stem, pod and petiole.

In another aspect, the present disclosure includes a tissue culture produced from a protoplast or cell from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar Narrow Leaf Madole (phph) SRC, where the cell or protoplast of the tissue culture is produced from a plant part selected from the group consisting of a leaf, pollen, embryo, cotyledon, hypocotyl, meristematic cell, root, root tip, pistil, anther, flower, shoot, stem, pod, and petiole.

In an aspect, the present disclosure includes a tobacco plant regenerated from a tissue culture produced from a protoplast or cell from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar Narrow Leaf Madole (phph) SRC, where the cell or protoplast of the tissue culture is produced from a plant part selected from the group consisting of a leaf, pollen, embryo, cotyledon, hypocotyl, meristematic cell, root, root tip, pistil, anther, flower, shoot, stem, pod, and petiole where the regenerated plant has all, or essentially all of the morphological and physiological characteristics of cultivar Narrow Leaf Madole (phph) SRC.

In an aspect, the present disclosure includes an F₁ progeny plant of tobacco cultivar Narrow Leaf Madole (phph) SRC.

In another aspect, the present disclosure includes an F₁ progeny plant of tobacco cultivar Narrow Leaf Madole (phph) SRC, where the F₁ plant is cytoplasmic male sterile (CMS).

5 In another aspect, the present disclosure includes an F₁ progeny seed produced crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar Narrow Leaf Madole (phph) SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

10 In another aspect, the present disclosure includes a method for producing a tobacco seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar Narrow Leaf Madole (phph) SRC.

15 In another aspect, the present disclosure includes a method for producing a tobacco seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar Narrow Leaf Madole (phph) SRC, where the plant of tobacco cultivar Narrow Leaf Madole (phph) SRC is the male parent.

20 In another aspect, the present disclosure includes a method for producing a tobacco seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar Narrow Leaf Madole (phph) SRC, where the plant of tobacco cultivar Narrow Leaf Madole (phph) SRC is the female parent.

25 In another aspect, the present disclosure includes a method for producing a tobacco seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar Narrow Leaf Madole (phph) SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

30 In an aspect, the present disclosure also includes a container of F₁ progeny seeds produced by crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar Narrow Leaf Madole (phph) SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present disclosure includes an F₁ progeny plant produced by growing a seed produced by crossing two tobacco plants and harvesting the resultant tobacco seed,

where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar Narrow Leaf Madole (phph) SRC, and where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present disclosure also includes a harvested leaf of an F₁ progeny plant produced by growing a seed produced by a method of comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar Narrow Leaf Madole (phph) SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In another aspect, the present disclosure further includes a harvested leaf of an F₁ progeny plant having a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) produced by growing a seed produced by a method of comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar Narrow Leaf Madole (phph) SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present disclosure also includes a harvested leaf of an F₁ progeny plant having a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) in a smoke stream produced from the leaf, where the plant is produced by growing a seed produced by crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar Narrow Leaf Madole (phph) SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present disclosure includes a tobacco product prepared from an F₁ progeny tobacco plant, or part thereof, where the plant or part thereof is produced by growing a F₁ progeny seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is produced by growing a seed of tobacco cultivar Narrow Leaf Madole (phph) SRC, where at least one tobacco plant is cytoplasmic male sterile, and where the tobacco product is selected from the group consisting of pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, shredded tobacco, cut tobacco and any combination thereof.

In an aspect, the present disclosure further includes a tobacco product prepared from an F₁ progeny tobacco plant, or part thereof, where the plant or part thereof is produced by growing a F₁ progeny seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is produced by growing a seed of tobacco cultivar Narrow Leaf Madole (phph) SRC, where at least one

tobacco plant is cytoplasmic male sterile, and where the tobacco product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, plug
5 chewing tobacco, moist snuff, and nasal snuff.

In an aspect, the present disclosure further includes a tobacco product prepared from an F₁ progeny tobacco plant, or part thereof, where the plant or part thereof is produced by growing a F₁ progeny seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is produced by
10 growing a seed of tobacco cultivar Narrow Leaf Madole (phph) SRC, where at least one tobacco plant is cytoplasmic male sterile, and where the tobacco product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, and
15 further where the product has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN).

In an aspect, the present disclosure includes a seed of tobacco cultivar Narrow Leaf Madole SRC, a representative sample seed of the cultivar having been deposited with the American Type Culture Collection (ATCC) under ATCC Accession No. PTA-123663.

20 In another aspect, the present disclosure includes a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar Narrow Leaf Madole SRC.

In a further aspect, the present disclosure includes a harvested leaf, or part thereof, of a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar Narrow Leaf Madole SRC.

25 In an aspect, the present disclosure includes a harvested leaf, or part thereof, of a tobacco plant, produced by growing the seed of tobacco cultivar Narrow Leaf Madole SRC, where the leaf has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN).

In an aspect, the present disclosure includes a harvested leaf, or part thereof, produced by growing the seed of tobacco cultivar Narrow Leaf Madole SRC, where the leaf has a
30 reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN), where the reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) is reduced in a smoke stream produced from the leaf.

In a further aspect, the present disclosure includes a tobacco product, prepared from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar Narrow Leaf

Madole SRC, where the product is selected from the group consisting of pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, shredded tobacco, cut tobacco, and any combination thereof.

5 In an aspect, the present disclosure includes a tobacco product, prepared from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar Narrow Leaf Madole SRC, where the product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, plug chewing tobacco, moist snuff, and
10 nasal snuff.

In another aspect, the present disclosure includes a tobacco product, prepared from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar Narrow Leaf Madole SRC, where the product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco,
15 cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, and further where the product has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN).

In a further aspect, the present disclosure includes a second tobacco product prepared or produced from a first tobacco product prepared from a tobacco plant, or part thereof,
20 produced by growing the seed of tobacco cultivar Narrow Leaf Madole SRC, where first product is selected from the group consisting of pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, shredded tobacco, cut tobacco and any combination thereof, and the second tobacco product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe
25 tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, plug chewing tobacco, moist snuff, and nasal snuff. In some aspects, the first and/or second product comprises a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN).

In an aspect, the present disclosure includes a part of a tobacco plant, produced by
30 growing the seed of tobacco cultivar Narrow Leaf Madole SRC, where the part is selected from the group consisting of leaf, pollen, ovule, embryo, cotyledon, hypocotyl, meristematic cell, protoplast, root, root tip, pistil, anther, flower, shoot, stem, pod and petiole.

In another aspect, the present disclosure includes a tissue culture produced from a protoplast or cell from a tobacco plant, or part thereof, produced by growing the seed of

tobacco cultivar Narrow Leaf Madole SRC, where the cell or protoplast of the tissue culture is produced from a plant part selected from the group consisting of a leaf, pollen, embryo, cotyledon, hypocotyl, meristematic cell, root, root tip, pistil, anther, flower, shoot, stem, pod, and petiole.

5 In an aspect, the present disclosure includes a tobacco plant regenerated from a tissue culture produced from a protoplast or cell from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar Narrow Leaf Madole SRC, where the cell or protoplast of the tissue culture is produced from a plant part selected from the group consisting of a leaf, pollen, embryo, cotyledon, hypocotyl, meristematic cell, root, root tip, pistil, anther, flower, 10 shoot, stem, pod, and petiole where the regenerated plant has all, or essentially all of the morphological and physiological characteristics of cultivar Narrow Leaf Madole SRC.

In an aspect, the present disclosure includes an F₁ progeny plant of tobacco cultivar Narrow Leaf Madole SRC.

15 In another aspect, the present disclosure includes an F₁ progeny plant of tobacco cultivar Narrow Leaf Madole SRC, where the F₁ plant is cytoplasmic male sterile (CMS).

In another aspect, the present disclosure includes an F₁ progeny seed produced crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar Narrow Leaf Madole SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

20 In another aspect, the present disclosure includes a method for producing a tobacco seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar Narrow Leaf Madole SRC.

25 In another aspect, the present disclosure includes a method for producing a tobacco seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar Narrow Leaf Madole SRC, where the plant of tobacco cultivar Narrow Leaf Madole SRC is the male parent.

30 In another aspect, the present disclosure includes a method for producing a tobacco seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar Narrow Leaf Madole SRC, where the plant of tobacco cultivar Narrow Leaf Madole SRC is the female parent.

In another aspect, the present disclosure includes a method for producing a tobacco seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar Narrow Leaf Madole SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present disclosure also includes a container of F₁ progeny seeds produced by crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar Narrow Leaf Madole SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present disclosure includes an F₁ progeny plant produced by growing a seed produced by crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar Narrow Leaf Madole SRC, and where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present disclosure also includes a harvested leaf of an F₁ progeny plant produced by growing a seed produced by a method of comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar Narrow Leaf Madole SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In another aspect, the present disclosure further includes a harvested leaf of an F₁ progeny plant having a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) produced by growing a seed produced by a method of comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar Narrow Leaf Madole SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present disclosure also includes a harvested leaf of an F₁ progeny plant having a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) in a smoke stream produced from the leaf, where the plant is produced by growing a seed produced by crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar Narrow Leaf Madole SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present disclosure includes a tobacco product prepared from an F₁ progeny tobacco plant, or part thereof, where the plant or part thereof is produced by growing a F₁ progeny seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is produced by growing a seed of tobacco cultivar Narrow Leaf Madole SRC, where at least one tobacco plant is cytoplasmic male sterile, and where the tobacco product is selected from the group consisting of pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, shredded tobacco, cut tobacco and any combination thereof.

In an aspect, the present disclosure further includes a tobacco product prepared from an F₁ progeny tobacco plant, or part thereof, where the plant or part thereof is produced by growing a F₁ progeny seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is produced by growing a seed of tobacco cultivar Narrow Leaf Madole SRC, where at least one tobacco plant is cytoplasmic male sterile, and where the tobacco product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, plug chewing tobacco, moist snuff, and nasal snuff.

In an aspect, the present disclosure further includes a tobacco product prepared from an F₁ progeny tobacco plant, or part thereof, where the plant or part thereof is produced by growing a F₁ progeny seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is produced by growing a seed of tobacco cultivar Narrow Leaf Madole SRC, where at least one tobacco plant is cytoplasmic male sterile, and where the tobacco product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, and further where the product has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN).

In an aspect, the present disclosure includes a seed of tobacco cultivar CMS TND950 SRC, a representative sample seed of the cultivar having been deposited with the American Type Culture Collection (ATCC) under ATCC Accession No. PTA-123662.

In another aspect, the present disclosure includes a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar CMS TND950 SRC.

In a further aspect, the present disclosure includes a harvested leaf, or part thereof, of a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar CMS TND950 SRC.

5 In an aspect, the present disclosure includes a harvested leaf, or part thereof, of a tobacco plant, produced by growing the seed of tobacco cultivar CMS TND950 SRC, where the leaf has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN).

10 In an aspect, the present disclosure includes a harvested leaf, or part thereof, produced by growing the seed of tobacco cultivar CMS TND950 SRC, where the leaf has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN), where the reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) is reduced in a smoke stream produced from the leaf.

15 In a further aspect, the present disclosure includes a tobacco product, prepared from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar CMS TND950 SRC, where the product is selected from the group consisting of pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, shredded tobacco, cut tobacco, and any combination thereof.

20 In an aspect, the present disclosure includes a tobacco product, prepared from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar CMS TND950 SRC, where the product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, plug chewing tobacco, moist snuff, and nasal snuff.

25 In another aspect, the present disclosure includes a tobacco product, prepared from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar CMS TND950 SRC, where the product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, and further where the product has a
30 reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN).

In a further aspect, the present disclosure includes a second tobacco product prepared or produced from a first tobacco product prepared from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar CMS TND950 SRC, where first product is selected from the group consisting of pipe tobacco, cigar tobacco, cigarette tobacco, chewing

tobacco, leaf tobacco, shredded tobacco, cut tobacco and any combination thereof, and the second tobacco product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, plug chewing tobacco, moist snuff, and nasal snuff. In some aspects, the first and/or second product comprises a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN).

In an aspect, the present disclosure includes a part of a tobacco plant, produced by growing the seed of tobacco cultivar CMS TND950 SRC, where the part is selected from the group consisting of leaf, pollen, ovule, embryo, cotyledon, hypocotyl, meristematic cell, protoplast, root, root tip, pistil, anther, flower, shoot, stem, pod and petiole.

In another aspect, the present disclosure includes a tissue culture produced from a protoplast or cell from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar CMS TND950 SRC, where the cell or protoplast of the tissue culture is produced from a plant part selected from the group consisting of a leaf, pollen, embryo, cotyledon, hypocotyl, meristematic cell, root, root tip, pistil, anther, flower, shoot, stem, pod, and petiole.

In an aspect, the present disclosure includes a tobacco plant regenerated from a tissue culture produced from a protoplast or cell from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar CMS TND950 SRC, where the cell or protoplast of the tissue culture is produced from a plant part selected from the group consisting of a leaf, pollen, embryo, cotyledon, hypocotyl, meristematic cell, root, root tip, pistil, anther, flower, shoot, stem, pod, and petiole where the regenerated plant has all, or essentially all of the morphological and physiological characteristics of cultivar CMS TND950 SRC.

In an aspect, the present disclosure includes an F₁ progeny plant of tobacco cultivar CMS TND950 SRC.

In another aspect, the present disclosure includes an F₁ progeny plant of tobacco cultivar CMS TND950 SRC, where the F₁ plant is cytoplasmic male sterile (CMS).

In another aspect, the present disclosure includes an F₁ progeny seed produced crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar CMS TND950 SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In another aspect, the present disclosure includes a method for producing a tobacco seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where

at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar CMS TND950 SRC.

In another aspect, the present disclosure includes a method for producing a tobacco seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where
5 at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar CMS TND950 SRC, where the plant of tobacco cultivar CMS TND950 SRC is the male parent.

In another aspect, the present disclosure includes a method for producing a tobacco seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where
10 at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar CMS TND950 SRC, where the plant of tobacco cultivar CMS TND950 SRC is the female parent.

In another aspect, the present disclosure includes a method for producing a tobacco seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where
15 at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar CMS TND950 SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present disclosure also includes a container of F₁ progeny seeds produced by crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar
20 CMS TND950 SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present disclosure includes an F₁ progeny plant produced by growing a seed produced by crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar CMS TND950 SRC, and where at least one tobacco plant is cytoplasmic male sterile
25 (CMS).

In an aspect, the present disclosure also includes a harvested leaf of an F₁ progeny plant produced by growing a seed produced by a method of comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar CMS TND950 SRC, where at least
30 one tobacco plant is cytoplasmic male sterile (CMS).

In another aspect, the present disclosure further includes a harvested leaf of an F₁ progeny plant having a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) produced by growing a seed produced by a method of comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant

produced by growing the seed of tobacco cultivar CMS TND950 SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present disclosure also includes a harvested leaf of an F₁ progeny plant having a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) in a smoke stream produced from the leaf, where the plant is produced by growing a seed produced by crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar CMS TND950 SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present disclosure includes a tobacco product prepared from an F₁ progeny tobacco plant, or part thereof, where the plant or part thereof is produced by growing a F₁ progeny seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is produced by growing a seed of tobacco cultivar CMS TND950 SRC, where at least one tobacco plant is cytoplasmic male sterile, and where the tobacco product is selected from the group consisting of pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, shredded tobacco, cut tobacco and any combination thereof.

In an aspect, the present disclosure further includes a tobacco product prepared from an F₁ progeny tobacco plant, or part thereof, where the plant or part thereof is produced by growing a F₁ progeny seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is produced by growing a seed of tobacco cultivar CMS TND950 SRC, where at least one tobacco plant is cytoplasmic male sterile, and where the tobacco product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, plug chewing tobacco, moist snuff, and nasal snuff.

In an aspect, the present disclosure further includes a tobacco product prepared from an F₁ progeny tobacco plant, or part thereof, where the plant or part thereof is produced by growing a F₁ progeny seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is produced by growing a seed of tobacco cultivar CMS TND950 SRC, where at least one tobacco plant is cytoplasmic male sterile, and where the tobacco product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah

tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, and further where the product has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN).

In an aspect, the present disclosure includes a method for producing a tobacco seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at
5 least one tobacco plant is cytoplasmic male sterile, where the cytoplasmic male sterile plant is a plant of tobacco cultivar CMS TND950 SRC.

In an aspect, the present disclosure includes a seed of tobacco cultivar CMS Narrow Leaf Madole SRC, a representative sample seed of the cultivar having been deposited with the American Type Culture Collection (ATCC) under ATCC Accession No. PTA-123661.

10 In another aspect, the present disclosure includes a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar CMS Narrow Leaf Madole SRC.

In a further aspect, the present disclosure includes a harvested leaf, or part thereof, of a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar CMS Narrow Leaf Madole SRC.

15 In an aspect, the present disclosure includes a harvested leaf, or part thereof, of a tobacco plant, produced by growing the seed of tobacco cultivar CMS Narrow Leaf Madole SRC, where the leaf has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN).

In an aspect, the present disclosure includes a harvested leaf, or part thereof, produced
20 by growing the seed of tobacco cultivar CMS Narrow Leaf Madole SRC, where the leaf has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN), where the reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) is reduced in a smoke stream produced from the leaf.

In a further aspect, the present disclosure includes a tobacco product, prepared from a
25 tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar CMS Narrow Leaf Madole SRC, where the product is selected from the group consisting of pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, shredded tobacco, cut tobacco, and any combination thereof.

In an aspect, the present disclosure includes a tobacco product, prepared from a
30 tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar CMS Narrow Leaf Madole SRC, where the product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco,

shredded tobacco, cut tobacco, loose leaf chewing tobacco, plug chewing tobacco, moist snuff, and nasal snuff.

In another aspect, the present disclosure includes a tobacco product, prepared from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar CMS Narrow Leaf Madole SRC, where the product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, and further where the product has a reduced amount of normicotine and/or N'-nitrosonornicotine (NNN).

In a further aspect, the present disclosure includes a second tobacco product prepared or produced from a first tobacco product prepared from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar CMS Narrow Leaf Madole SRC, where first product is selected from the group consisting of pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, shredded tobacco, cut tobacco and any combination thereof, and the second tobacco product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, plug chewing tobacco, moist snuff, and nasal snuff. In some aspects, the first and/or second product comprises a reduced amount of normicotine and/or N'-nitrosonornicotine (NNN).

In an aspect, the present disclosure includes a part of a tobacco plant, produced by growing the seed of tobacco cultivar CMS Narrow Leaf Madole SRC, where the part is selected from the group consisting of leaf, pollen, ovule, embryo, cotyledon, hypocotyl, meristematic cell, protoplast, root, root tip, pistil, anther, flower, shoot, stem, pod and petiole.

In another aspect, the present disclosure includes a tissue culture produced from a protoplast or cell from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar CMS Narrow Leaf Madole SRC, where the cell or protoplast of the tissue culture is produced from a plant part selected from the group consisting of a leaf, pollen, embryo, cotyledon, hypocotyl, meristematic cell, root, root tip, pistil, anther, flower, shoot, stem, pod, and petiole.

In an aspect, the present disclosure includes a tobacco plant regenerated from a tissue culture produced from a protoplast or cell from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar CMS Narrow Leaf Madole SRC, where the cell or protoplast of the tissue culture is produced from a plant part selected from the group

consisting of a leaf, pollen, embryo, cotyledon, hypocotyl, meristematic cell, root, root tip, pistil, anther, flower, shoot, stem, pod, and petiole where the regenerated plant has all, or essentially all of the morphological and physiological characteristics of cultivar CMS Narrow Leaf Madole SRC.

5 In an aspect, the present disclosure includes an F₁ progeny plant of tobacco cultivar CMS Narrow Leaf Madole SRC.

 In another aspect, the present disclosure includes an F₁ progeny plant of tobacco cultivar CMS Narrow Leaf Madole SRC, where the F₁ plant is cytoplasmic male sterile (CMS).

10 In another aspect, the present disclosure includes an F₁ progeny seed produced crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar CMS Narrow Leaf Madole SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

15 In another aspect, the present disclosure includes a method for producing a tobacco seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar CMS Narrow Leaf Madole SRC.

 In another aspect, the present disclosure includes a method for producing a tobacco
20 seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar CMS Narrow Leaf Madole SRC, where the plant of tobacco cultivar CMS Narrow Leaf Madole SRC is the male parent.

 In another aspect, the present disclosure includes a method for producing a tobacco
25 seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar CMS Narrow Leaf Madole SRC, where the plant of tobacco cultivar CMS Narrow Leaf Madole SRC is the female parent.

 In another aspect, the present disclosure includes a method for producing a tobacco
30 seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar CMS Narrow Leaf Madole SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present disclosure also includes a container of F₁ progeny seeds produced by crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar CMS Narrow Leaf Madole SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present disclosure includes an F₁ progeny plant produced by growing a seed produced by crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar CMS Narrow Leaf Madole SRC, and where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present disclosure also includes a harvested leaf of an F₁ progeny plant produced by growing a seed produced by a method of comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar CMS Narrow Leaf Madole SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In another aspect, the present disclosure further includes a harvested leaf of an F₁ progeny plant having a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) produced by growing a seed produced by a method of comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar CMS Narrow Leaf Madole SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present disclosure also includes a harvested leaf of an F₁ progeny plant having a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) in a smoke stream produced from the leaf, where the plant is produced by growing a seed produced by crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar CMS Narrow Leaf Madole SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present disclosure includes a tobacco product prepared from an F₁ progeny tobacco plant, or part thereof, where the plant or part thereof is produced by growing a F₁ progeny seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is produced by growing a seed of tobacco cultivar CMS Narrow Leaf Madole SRC, where at least one tobacco plant is cytoplasmic male sterile, and where the tobacco product is selected from the

group consisting of pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, shredded tobacco, cut tobacco and any combination thereof..

5 In an aspect, the present disclosure further includes a tobacco product prepared from an F₁ progeny tobacco plant, or part thereof, where the plant or part thereof is produced by growing a F₁ progeny seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is produced by growing a seed of tobacco cultivar CMS Narrow Leaf Madole SRC, where at least one tobacco plant is cytoplasmic male sterile, and where the tobacco product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter
10 cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, plug chewing tobacco, moist snuff, and nasal snuff.

In an aspect, the present disclosure further includes a tobacco product prepared from an F₁ progeny tobacco plant, or part thereof, where the plant or part thereof is produced by
15 growing a F₁ progeny seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is produced by growing a seed of tobacco cultivar CMS Narrow Leaf Madole SRC, where at least one tobacco plant is cytoplasmic male sterile, and where the tobacco product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter
20 cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, and further where the product has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN).

In an aspect, the present disclosure includes a method for producing a tobacco seed
25 comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is cytoplasmic male sterile, where the cytoplasmic male sterile plant is a plant of tobacco cultivar CMS Narrow Leaf Madole SRC.

In an aspect, the present disclosure includes a seed of tobacco cultivar CMS KY171 SRC, a representative sample seed of the cultivar having been deposited with the American
30 Type Culture Collection (ATCC) under ATCC Accession No. PTA-123660.

In another aspect, the present disclosure includes a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar CMS KY171 SRC.

In a further aspect, the present disclosure includes a harvested leaf, or part thereof, of a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar CMS KY171 SRC.

5 In an aspect, the present disclosure includes a harvested leaf, or part thereof, of a tobacco plant, produced by growing the seed of tobacco cultivar CMS KY171 SRC, where the leaf has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN).

10 In an aspect, the present disclosure includes a harvested leaf, or part thereof, produced by growing the seed of tobacco cultivar CMS KY171 SRC, where the leaf has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN), where the reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) is reduced in a smoke stream produced from the leaf.

15 In a further aspect, the present disclosure includes a tobacco product, prepared from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar CMS KY171 SRC, where the product is selected from the group consisting of pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, shredded tobacco, cut tobacco, and any combination thereof.

20 In an aspect, the present disclosure includes a tobacco product, prepared from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar CMS KY171 SRC, where the product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, plug chewing tobacco, moist snuff, and nasal snuff.

25 In another aspect, the present disclosure includes a tobacco product, prepared from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar CMS KY171 SRC, where the product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, and where the product has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN).

30 In a further aspect, the present disclosure includes a second tobacco product prepared or produced from a first tobacco product prepared from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar CMS KY171 SRC, where first product is selected from the group consisting of pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, shredded tobacco, cut tobacco and any combination thereof, and the

second tobacco product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, plug chewing tobacco, moist snuff, and nasal snuff.

5 In some aspects, the first and/or second product comprises a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN).

In an aspect, the present disclosure includes a part of a tobacco plant, produced by growing the seed of tobacco cultivar CMS KY171 SRC, where the part is selected from the group consisting of leaf, pollen, ovule, embryo, cotyledon, hypocotyl, meristematic cell,
10 protoplast, root, root tip, pistil, anther, flower, shoot, stem, pod and petiole.

In another aspect, the present disclosure includes a tissue culture produced from a protoplast or cell from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar CMS KY171 SRC, where the cell or protoplast of the tissue culture is produced from a plant part selected from the group consisting of a leaf, pollen, embryo,
15 cotyledon, hypocotyl, meristematic cell, root, root tip, pistil, anther, flower, shoot, stem, pod, and petiole.

In an aspect, the present disclosure includes a tobacco plant regenerated from a tissue culture produced from a protoplast or cell from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar CMS KY171 SRC, where the cell or protoplast of the
20 tissue culture is produced from a plant part selected from the group consisting of a leaf, pollen, embryo, cotyledon, hypocotyl, meristematic cell, root, root tip, pistil, anther, flower, shoot, stem, pod, and petiole where the regenerated plant has all, or essentially all of the morphological and physiological characteristics of cultivar CMS KY171 SRC.

In an aspect, the present disclosure includes an F₁ progeny plant of tobacco cultivar
25 CMS KY171 SRC.

In another aspect, the present disclosure includes an F₁ progeny plant of tobacco cultivar CMS KY171 SRC, where the F₁ plant is cytoplasmic male sterile (CMS).

In another aspect, the present disclosure includes an F₁ progeny seed produced crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one
30 tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar CMS KY171 SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In another aspect, the present disclosure includes a method for producing a tobacco seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where

at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar CMS KY171 SRC.

In another aspect, the present disclosure includes a method for producing a tobacco seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where
5 at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar CMS KY171 SRC, where the plant of tobacco cultivar CMS KY171 SRC is the male parent.

In another aspect, the present disclosure includes a method for producing a tobacco seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where
10 at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar CMS KY171 SRC, where the plant of tobacco cultivar CMS KY171 SRC is the female parent.

In another aspect, the present disclosure includes a method for producing a tobacco seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where
15 at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar CMS KY171 SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present disclosure also includes a container of F₁ progeny seeds produced by crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar CMS KY171 SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

20 In an aspect, the present disclosure includes an F₁ progeny plant produced by growing a seed produced by crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar CMS KY171 SRC, and where at least one tobacco plant is cytoplasmic male sterile (CMS).

25 In an aspect, the present disclosure also includes a harvested leaf of an F₁ progeny plant produced by growing a seed produced by a method of comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar CMS KY171 SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

30 In another aspect, the present disclosure further includes a harvested leaf of an F₁ progeny plant having a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) produced by growing a seed produced by a method of comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant

produced by growing the seed of tobacco cultivar CMS KY171 SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present disclosure also includes a harvested leaf of an F₁ progeny plant having a reduced amount of nornicotine and/or N'-nitrosornicotine (NNN) in a smoke stream produced from the leaf, where the plant is produced by growing a seed produced by crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar CMS KY171 SRC, where at least one tobacco plant is cytoplasmic male sterile (CMS).

In an aspect, the present disclosure includes a tobacco product prepared from an F₁ progeny tobacco plant, or part thereof, where the plant or part thereof is produced by growing a F₁ progeny seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is produced by growing a seed of tobacco cultivar CMS KY171 SRC, where at least one tobacco plant is cytoplasmic male sterile, and where the tobacco product is selected from the group consisting of pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, shredded tobacco, cut tobacco and any combination thereof.

In an aspect, the present disclosure further includes a tobacco product prepared from an F₁ progeny tobacco plant, or part thereof, where the plant or part thereof is produced by growing a F₁ progeny seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is produced by growing a seed of tobacco cultivar CMS KY171 SRC, where at least one tobacco plant is cytoplasmic male sterile, and where the tobacco product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, plug chewing tobacco, moist snuff, and nasal snuff.

In an aspect, the present disclosure further includes a tobacco product prepared from an F₁ progeny tobacco plant, or part thereof, where the plant or part thereof is produced by growing a F₁ progeny seed produced by a method comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at least one tobacco plant is produced by growing a seed of tobacco cultivar CMS KY171 SRC, where at least one tobacco plant is cytoplasmic male sterile, and where the tobacco product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah

tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, and further where the product has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN).

In an aspect, the present disclosure includes a method for producing a tobacco seed comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where at
5 least one tobacco plant is cytoplasmic male sterile, where the cytoplasmic male sterile plant is a plant of tobacco cultivar CMS KY171 SRC.

In another aspect, the present disclosure includes seed of hybrid tobacco cultivars PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, and PD7319 SRC. Seeds of hybrid cultivar PD7305 SRC are obtainable by crossing CMS TND950 SRC dark tobacco
10 with pollen of TND950 (phph) SRC dark tobacco. Seeds of hybrid cultivar PD7309 SRC are obtainable by crossing CMS Narrow Leaf Madole SRC dark tobacco with pollen of Narrow Leaf Madole (phph) SRC dark tobacco. Seeds of hybrid cultivar PD7312 SRC are obtainable by crossing CMS KY171 SRC dark tobacco with pollen of Narrow Leaf Madole SRC dark tobacco. Seeds of hybrid cultivar PD7318 SRC are obtainable by crossing CMS KY171 SRC
15 dark tobacco with pollen of Narrow Leaf Madole (phph) SRC dark tobacco. Seeds of hybrid cultivar PD7319 SRC are obtainable by crossing CMS TND950 SRC dark tobacco with pollen of Narrow Leaf Madole (phph) SRC dark tobacco, and collecting the seeds.

In an aspect, the present disclosure includes a tobacco plant, or part thereof, produced by growing a seed of hybrid tobacco cultivar PD7305 SRC, PD7309 SRC, PD7312 SRC,
20 PD7318 SRC, or PD7319 SRC.

In a further aspect, the present disclosure includes a harvested leaf, or part thereof, of a tobacco plant produced by growing the seed of hybrid tobacco cultivar PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC.

In another aspect, the present disclosure includes a harvested leaf, or part thereof, of a
25 tobacco plant, produced by growing the seed of hybrid tobacco cultivar PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC, where the leaf has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN).

In an aspect, the present disclosure includes a harvested leaf, or part thereof, produced by growing the seed of hybrid tobacco cultivar PD7305 SRC, PD7309 SRC, PD7312 SRC,
30 PD7318 SRC, or PD7319 SRC, where the leaf has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN), where the reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN) is reduced in a smoke stream produced from the leaf.

In a further aspect, the present disclosure includes a tobacco product, prepared from a tobacco plant, or part thereof, produced by growing the seed of hybrid tobacco cultivar

PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC, where the product is selected from the group consisting of pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, shredded tobacco, cut tobacco, and any combination thereof.

5 In an aspect, the present disclosure includes a tobacco product, prepared from a tobacco plant, or part thereof, produced by growing the seed of hybrid tobacco cultivar PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC, where the product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette
10 tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, plug chewing tobacco, moist snuff, and nasal snuff.

In another aspect, the present disclosure includes a tobacco product, prepared from a tobacco plant, or part thereof, produced by growing the seed of hybrid tobacco cultivar PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC, where the
15 product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, and further where the product has a reduced amount of
nornicotine and/or N'-nitrosonornicotine (NNN).

20 In a further aspect, the present disclosure includes a second tobacco product prepared or produced from a first tobacco product prepared from a tobacco plant, or part thereof, produced by growing the seed of tobacco cultivar PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC, where the first tobacco product is selected from the group consisting of pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco,
25 shredded tobacco, cut tobacco and any combination thereof, and the second tobacco product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, plug chewing tobacco, moist snuff, and nasal snuff. In some aspects, the
30 first and/or second product comprises a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN).

In an aspect, the present disclosure includes a part of a tobacco plant, produced by growing a seed of hybrid tobacco cultivar PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC, where the part is selected from the group consisting of leaf,

pollen, ovule, embryo, cotyledon, hypocotyl, meristematic cell, protoplast, root, root tip, pistil, anther, flower, shoot, stem, pod, and petiole.

In a further aspect, the present disclosure includes a tissue culture produced from a protoplast or cell from a tobacco plant, or part thereof, produced by growing the seed of
5 hybrid tobacco cultivar PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC, where the cell or protoplast of the tissue culture is produced from a plant part selected from the group consisting of a leaf, pollen, embryo, cotyledon, hypocotyl, meristematic cell, root, root tip, pistil, anther, flower, shoot, stem, pod, and petiole.

In an aspect, the present disclosure includes a tobacco plant regenerated from a tissue
10 culture produced from a protoplast or cell from a tobacco plant, or part thereof, produced by growing the seed of hybrid tobacco cultivar PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC, where the cell or protoplast of the tissue culture can be produced from a plant part selected from the group consisting of a leaf, pollen, embryo, cotyledon, hypocotyl, meristematic cell, root, root tip, pistil, anther, flower, shoot, stem, pod,
15 and petiole, where the regenerated plant has all, or essentially all, of the morphological and physiological characteristics of hybrid cultivar PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC.

In an aspect, the present disclosure includes an F₁ progeny plant of hybrid tobacco cultivar PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC.

20 In an aspect, the present disclosure includes an F₁ progeny plant of hybrid tobacco cultivar PD7305 SRC or PD7319 SRC, where the plant of tobacco cultivar CMS TND950 SRC is the female parent.

In an aspect, the present disclosure includes an F₁ progeny plant of hybrid tobacco cultivar PD7309 SRC, where the plant of tobacco cultivar CMS Narrow Leaf Madole SRC is
25 the female parent.

In an aspect, the present disclosure includes an F₁ progeny plant of hybrid tobacco cultivar PD7312 SRC or PD7318 SRC, where the plant of tobacco cultivar CMS KY171 SRC is the female parent.

In an aspect, the present disclosure includes an F₁ progeny plant of hybrid tobacco
30 cultivar PD7305 SRC, where the plant of tobacco cultivar CMS TND950 SRC is the male parent.

In an aspect, the present disclosure includes an F₁ progeny plant of hybrid tobacco cultivar PD7309 SRC, PD7318 SRC, or PD7319 SRC where the plant of tobacco cultivar Narrow Leaf Madole (phph) SRC is the male parent.

In an aspect, the present disclosure includes an F₁ progeny plant of hybrid tobacco cultivar PD7312 SRC, where the plant of tobacco cultivar Narrow Leaf Madole SRC is the male parent.

5 In another aspect, the present disclosure includes an F₁ progeny plant of hybrid tobacco cultivar PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC where the F₁ plant is cytoplasmic male sterile (CMS).

10 In another aspect, the present disclosure includes a method for producing a tobacco seed of PD7305 SRC or PD7319 SRC comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar CMS TND950 SRC.

In another aspect, the present disclosure includes a method for producing a tobacco seed of PD7309 SRC comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar CMS Narrow Leaf Madole SRC.

15 In another aspect, the present disclosure includes a method for producing a tobacco seed of PD7312 SRC or PD7318 SRC comprising crossing two tobacco plants and harvesting the resultant tobacco seed, where one tobacco plant is a tobacco plant produced by growing the seed of tobacco cultivar CMS KY171 SRC.

20 In an aspect, the present disclosure includes a method of vegetatively propagating a plant of a tobacco cultivar comprising the steps of (a) collecting tissue capable of being propagated from a plant of a tobacco cultivar selected from the group consisting of TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, and CMS KY171 SRC, (b) cultivating the tissue to obtain a proliferated shoot; and (c) rooting the proliferated shoots to obtain a rooted plantlet.

25 In an aspect, the present disclosure includes a method of vegetatively propagating a plant of a tobacco cultivar comprising the steps of (a) collecting tissue capable of being propagated from a plant of a tobacco cultivar selected from the group consisting of TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, and CMS KY171 SRC; (b) cultivating the tissue to
30 obtain a proliferated shoot; (c) rooting the proliferated shoots to obtain a rooted plantlet; and (d) growing a plant from the rooted plantlet.

In an aspect, the present disclosure includes a method of introducing a desired trait into a tobacco cultivar comprising: (a) crossing a plant of a first tobacco cultivar selected from the group consisting of TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC,

Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, and CMS KY171 SRC, with a second tobacco plant that comprises a desired trait to produce an F₁ progeny seed; (b) growing the F₁ progeny seed and selecting an F₁ progeny plant that comprises the desired trait; (c) crossing the selected F₁ progeny plant with a plant of said first tobacco cultivar to produce a backcross BC₁F₁ progeny seed; (d) growing the BC₁F₁ progeny seed and selecting a backcross BC₁F₁ progeny plant comprising the desired trait and essentially all of the physiological and morphological characteristics of said first tobacco cultivar, TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, or CMS KY171 SRC; and e) repeating steps (c) and (d) three or more times (e.g., 3, 4, 5, 6, 7, 8, 9, 10, and the like) in succession to produce selected fourth or higher backcross progeny that comprise the desired trait. In additional aspects, steps (c) and (d) can be repeated one or more times (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and the like) in succession to produce second or higher backcross progeny comprising the desired trait.

In an aspect, the present disclosure includes a method of introducing a desired trait into a tobacco cultivar comprising: (a) crossing a plant of a first tobacco cultivar selected from the group consisting of TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, and CMS KY171 SRC, with a second tobacco plant that comprises a desired trait to produce an F₁ progeny seed; (b) growing the F₁ progeny seed and selecting an F₁ progeny plant that comprises the desired trait; (c) crossing the selected F₁ progeny plant with a plant of said first tobacco cultivar to produce a backcross BC₁F₁ progeny seed; (d) growing the BC₁F₁ progeny seed and selecting a backcross BC₁F₁ progeny plant comprising the desired trait and essentially all of the physiological and morphological characteristics of the first tobacco cultivar, TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, or CMS KY171 SRC; and (e) repeating steps (c) and (d) three or more times in succession to produce selected fourth or higher backcross progeny that comprise the desired trait, where the trait is cytoplasmic male sterility (CMS). In additional aspects, steps (c) and (d) can be repeated one or more times in succession to produce second or higher backcross progeny comprising the desired trait, where the trait is CMS.

In an aspect, the present disclosure includes a method of introducing a desired trait into a tobacco cultivar comprising: (a) crossing a plant of a first tobacco cultivar selected from the group consisting of TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC,

Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, and CMS KY171 SRC, with a second tobacco plant that comprises a desired trait to produce an F₁ progeny seed; (b) growing the F₁ progeny seed and selecting an F₁ progeny plant that comprises the desired trait; (c) crossing the selected F₁ progeny plant with a plant of the first tobacco cultivar selected from the group consisting of TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, and Narrow Leaf Madole SRC, to produce a backcross BC₁F₁ progeny seed; (d) growing the BC₁F₁ progeny seed and selecting a backcross BC₁F₁ progeny plant comprising the desired trait and the physiological and essentially all of morphological characteristics of the first tobacco cultivar TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, or Narrow Leaf Madole SRC; and (e) repeating steps (c) and (d) three or more times in succession to produce selected fourth or higher backcross progeny that comprise the desired trait, where the trait is cytoplasmic male sterility (CMS) and the CMS trait is obtained from the cytoplasm of *Nicotiana suaveolens* or *Nicotiana glauca*. In additional aspects, steps (c) and (d) can be repeated one or more times in succession to produce second or higher backcross progeny comprising the desired trait, where the trait is CMS and the CMS trait is obtained from the cytoplasm of *Nicotiana suaveolens* or *Nicotiana glauca*.

In an aspect, the present disclosure includes a method of introducing a desired trait into a tobacco cultivar comprising: (a) crossing a plant of a first tobacco cultivar selected from the group TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, and Narrow Leaf Madole SRC with a second tobacco plant that comprises a desired trait to produce an F₁ progeny seed; (b) growing the F₁ progeny seed and selecting an F₁ progeny plant that comprises the desired trait; (c) crossing the selected F₁ progeny plant with a plant of said first tobacco cultivar to produce a backcross BC₁F₁ progeny seed; (d) growing the BC₁F₁ progeny seed and selecting a backcross BC₁F₁ progeny plant comprising the desired trait and essentially all of the physiological and morphological characteristics of the first tobacco cultivar TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, or Narrow Leaf Madole SRC; and (e) repeating steps (c) and (d) three or more times in succession to produce selected fourth or higher backcross progeny that comprise the desired trait, where the trait is cytoplasmic male sterility (CMS) and the CMS trait is obtained from the cytoplasm of *Nicotiana suaveolens*, and where the second tobacco plant is selected from the group consisting of CMS TND950 SRC, CMS Narrow Leaf Madole SRC, or CMS KY171 SRC. In additional aspects, steps (c) and (d) can be repeated one or more times in succession to produce second or higher backcross progeny comprising the CMS trait obtained from the cytoplasm of *Nicotiana suaveolens*, and where the second tobacco plant is selected from the

group consisting of CMS TND950 SRC, CMS Narrow Leaf Madole SRC, and CMS KY171 SRC.

In an aspect, the present disclosure includes a tobacco plant produced by a method comprising introducing a desired trait into a tobacco cultivar comprising: (a) crossing a plant of a first tobacco cultivar selected from the group consisting of TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, and CMS KY171 SRC, with a second tobacco plant that comprises a desired trait to produce an F_1 progeny seed; (b) growing the F_1 progeny seed and selecting an F_1 progeny plant that comprises the desired trait; (c) crossing the selected F_1 progeny plant with a plant of said first tobacco cultivar to produce a backcross BC_1F_1 progeny seed; (d) growing the BC_1F_1 progeny seed and selecting a backcross BC_1F_1 progeny plant comprising the desired trait and essentially all of the physiological and morphological characteristics of the first tobacco cultivar TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, or CMS KY171 SRC; and (e) repeating steps (c) and (d) three or more times in succession to produce selected fourth or higher backcross progeny that comprise the desired trait. In additional aspects, steps (c) and (d) can be repeated one or more times in succession to produce second or higher backcross progeny comprising the desired trait.

In another aspect, the present disclosure includes a method of introducing a desired trait into a tobacco cultivar comprising: (a) crossing a plant of a first tobacco cultivar selected from the group consisting of TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, and CMS KY171 SRC, with a plant of a second tobacco cultivar that comprises a desired trait to produce a progeny plant where the desired trait is selected from the group consisting of herbicide resistance, pest resistance, disease resistance, high yield, high grade index, curability, curing quality, mechanical harvestability, holding ability, leaf quality, height, plant maturation, early maturing, early to medium maturing, medium maturing, medium to late maturing, late maturing, small stalk, medium stalk, large stalk, leaf number per plant, 5-10 leaves per plant, 11-15 leaves per plant, 16-21 leaves per plant, and any combination thereof, to produce an F_1 progeny seed; (b) growing the F_1 progeny seed into an F_1 progeny plant and selecting the F_1 progeny plant having the desired trait; (c) crossing the selected F_1 progeny plant with a plant of said first tobacco cultivar, to produce a backcross progeny plant seed; (d) growing the backcross progeny plant seed into a backcross progeny plant and selecting the backcross progeny plant comprising the desired trait and essentially all of the

physiological and morphological characteristics of the first tobacco cultivar, TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, or CMS KY171 SRC; and (e) repeating steps (c) and (d) one or more times in succession to produce a selected second, third, fourth or higher

- 5 backcross progeny plant that comprises the desired trait and essentially all of the physiological and morphological characteristics of the first tobacco cultivar, TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, or CMS KY171 SRC.

- 10 In another aspect, the present disclosure includes a method of introducing a desired trait into a tobacco cultivar comprising: (a) crossing a plant of a first tobacco cultivar selected from the group consisting of TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, and CMS KY171 SRC, with a plant of a second tobacco cultivar that comprises a desired trait to produce a progeny plant where the desired trait is selected from the group consisting of
- 15 herbicide resistance, pest resistance, disease resistance, high yield, high grade index, curability, curing quality, mechanical harvestability, holding ability, leaf quality, height, plant maturation, early maturing, early to medium maturing, medium maturing, medium to late maturing, late maturing, small stalk, medium stalk, large stalk, leaf number per plant, 5-10 leaves per plant, 11-15 leaves per plant, 16-21 leaves per plant, and any combination thereof,
- 20 to produce an F₁ progeny seed; (b) growing the F₁ progeny seed into an F₁ progeny plant and selecting the F₁ progeny plant having the desired trait; (c) crossing the selected F₁ progeny plant with a plant of said first tobacco cultivar to produce a backcross progeny plant seed; (d) growing the backcross progeny plant seed into a backcross progeny plant and selecting the backcross progeny plant comprising the desired trait and essentially all of the
- 25 physiological and morphological characteristics of said first tobacco cultivar, TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, or CMS KY171 SRC; and (e) repeating steps (c) and (d) one or more times in succession to produce a selected second, third, fourth or higher backcross progeny plant that comprises the desired trait and essentially all of the
- 30 physiological and morphological characteristics of the first tobacco cultivar, TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, or CMS KY171 SRC.

In another aspect, the present disclosure includes a method of introducing a desired trait into a tobacco cultivar comprising: (a) crossing a plant of a first tobacco cultivar selected

from the group consisting of TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, and CMS KY171 SRC, with a plant of a second tobacco cultivar that comprises a desired trait to produce a progeny plant where the desired trait is selected from the group consisting of

5 herbicide resistance, pest resistance, disease resistance, high yield, high grade index, curability, curing quality, mechanical harvestability, holding ability, leaf quality, height, plant maturation, early maturing, early to medium maturing, medium maturing, medium to late maturing, late maturing, small stalk, medium stalk, large stalk, leaf number per plant, 5-10 leaves per plant, 11-15 leaves per plant, 16-21 leaves per plant, and any combination thereof,

10 to produce an F₁ progeny seed; (b) growing the F₁ progeny seed into an F₁ progeny plant and selecting the F₁ progeny plant having the desired trait; (c) crossing the selected F₁ progeny plant with a plant of said first tobacco cultivar to produce a backcross progeny plant seed; (d) growing the backcross progeny plant seed into a backcross progeny plant and selecting the backcross progeny plant comprising the desired trait and essentially all of the

15 physiological and morphological characteristics of the first tobacco cultivar, TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, or CMS KY171 SRC; and (e) repeating steps (c) and (d) one or more times in succession to produce a selected second, third, fourth or higher backcross progeny plant that comprises the desired trait and essentially all of the

20 physiological and morphological characteristics of said first tobacco cultivar, TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, or CMS KY171 SRC, where the desired trait is disease resistance.

In another aspect, the present disclosure includes a method for producing a tobacco

25 plant having decreased nicotine conversion comprising: identifying a first tobacco plant comprising a nucleotide sequence selected from the group consisting of the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 11, and any combination thereof; crossing the first tobacco plant with a second tobacco plant and collecting an F₁ seed; crossing a plant grown from the F₁ seed to a third tobacco plant and collecting a second

30 tobacco seed; and identifying a second tobacco seed or a plant grown from the second seed that is homozygous for the nucleotide sequence selected from the group consisting of the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 11, and any combination thereof.

In an aspect, the present disclosure includes a method for producing a tobacco plant having decreased nicotine conversion comprising: identifying a first tobacco plant comprising a nucleotide sequence selected from the group consisting of the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 11, and any combination thereof; 5 crossing the first tobacco plant with a second tobacco plant and collecting an F₁ seed; crossing a plant grown from the F₁ seed to a third tobacco plant and collecting a second tobacco seed; and identifying a second tobacco seed or a plant grown from the second seed that is homozygous for the nucleotide sequence selected from the group consisting of the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 11, and any combination 10 thereof, where the second tobacco plant comprises a nucleotide sequence selected from the group consisting of the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 11, and any combination thereof.

In an aspect, the present disclosure includes a method for producing a tobacco plant having decreased nicotine conversion comprising: identifying a first tobacco plant 15 comprising a nucleotide sequence selected from the group consisting of the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 11, and any combination thereof; crossing the first tobacco plant with a second tobacco plant and collecting an F₁ seed; crossing a plant grown from the F₁ seed to a third tobacco plant and collecting a second tobacco seed; and identifying a second tobacco seed or a plant grown from the second seed 20 that is homozygous for the nucleotide sequence selected from the group consisting of the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 11, and any combination thereof, where the second tobacco plant does not have the nucleotide sequence selected from the group consisting of the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 11, and any combination thereof, and the third tobacco plant is a tobacco plant 25 comprising a nucleotide sequence selected from the group consisting of the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 11, and any combination thereof.

In an aspect, the present disclosure includes a method for producing a tobacco plant having decreased nicotine conversion comprising: identifying a first tobacco plant comprising a nucleotide sequence selected from the group consisting of the nucleotide 30 sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 11, and any combination thereof; crossing the first tobacco plant with a second tobacco plant and collecting an F₁ seed; crossing a plant grown from the F₁ seed to a third tobacco plant and collecting a second tobacco seed; and identifying a second tobacco seed or a plant grown from the second seed that is homozygous for the nucleotide sequence selected from the group consisting of the

nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 11, and any combination thereof, where the first tobacco plant comprises the sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2.

5 In an aspect, the present disclosure includes a method for producing a tobacco plant having decreased nicotine conversion comprising: identifying a first tobacco plant comprising a nucleotide sequence selected from the group consisting of the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 11, and any combination thereof; crossing the first tobacco plant with a second tobacco plant and collecting an F₁ seed; crossing a plant grown from the F₁ seed to a third tobacco plant and collecting a second
10 tobacco seed; and identifying a second tobacco seed or a plant grown from the second seed that is homozygous for the nucleotide sequence selected from the group consisting of the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 11, and any combination thereof, where the third tobacco plant is a tobacco plant comprising a nucleotide sequence selected from the group consisting of the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO:
15 2, SEQ ID NO: 11, and any combination thereof.

In an aspect, the present disclosure includes a method for producing a tobacco plant having decreased nicotine conversion comprising: identifying a first tobacco plant comprising a nucleotide sequence selected from the group consisting of the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 11, and any combination thereof;
20 crossing the first tobacco plant with a second tobacco plant and collecting an F₁ seed; crossing a plant grown from the F₁ seed to a third tobacco plant and collecting a second tobacco seed; and identifying a second tobacco seed or a plant grown from the second seed that is homozygous for the nucleotide sequence selected from the group consisting of the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 11, and any combination
25 thereof, where the first tobacco plant is a plant of a tobacco cultivar TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, or CMS KY171 SRC and hybrid cultivars PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC.

In an aspect, the present disclosure includes a method for producing a tobacco plant
30 having decreased nicotine conversion comprising: identifying a first tobacco plant comprising a nucleotide sequence selected from the group consisting of the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 11, and any combination thereof; crossing the first tobacco plant with a second tobacco plant and collecting an F₁ seed; crossing a plant grown from the F₁ seed to a third tobacco plant and collecting a second

tobacco seed; and identifying a second tobacco seed or a plant grown from the second seed that is homozygous for the nucleotide sequence selected from the group consisting of the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 11, and any combination thereof, where the third tobacco plant is a plant of tobacco cultivar TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, or CMS KY171 SRC and hybrid cultivars PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC.

In another aspect, the present disclosure includes a method of producing a plant of a tobacco cultivar selected from the group consisting of TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, and CMS KY171 SRC, and hybrid cultivars PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, and PD7319 SRC comprising at least one (e.g., 1, 2, 3, 4, 5, 6, 7, 8, and the like) additional desired trait comprising the steps of: (a) collecting tissue capable of being propagated from a plant of a tobacco cultivar selected from the group consisting of TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, and CMS KY171 SRC, and hybrid cultivars PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, and PD7319 SRC; and (b) introducing a transgene (nucleic acid construct) conferring at least one desired trait into the tissue.

In another aspect, the present disclosure includes a method of producing an herbicide resistant tobacco plant comprising transforming a tobacco plant, or part thereof, produced by growing a seed of a tobacco cultivar selected from the group consisting of tobacco cultivar TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, and CMS KY171 SRC, and hybrid cultivars PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, and PD7319 SRC with at least one transgene (nucleic acid construct), where the at least one transgene (nucleic acid construct) confers resistance to an herbicide selected from the group consisting of imidazolinone, cyclohexanedione, sulfonylurea, glyphosate, glufosinate, phenoxy propionic acid, L-phosphinothricin, triazine, benzonitrile and any combination thereof.

In another aspect, the present disclosure includes an herbicide resistant tobacco plant produced by a method comprising transforming a tobacco plant, or part thereof, produced by growing a seed of a tobacco cultivar selected from the group consisting of tobacco cultivar TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, and CMS KY171 SRC, and hybrid cultivars

PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, and PD7319 SRC, with at least one transgene (nucleic acid construct), where the at least one transgene (nucleic acid construct) confers resistance to an herbicide selected from the group consisting of imidazolinone, cyclohexanedione, sulfonylurea, glyphosate, glufosinate, phenoxy propionic acid, L-phosphinothricin, triazine, benzonitrile, and any combination thereof.

In another aspect, the present disclosure includes a method of producing a pest and/or insect resistant tobacco plant where the method comprises transforming a tobacco plant produced by growing a seed of a tobacco cultivar selected from the group consisting of TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, and CMS KY171 SRC, and hybrid cultivars PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, and PD7319 SRC, with at least one transgene (nucleic acid construct) that confers pest and/or insect resistance.

In a further aspect, the present disclosure includes a pest and/or insect resistant tobacco plant produced by a method comprising transforming a tobacco plant produced by growing a seed of a tobacco cultivar selected from the group consisting of TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, and CMS KY171 SRC, and hybrid cultivars PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, and PD7319 SRC with at least one transgene (nucleic acid construct) that confers pest and/or insect resistance.

In a further aspect, the present disclosure includes a pest and/or insect resistant tobacco plant produced by a method comprising transforming a tobacco plant produced by growing a seed of a tobacco cultivar selected from the group consisting of TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, and CMS KY171 SRC, and hybrid cultivars PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, and PD7319 SRC, with at least one transgene (nucleic acid construct) that confers pest and/or insect resistance, where the transgene (nucleic acid construct) encodes a *Bacillus thuringiensis* (BT) endotoxin.

In another aspect, the present disclosure includes a method of producing a disease resistant tobacco plant, the method comprising transforming a tobacco plant produced by growing a seed of a tobacco cultivar selected from the group consisting of TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, and CMS KY171 SRC, and hybrid cultivars PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, and PD7319 SRC with at least one transgene (nucleic acid construct) that confers disease resistance.

In a further aspect, the present disclosure includes a disease resistant tobacco plant produced by transforming a tobacco plant produced by growing a seed of a tobacco cultivar selected from the group consisting of TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, and CMS KY171 SRC, and hybrid cultivars PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, and PD7319 SRC with at least one transgene (nucleic acid construct) that confers disease resistance.

BRIEF DESCRIPTION OF THE SEQUENCES

SEQ ID NO: 1 sets forth a *cyp82e4* W329Stop nucleotide sequence.

SEQ ID NO: 2 sets forth a *cyp82e5v2* W422Stop nucleotide sequence.

SEQ ID NO: 3 sets forth a *cyp82e4* W329Stop amino acid sequence.

SEQ ID NO: 4 sets forth a *cyp82e5v2* W422Stop amino acid sequence.

SEQ ID NO: 5 sets forth a *CYP82E4* wild-type nucleotide sequence.

SEQ ID NO: 6 sets forth a *CYP82E5v2* wild-type nucleotide sequence.

SEQ ID NO: 7 sets forth a *CYP82E4* wild-type amino acid sequence.

SEQ ID NO: 8 sets forth a *CYP82E5v2* wild-type amino acid sequence.

SEQ ID NO: 9 sets forth a *CYP82E10* wild-type nucleotide sequence.

SEQ ID NO: 10 sets forth a *CYP82E10* wild-type amino acid sequence.

SEQ ID NO: 11 sets forth a *CYP82E10* P381S nucleotide sequence.

SEQ ID NO: 12 sets forth a *CYP82E10* P381S amino acid sequence.

All three tobacco Nicotine Demethylase genes (*CYP82E4*, *CYP82E5v2*, *CYP82E10*) share a common structure: a 939 bp exon 1 and a 612 bp exon 2 separated by a large intron, whose length varies among the three genes. See Lewis *et al.*, "Three nicotine demethylase genes mediate nornicotine biosynthesis in *Nicotiana tabacum* L.: Functional characterization of the CYP82E10 gene," *Phytochemistry*, 71 (2010), 1988-1998. SEQ ID NOs: 1, 2, 5, 6, 9, and 11 set forth wild-type or mutant versions of coding sequences of *CYP82E4*, *CYP82E5v2*, and *CYP82E10*. It is understood that, used herein, a plant comprising, having, or homozygous for a sequence selected from SEQ ID NOs: 1, 2, 5, 6, 9, and 11 refers to a plant comprising at the *CYP82E4*, *CYP82E5v2*, or *CYP82E10* endogenous locus a genomic sequence comprising the coding sequence of SEQ ID NO: 1, 2, 5, 6, 9, or 11.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts the amount of nornicotine, as a percentage of total alkaloids, found in LC varieties compared to the same varieties comprising SRC technology when grown and cured under normal conditions as compared to growth under normal conditions and curing under high-temperature stressed curing conditions.

Figure 2 depicts the amount of NNN, in parts per million, found in LC varieties compared to the same varieties comprising SRC technology when grown and cured under normal conditions as compared to growth under normal conditions and curing under high-temperature stressed curing conditions.

Figure 3 depicts the maximum temperature reached (in degrees Fahrenheit) during the four firings used to fire-cure LC and SRC dark tobacco varieties in both the control curing barn and the high-temperature stressed curing barn.

Figure 4 depicts the total TSNA amounts, in parts per million, detected in dark tobacco hybrid cultivars PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, and PD7319 SRC after curing.

Figure 5 depicts the NNN amounts, in parts per million, detected in dark tobacco hybrid cultivars PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, and PD7319 SRC after curing.

Figure 6 depicts the total TSNA amounts, in parts per million, detected in the various component tobaccos used to create dark tobacco blends for smokeless products prototypes including both LC (control) and SRC varieties of dark-fired cured tobacco (DFC), dark air-cured tobacco (DAC), and Burley stems.

Figure 7 depicts the NNN amounts, in parts per million, detected in the various component tobaccos used to create dark tobacco blends for smokeless products prototypes including both LC (control) and SRC varieties of dark-fired cured tobacco (DFC), dark air-cured tobacco (DAC), and Burley stems.

Figure 8 depicts the total TSNA amounts, in parts per million, detected in blended dark tobacco used for smokeless products prototypes at three stages of the production process: the dry flour blending stage, the out of cure stage, and the finished product.

Figure 9 depicts the NNN amounts, in parts per million, detected in blended dark tobacco used for smokeless products prototypes at three stages of the production process: the dry flour blending stage, the out of cure stage, and the finished product.

DETAILED DESCRIPTION OF THE DISCLOSURE

5 This description is not intended to be a detailed catalog of all the different ways in which the disclosure may be implemented, or all the features that may be added to the instant disclosure. For example, features illustrated with respect to one embodiment may be incorporated into other embodiments, and features illustrated with respect to a particular embodiment may be deleted from that embodiment. Thus, the disclosure contemplates that in
10 some embodiments of the disclosure, any feature or combination of features set forth herein can be excluded or omitted. In addition, numerous variations and additions to the various embodiments suggested herein will be apparent to those skilled in the art in light of the instant disclosure, which do not depart from the instant disclosure. Hence, the following descriptions are intended to illustrate some particular embodiments of the disclosure, and not
15 to exhaustively specify all permutations, combinations and variations thereof.

 Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. The terminology used in the description of the disclosure herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the disclosure.

20 All publications, patent applications, patents and other references cited herein are incorporated by reference in their entireties for the teachings relevant to the sentence and/or paragraph in which the reference is presented. References to techniques employed herein are intended to refer to the techniques as commonly understood in the art, including variations on those techniques or substitutions of equivalent techniques that would be apparent to one of
25 skill in the art.

 Unless the context indicates otherwise, it is specifically intended that the various features of the disclosure described herein can be used in any combination. Moreover, the present disclosure also contemplates that in some embodiments of the disclosure, any feature or combination of features set forth herein can be excluded or omitted. To illustrate, if the
30 specification states that a composition comprises components A, B and C, it is specifically intended that any of A, B or C, or a combination thereof, can be omitted and disclaimed singularly or in any combination.

 As used in the description of the disclosure and the appended claims, the singular

forms “a,” “an” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise.

As used herein, “and/or” refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (“or”).

The term “about,” as used herein when referring to a measurable value such as a dosage or time period and the like, is meant to encompass variations of $\pm 20\%$, $\pm 10\%$, $\pm 5\%$, $\pm 1\%$, $\pm 0.5\%$, or even $\pm 0.1\%$ of the specified amount.

As used herein, phrases such as “between X and Y” and “between about X and Y” should be interpreted to include X and Y. As used herein, phrases such as “between about X and Y” mean “between about X and about Y” and phrases such as “from about X to Y” mean “from about X to about Y.”

The terms “comprise,” “comprises” and “comprising” as used herein, specify the presence of the stated features, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups thereof.

As used herein, the transitional phrase “consisting essentially of” means that the scope of a claim is to be interpreted to encompass the specified materials or steps recited in the claim and those that do not materially affect the basic and novel characteristic(s) of the claimed disclosure. Thus, the term “consisting essentially of” when used in a claim of this disclosure is not intended to be interpreted to be equivalent to “comprising.”

“Introducing,” in the context of a polynucleotide sequence (e.g., a recombinant polynucleotide and/or expression cassette of the disclosure), means presenting a polynucleotide sequence to the plant, plant part, and/or plant cell in such a manner that the polynucleotide sequence gains access to the interior of a cell. Where more than one polynucleotide sequence is to be introduced these polynucleotide sequences can be assembled as part of a single polynucleotide or nucleic acid construct, or as separate polynucleotide or nucleic acid constructs, and can be located on the same or different transformation vectors. Accordingly, these polynucleotides can be introduced into plant cells in a single transformation event, in separate transformation events, or, e.g., as part of a breeding protocol. Thus, the term “transformation” as used herein refers to the introduction of a heterologous nucleic acid into a cell. Transformation of a cell may be stable or transient. Thus, in some embodiments, a plant cell, plant part or plant of this disclosure can be stably

transformed with a recombinant polynucleotide of the disclosure. In other embodiments, a plant cell, plant part or plant of this disclosure can be transiently transformed with a recombinant polynucleotide of the disclosure.

“Tobacco product” is defined as “any product made or derived from tobacco that is intended for human use or consumption, including any component, part, or accessory of a tobacco product (except for raw materials other than tobacco used in manufacturing a component, part, or accessory of a tobacco product)” (section 201 of the FD&C Act; 21 U.S.C. 321).

Terms “nicotine conversion rate,” “percent nicotine conversion,” and “percentage nicotine conversion” are used interchangeably. Percent nicotine demethylation in a sample is calculated by dividing the level of nornicotine by the combined level of nicotine and nornicotine as measured in the sample, and multiplying by 100.

PD7305 SRC

In some aspects, the present disclosure provides tobacco cultivars, and parts thereof, from PD7305 SRC. In other aspects, the present disclosure provides a tobacco plant, or part thereof, produced by growing a seed of PD7305 SRC. In further aspects, a plant of the present disclosure can include a plant with all, or essentially all, of the morphological and physiological characteristics of cultivar PD7305 SRC.

While not being limited by process, PD7305 SRC is produced by pollinating plants of CMS TND950 SRC dark tobacco with pollen of TND950 (phph)SRC dark tobacco. The original tobacco cultivar PD7305 LC is a hybrid generated by pollinating plants of a male-sterile breeding line CMS TND950 with pollen produced by fertile breeding line TND950 (phph). TND950 SRC dark tobacco and TND950 (phph) SRC dark tobacco are created through the introduction of three mutated *CYP82E* genes into the male-sterile breeding line CMS TND950 and the fertile breeding line TND950 (phph). The three genes are a mutated *CYP82E4* gene recited as 325-6 #775 in Lewis *et al.* (“Three nicotine demethylase genes mediate nornicotine biosynthesis in *Nicotiana tabacum* L.: Functional characterization of the CYP82E10 gene,” *Phytochemistry*, 71 (2010), 1988-1998 (SEQ ID NO: 1, which sets forth a *cyp82e4* W329Stop, hereby incorporated by reference in its entirety)), a mutated *CYP82E5v2* recited in Lewis *et al. (supra)* as 325-6 #1-13 (SEQ ID NO: 2, which sets forth a *cyp82e5v2* W422Stop, hereby incorporated by reference in its entirety), and a mutated *CYP82E10* recited in Lewis *et al. (supra)* as 325-6 #1041 (SEQ ID NO: 11, which sets forth a *cyp82e10* P381S, hereby incorporated by reference in its entirety). Mutations *cyp82e4* W329Stop and

cyp82e5v2 W422Stop result in truncated proteins while *cyp82E10* P381S results in a nonfunctional protein. A *cyp82e4* W329Stop (“e4”), a *cyp82e5v2* W422Stop (“e5”), and a *cyp82e10* P381S (“e10”) mutation are introduced from a *e4e4|e5e5|e10e10* triple mutant from a strong converter burley background, line DH98-325-6, as listed in Table 4 of Lewis *et al.*

(*supra*) into both the male-sterile breeding line CMS TND950 and a fertile breeding line TND950 (phph) dark tobacco.

TND950 SRC dark tobacco and TND950 (phph) SRC dark tobacco are the result of seven backcrosses with TND950 or TND950 (phph) as the recurrent parents, followed by two rounds of selfing with selection for homozygosity for the *cyp82e4* W329Stop, the *cyp82e5v2* W422Stop, and *cyp82e10* P381S mutations to yield BC₇F₃ plants (TND950 SRC and TND950 (phph) SRC) in which the wild-type CYP82E4, CYP82E5v2 and CYP82E10 alleles of KY171 and TND950 (phph) are replaced by the mutant alleles (e.g., *cyp82e4* W329Stop, *cyp82e5v2* W422Stop, and *cyp82e10* P381S).

To develop CMS TND950 SRC dark tobacco, a plant of CMS TND950 was crossed with TND950 SRC dark tobacco to produce male-sterile plants heterozygous for all three mutations. A single male-sterile plant resulting from this cross was then subsequently backcrossed as a female to TND950 SRC dark tobacco to produce progeny that were segregating for individuals homozygous for all three mutations. Triple homozygous individuals were identified by DNA genotyping to produce the CMS TND950 SRC dark tobacco line. Because the line is male-sterile, it is maintained via pollination with TND950 SRC dark tobacco. PD7305 SRC dark tobacco hybrid is produced by pollinating plants of CMS TND950 SRC dark tobacco with pollen of TND950 (phph) SRC dark tobacco.

CMS TND950 SRC dark tobacco, TND950 (phph) SRC dark tobacco, and PD7305 SRC dark tobacco progeny plants have genetic backgrounds that are at least 95%, at least 97%, at least 98%, or at least 99% similar to CMS TND950, TND950 (phph), and PD7305 LC respectively. PD7305 SRC plants exhibit low normicotine levels and produce leaves with reduced potential for accumulating derived NNN during curing, storage, and smoking.

PD7309 SRC

In some aspects, the present disclosure provides tobacco cultivars, and parts thereof, from PD7309 SRC. In other aspects, the present disclosure provides a tobacco plant, or part thereof, produced by growing a seed of PD7309 SRC. In further aspects, a plant of the present disclosure can include a plant with all, or essentially all, of the morphological and physiological characteristics of cultivar PD7309 SRC.

While not being limited by process, PD7309 SRC is produced by pollinating plants of CMS Narrow Leaf Madole SRC dark tobacco with pollen of Narrow Leaf Madole (phph) SRC dark tobacco. The original tobacco cultivar PD7309 LC is a hybrid generated by pollinating plants of a male-sterile breeding line CMS Narrow Leaf Madole with pollen produced by fertile breeding line Narrow Leaf Madole (phph). Narrow Leaf Madole SRC dark tobacco and Narrow Leaf Madole (phph) SRC dark tobacco are created through the introduction of three mutated *CYP82E* genes into the male-sterile breeding line CMS Narrow Leaf Madole and the fertile breeding line Narrow Leaf Madole (phph). The three genes are a mutated *CYP82E4* gene recited as 325-6 #775 in Lewis *et al.* (“Three nicotine demethylase genes mediate nornicotine biosynthesis in *Nicotiana tabacum* L.: Functional characterization of the CYP82E10 gene,” *Phytochemistry*, 71 (2010), 1988-1998 (SEQ ID NO: 1, which sets forth a *cyp82e4* W329Stop, hereby incorporated by reference in its entirety)), a mutated *CYP82E5v2* recited in Lewis *et al. (supra)* as 325-6 #1-13 (SEQ ID NO: 2, which sets forth a *cyp82e5v2* W422Stop, hereby incorporated by reference in its entirety), and a mutated *CYP82E10* recited in Lewis *et al. (supra)* as 325-6 #1041 (SEQ ID NO: 11, which sets forth a *cyp82e10* P381S, hereby incorporated by reference in its entirety). Mutations *cyp82e4* W329Stop and *cyp82e5v2* W422Stop result in truncated proteins while *cyp82e10* P381S results in a nonfunctional protein. A *cyp82e4* W329Stop (“*e4*”), a *cyp82e5v2* W422Stop (“*e5*”), and a *cyp82e10* P381S (“*e10*”) mutation are introduced from a *e4e4|e5e5|e10e10* triple mutant from a strong converter burley background, line DH98-325-6, as listed in Table 4 of Lewis *et al. (supra)* into both the male-sterile breeding line CMS Narrow Leaf Madole and a fertile breeding line Narrow Leaf Madole (phph) dark tobacco.

Narrow Leaf Madole SRC dark tobacco and Narrow Leaf Madole (phph) SRC dark tobacco are the result of seven backcrosses with Narrow Leaf Madole or Narrow Leaf Madole (phph) as the recurrent parents, followed by two rounds of selfing with selection for homozygosity for the *cyp82e4* W329Stop, the *cyp82e5v2* W422Stop, and *cyp82e10* P381S mutations to yield BC₇F₃ plants (Narrow Leaf Madole SRC and Narrow Leaf Madole (phph) SRC) in which the wild-type CYP82E4, CYP82E5v2 and CYP82E10 alleles of Narrow Leaf Madole and Narrow Leaf Madole (phph) are replaced by the mutant alleles (*e.g.*, *cyp82e4* W329Stop, *cyp82e5v2* W422Stop, and *cyp82e10* P381S).

To develop CMS Narrow Leaf Madole SRC dark tobacco, a plant of CMS Narrow Leaf Madole was crossed with Narrow Leaf Madole SRC dark tobacco to produce male-sterile plants heterozygous for all three mutations. A single male-sterile plant resulting from this cross was then subsequently backcrossed as a female to Narrow Leaf Madole SRC dark

tobacco to produce progeny that were segregating for individuals homozygous for all three mutations. Triple homozygous individuals were identified by DNA genotyping to produce the CMS Narrow Leaf Madole SRC dark tobacco line. Because the line is male-sterile, it is maintained via pollination with Narrow Leaf Madole SRC dark tobacco. PD7309 SRC dark tobacco hybrid is produced by pollinating plants of CMS Narrow Leaf Madole SRC dark tobacco with pollen of Narrow Leaf Madole (phph) SRC dark tobacco.

CMS Narrow Leaf Madole SRC dark tobacco, Narrow Leaf Madole (phph) SRC dark tobacco, and PD7309 SRC dark tobacco progeny plants have genetic backgrounds that are at least 95%, at least 97%, at least 98%, or at least 99% similar to CMS Narrow Leaf Madole, Narrow Leaf Madole (phph), and PD7309 LC respectively. PD7309 SRC plants exhibit low normicotine levels and produce leaves with reduced potential for accumulating derived NNN during curing, storage, and smoking.

PD7312 SRC

In some aspects, the present disclosure provides tobacco cultivars, and parts thereof, from PD7312 SRC. In other aspects, the present disclosure provides a tobacco plant, or part thereof, produced by growing a seed of PD7312 SRC. In further aspects, a plant of the present disclosure can include a plant with all, or essentially all, of the morphological and physiological characteristics of cultivar PD7312 SRC.

While not being limited by process, PD7312 SRC is produced by pollinating plants of CMS KY171 SRC dark tobacco with pollen of Narrow Leaf Madole SRC dark tobacco. The original tobacco cultivar PD7312 LC is a hybrid generated by pollinating plants of a male-sterile breeding line CMS KY171 with pollen produced by fertile breeding line Narrow Leaf Madole. KY171 SRC dark tobacco and Narrow Leaf Madole SRC dark tobacco are created through the introduction of three mutated *CYP82E* genes into the male-sterile breeding line CMS KY171 and the fertile breeding line Narrow Leaf Madole. The three genes are a mutated *CYP82E4* gene recited as 325-6 #775 in Lewis *et al.* ("Three nicotine demethylase genes mediate normicotine biosynthesis in *Nicotiana tabacum* L.: Functional characterization of the CYP82E10 gene," *Phytochemistry*, 71 (2010), 1988-1998 (SEQ ID NO: 1, which sets forth a *cyp82e4* W329Stop, hereby incorporated by reference in its entirety)), a mutated *CYP82E5v2* recited in Lewis *et al. (supra)* as 325-6 #1-13 (SEQ ID NO: 2, which sets forth a *cyp82e5v2* W422Stop, hereby incorporated by reference in its entirety), and a mutated *CYP82E10* recited in Lewis *et al. (supra)* as 325-6 #1041 (SEQ ID NO: 11, which sets forth a *cyp82e10* P381S, hereby incorporated by reference in its entirety). Mutations *cyp82e4*

W329Stop and *cyp82e5v2* W422Stop result in truncated proteins while *cyp82E10* P381S results in a nonfunctional protein. A *cyp82e4* W329Stop (“*e4*”), a *cyp82e5v2* W422Stop (“*e5*”), and a *cyp82e10* P381S (“*e10*”) mutation are introduced from a *e4e4|e5e5|e10e10* triple mutant from a strong converter burley background, line DH98-325-6, as listed in Table 4 of Lewis *et al.* (*supra*) into both the male-sterile breeding line CMS KY171 and a fertile breeding line Narrow Leaf Madole dark tobacco.

KY171 SRC dark tobacco and Narrow Leaf Madole SRC dark tobacco are the result of seven backcrosses with KY171 or Narrow Leaf Madole as the recurrent parents, followed by two rounds of selfing with selection for homozygosity for the *cyp82e4* W329Stop, the *cyp82e5v2* W422Stop, and *cyp82e10* P381S mutations to yield BC₇F₃ plants (KY171 SRC and Narrow Leaf Madole SRC) in which the wild-type CYP82E4, CYP82E5v2 and CYP82E10 alleles of KY171 and Narrow Leaf Madole are replaced by the mutant alleles (*e.g.*, *cyp82e4* W329Stop, *cyp82e5v2* W422Stop, and *cyp82e10* P381S).

To develop CMS KY171 SRC dark tobacco, a plant of CMS KY171 was crossed with KY171 SRC dark tobacco to produce male-sterile plants heterozygous for all three mutations. A single male-sterile plant resulting from this cross was then subsequently backcrossed as a female to KY171 SRC dark tobacco to produce progeny that were segregating for individuals homozygous for all three mutations. Triple homozygous individuals were identified by DNA genotyping to produce the CMS KY171 SRC dark tobacco line. Because the line is male-sterile, it is maintained via pollination with KY171 SRC dark tobacco. PD7312 SRC dark tobacco hybrid is produced by pollinating plants of CMS KY171 SRC dark tobacco with pollen of Narrow Leaf Madole SRC dark tobacco.

CMS KY171 SRC dark tobacco, Narrow Leaf Madole SRC dark tobacco, and PD7312 SRC dark tobacco progeny plants have genetic backgrounds that are at least 95%, at least 97%, at least 98%, or at least 99% similar to CMS KY171, Narrow Leaf Madole, and PD7312 LC respectively. PD7312 SRC plants exhibit low nornicotine levels and produce leaves with reduced potential for accumulating derived NNN during curing, storage, and smoking.

PD7318 SRC

In some aspects, the present disclosure provides tobacco cultivars, and parts thereof, from PD7318 SRC. In other aspects, the present disclosure provides a tobacco plant, or part thereof, produced by growing a seed of PD7318 SRC. In further aspects, a plant of the

present disclosure can include a plant with all, or essentially all, of the morphological and physiological characteristics of cultivar PD7318 SRC.

While not being limited by process, PD7318 SRC is produced by pollinating plants of CMS KY171 SRC dark tobacco with pollen of Narrow Leaf Madole (phph) SRC dark tobacco. The original tobacco cultivar PD7318 LC is a hybrid generated by pollinating plants of a male-sterile breeding line CMS KY171 with pollen produced by fertile breeding line Narrow Leaf Madole (phph). KY171 SRC dark tobacco and Narrow Leaf Madole (phph) SRC dark tobacco are created through the introduction of three mutated *CYP82E* genes into the male-sterile breeding line CMS KY171 and the fertile breeding line Narrow Leaf Madole (phph). The three genes are a mutated *CYP82E4* gene recited as 325-6 #775 in Lewis *et al.* (“Three nicotine demethylase genes mediate nornicotine biosynthesis in *Nicotiana tabacum* L.: Functional characterization of the CYP82E10 gene,” *Phytochemistry*, 71 (2010), 1988-1998 (SEQ ID NO: 1, which sets forth a *cyp82e4* W329Stop, hereby incorporated by reference in its entirety)), a mutated *CYP82E5v2* recited in Lewis *et al. (supra)* as 325-6 #1-13 (SEQ ID NO: 2, which sets forth a *cyp82e5v2* W422Stop, hereby incorporated by reference in its entirety), and a mutated CYP82E10 recited in Lewis *et al. (supra)* as 325-6 #1041 (SEQ ID NO: 11, which sets forth a *cyp82e10* P381S, hereby incorporated by reference in its entirety). Mutations *cyp82e4* W329Stop and *cyp82e5v2* W422Stop result in truncated proteins while *cyp82e10* P381S results in a nonfunctional protein. A *cyp82e4* W329Stop (“*e4*”), a *cyp82e5v2* W422Stop (“*e5*”), and a *cyp82e10* P381S (“*e10*”) mutation are introduced from a *e4e4|e5e5|e10e10* triple mutant from a strong converter burley background, line DH98-325-6, as listed in Table 4 of Lewis *et al. (supra)* into both the male-sterile breeding line CMS KY171 and a fertile breeding line Narrow Leaf Madole (phph) dark tobacco.

KY171 SRC dark tobacco and Narrow Leaf Madole (phph) SRC dark tobacco are the result of seven backcrosses with KY171 or Narrow Leaf Madole (phph) as the recurrent parents, followed by two rounds of selfing with selection for homozygosity for the *cyp82e4* W329Stop, the *cyp82e5v2* W422Stop, and *cyp82e10* P381S mutations to yield BC₇F₃ plants (KY171 SRC and Narrow Leaf Madole (phph) SRC) in which the wild-type CYP82E4, CYP82E5v2 and CYP82E10 alleles of KY171 and Narrow Leaf Madole (phph) are replaced by the mutant alleles (e.g., *cyp82e4* W329Stop, *cyp82e5v2* W422Stop, and *cyp82e10* P381S).

To develop CMS KY171 SRC dark tobacco, a plant of CMS KY171 was crossed with KY171 SRC dark tobacco to produce male-sterile plants heterozygous for all three mutations. A single male-sterile plant resulting from this cross was then subsequently backcrossed as a

female to KY171 SRC dark tobacco to produce progeny that were segregating for individuals homozygous for all three mutations. Triple homozygous individuals were identified by DNA genotyping to produce the CMS KY171 SRC dark tobacco line. Because the line is male-sterile, it is maintained via pollination with KY171 SRC dark tobacco. The PD7318 SRC dark tobacco hybrid is produced by pollinating plants of CMS KY171 SRC dark tobacco with pollen of Narrow Leaf Madole (phph) SRC dark tobacco

CMS KY171 SRC dark tobacco, Narrow Leaf Madole (phph) SRC dark tobacco, and PD7318 SRC dark tobacco progeny plants have genetic backgrounds that are at least 95%, at least 97%, at least 98%, or at least 99% similar to CMS KY171, Narrow Leaf Madole (phph), and PD7318 LC respectively. PD7318 SRC plants exhibit low normicotine levels and produce leaves with reduced potential for accumulating derived NNN during curing, storage, and smoking.

PD7319 SRC

In some aspects, the present disclosure provides tobacco cultivars, and parts thereof, from PD7319 SRC. In other aspects, the present disclosure provides a tobacco plant, or part thereof, produced by growing a seed of PD7319 SRC. In further aspects, a plant of the present disclosure can include a plant with all, or essentially all, of the morphological and physiological characteristics of cultivar PD7319 SRC.

While not being limited by process, PD7319 SRC is produced by pollinating plants of CMS TND950 SRC dark tobacco with pollen of Narrow Leaf Madole (phph) SRC dark tobacco. The original tobacco cultivar PD7319 LC is a hybrid generated by pollinating plants of a male-sterile breeding line CMS TND950 with pollen produced by fertile breeding line Narrow Leaf Madole (phph). TND950 SRC dark tobacco and Narrow Leaf Madole (phph) SRC dark tobacco are created through the introduction of three mutated *CYP82E* genes into the male-sterile breeding line CMS TND950 and the fertile breeding line Narrow Leaf Madole (phph). The three genes are a mutated *CYP82E4* gene recited as 325-6 #775 in Lewis *et al.* (“Three nicotine demethylase genes mediate normicotine biosynthesis in *Nicotiana tabacum* L.: Functional characterization of the CYP82E10 gene,” *Phytochemistry*, 71 (2010), 1988-1998 (SEQ ID NO: 1, which sets forth a *cyp82e4* W329Stop, hereby incorporated by reference in its entirety)), a mutated *CYP82E5v2* recited in Lewis *et al.* (*supra*) as 325-6 #1-13 (SEQ ID NO: 2, which sets forth a *cyp82e5v2* W422Stop, hereby incorporated by reference in its entirety), and a mutated CYP82E10 recited in Lewis *et al.* (*supra*) as 325-6 #1041 (SEQ ID NO:11, which sets forth a *cyp82e10* P381S, hereby

incorporated by reference in its entirety). Mutations *cyp82e4* W329Stop and *cyp82e5v2* W422Stop result in truncated proteins while *cyp82E10* P381S results in a nonfunctional protein. A *cyp82e4* W329Stop (“e4”), a *cyp82e5v2* W422Stop (“e5”), and a *cyp82e10* P381S (“e10”) mutation are introduced from a *e4e4|e5e5|e10e10* triple mutant from a strong
 5 converter burley background, line DH98-325-6, as listed in Table 4 of Lewis *et al.* (*supra*) into both the male-sterile breeding line CMS TND950 and a fertile breeding line Narrow Leaf Madole (phph) dark tobacco.

TND950 SRC dark tobacco and Narrow Leaf Madole (phph) SRC dark tobacco are the result of seven backcrosses with TND950 or Narrow Leaf Madole (phph) as the recurrent
 10 parents, followed by two rounds of selfing with selection for homozygosity for the *cyp82e4* W329Stop, the *cyp82e5v2* W422Stop, and *cyp82e10* P381S mutations to yield BC₇F₃ plants (TND950 SRC and Narrow Leaf Madole (phph) SRC) in which the wild-type CYP82E4, CYP82E5v2 and CYP82E10 alleles of KY171 and Narrow Leaf Madole (phph) are replaced by the mutant alleles (e.g., *cyp82e4* W329Stop, *cyp82e5v2* W422Stop, and *cyp82e10* P381S).

15 To develop CMS TND950 SRC dark tobacco, a plant of CMS TND950 was crossed with TND950 SRC dark tobacco to produce male-sterile plants heterozygous for all three mutations. A single male-sterile plant resulting from this cross was then subsequently backcrossed as a female to TND950 SRC dark tobacco to produce progeny that were segregating for individuals homozygous for all three mutations. Triple homozygous
 20 individuals were identified by DNA genotyping to produce the CMS TND950 SRC dark tobacco line. Because the line is male-sterile, it is maintained via pollination with TND950 SRC dark tobacco. The PD7319 SRC dark tobacco hybrid is produced by pollinating plants of CMS TND950 SRC dark tobacco with pollen of Narrow Leaf Madole (phph) SRC dark tobacco.

25 CMS TND950 SRC dark tobacco, Narrow Leaf Madole (phph) SRC dark tobacco, and PD7319 SRC dark tobacco progeny plants have genetic backgrounds that are at least 95%, at least 97%, at least 98%, or at least 99% similar to CMS TND950, Narrow Leaf Madole (phph), and PD7319 LC respectively. PD7319 SRC plants exhibit low nicotine levels and produce leaves with reduced potential for accumulating derived NNN during
 30 curing, storage, and smoking.

Other plants

In some aspects, the present disclosure provides a tobacco seed produced by crossing two parent tobacco plants and harvesting the resultant tobacco seed, where at least one parent

tobacco plant is TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, or CMS KY171 SRC.

In one aspect, the TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, or Narrow Leaf Madole SRC is the male parent plant. In another aspect, the CMS TND950 SRC, CMS

5 Narrow Leaf Madole SRC, or CMS KY171 SRC is the female parent plant. One aspect of the present disclosure provides tobacco plants that are homozygous at the *cyp82e4*,

cyp82e5v2, and *cyp82E10* loci for SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 11,

respectively, and which share a genetic background that is greater than about 75%, 80%,

85%, 90%, 95%, 98%, or 99% similar to TND950 (phph) SRC, Narrow Leaf Madole (phph)

10 SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, or

CMS KY171 SRC. In one aspect, approximately or greater than about 50%, 75%, or 100%

of a progeny's genetics is provided by a plant of the present disclosure that is homozygous at

the *cyp82e4*, *cyp82e5v2*, and *cyp82E10* loci for SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID

NO: 11, respectively. In one aspect, a plant of the present disclosure has a genetic

15 background that is at least 95%, at least 97%, at least 98%, or at least 99% similar to

TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS

TND950 SRC, CMS Narrow Leaf Madole SRC, or CMS KY171 SRC. In another aspect, a

plant of the present disclosure exhibits low nornicotine and is not subject to conversion to

high nornicotine. In one aspect, a plant of the present disclosure is the progeny plant of a

20 female or male parent plant that is *Fusarium* wilt resistant. In another aspect, a plant of

TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC has low

resistance to black shank and moderate resistance to bacterial wilt.

The present disclosure includes a tobacco seed produced by crossing two parent

tobacco plants and harvesting the resultant tobacco seed, where at least one parent tobacco

25 plant is TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, or Narrow Leaf Madole

SRC. In one aspect, the TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow

Leaf Madole SRC is the male parent plant. In another aspect, the CMS TND950 SRC, CMS

Narrow Leaf Madole SRC, or CMS KY171 SRC is the female parent plant. One aspect of

the present disclosure provides tobacco plants that are homozygous at the *cyp82e4*,

30 *cyp82e5v2*, and *cyp82E10* loci for SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 11,

respectively, and which share a genetic background that is greater than about 75%, 80%,

85%, 90%, 95%, 98%, or 99% similar to TND950 (phph) SRC, Narrow Leaf Madole (phph)

SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, or

CMS KY171 SRC. In one aspect, approximately or greater than about 50%, 75%, or 100%

of a progeny's genetics is provided by a plant of the present disclosure that is homozygous at the *cyp82e4*, *cyp82e5v2*, and *cyp82E10* loci for SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 11, respectively. In one aspect, a plant of the present disclosure has a genetic background that is at least 95%, at least 97%, at least 98%, or at least 99% similar to

5 TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, or CMS KY171 SRC. In another aspect, a plant of the present disclosure exhibits low nornicotine and is not subject to conversion to high nornicotine. In one aspect, a plant of the present disclosure is the progeny plant of a female or male parent plant that is *Fusarium* wilt resistant. In another aspect, a plant of
10 PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC has moderate resistance to black shank and moderate resistance to bacterial wilt.

In one aspect, a plant of the present disclosure is a medium-late maturing variety with moderately high yield potential. In another aspect, a plant of the present disclosure offers a broad range of important agronomic characteristics. In a further aspect, a plant of the present
15 disclosure has one, two, three, four or more of the traits including moderate resistance to black shank, some tolerance to blue mold, black root rot resistance, and resistance to common virus diseases. In another aspect, a plant of the present disclosure has blue mold tolerance and level 4 resistance to both races of black shank and high root rot resistance. In one aspect, a plant of the present disclosure, such as TND950 (phph) SRC, Narrow Leaf Madole (phph)
20 SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, CMS KY171 SRC or hybrid cultivars PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC, lacks *Fusarium* wilt resistance. In another aspect, a plant of the present disclosure is *Fusarium* wilt resistant. In another aspect, a plant of the present disclosure has low resistance to black shank and moderate resistance to bacterial wilt.

25 In an aspect, the plants of the present disclosure have reduced or eliminated ability to convert nicotine to nornicotine. In an aspect, the percentage nicotine conversion is less than about 75%, 70%, 60%, 50%, or 25% of that found in TND950 (phph), Narrow Leaf Madole (phph), Narrow Leaf Madole, CMS TND950, CMS Narrow Leaf Madole, CMS KY171 or hybrid cultivars PD7305 LC, PD7309 LC, PD7312 LC, PD7318 LC, or PD7319 LC. The
30 nicotine conversion in plants of the present disclosure, including TND950 (phph), Narrow Leaf Madole (phph), Narrow Leaf Madole, CMS TND950, CMS Narrow Leaf Madole, CMS KY171 or hybrid cultivars PD7305 LC, PD7309 LC, PD7312 LC, PD7318 LC, or PD7319 LC, can be less than about 4%, about 3.5%, about 3%, about 2.5%, about 2%, about 1.5%, or about 1%, or any range therein. In still other aspects, the nicotine conversion in plants of the

present disclosure, including TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, CMS KY171 SRC or hybrid cultivars PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC, can be in a range from about 3% to about 1%, about 3% to about 0.5%, or about 2% to about 0.5%. In a preferred aspect, the percentage nicotine conversion is less than about 25%, 10%, 5%, or 2% of that found in TND950 (phph), Narrow Leaf Madole (phph), Narrow Leaf Madole, CMS TND950, CMS Narrow Leaf Madole, CMS KY171 and hybrid cultivars PD7305 LC, PD7309 LC, PD7312 LC, PD7318 LC, or PD7319 LC without the *cyp82e4* W329Stop, the *cyp82e5v2* W422Stop, and *cyp82e10* P381S mutations. In an aspect, the tobacco plants of the present disclosure can have a nicotine conversion rate of about 3.5, 3.25, 3.0 or 2.75% or less. In another aspect, the nicotine conversion rate of tobacco plants of the present disclosure can be about 4.0, 3.9, 3.8, 3.7, 3.6, 3.5, 3.4, 3.3, 3.2, 3.1, 3.0, 2.9, 2.8, 2.7, 2.6, 2.5, 2.4, 2.3, 2.2, 2.1, 2.0, 1.9, 1.8, 1.7, 1.6, 1.5, 1.4, 1.3, 1.2, 1.1, 1.0, 0.9, 0.8, 0.7, 0.6, 0.5% or less or any range therein. In another aspect, the nicotine conversion rate of tobacco plants of the present disclosure can be about 2.9, 2.8, 2.7, 2.6, 2.5, 2.4, 2.3, 2.2, 2.1, 2.0, 1.9, 1.8, 1.7, 1.6, 1.5, 1.4, 1.3, 1.2, 1.1, 1.0, 0.9, 0.8, 0.7, 0.6 % or less or any range therein. In another aspect, the nicotine conversion rates can be in a range from about 0.5% to about 0.9%, about 0.5% to about 1.5%, about 0.5 % to about 2.0%, about 0.5 % to about 2.5%, about 0.5 % to about 2.75%, and about 0.5 % to about 3.0%. In another aspect, the nicotine conversion rates can be in a range from about 1.0 % to about 1.5%, about 1.0 % to about 1.75%, about 1.0 % to about 2.0%, about 1.0 % to about 2.5%, about 1.0 % to about 2.75%, or about 1.0 % to about 3.0%. In another aspect, the nicotine conversion rate in a plant of the present disclosure can be less than about 2.9, 2.75, 2.5, 2.25, 2.0, 1.9, 1.8, 1.7, 1.6, 1.5, 1.4, 1.3, 1.2, 1.1 or 1.0% or any range therein.

In another aspect, the tobacco plants of the present disclosure typically have a reduced amount of nornicotine of less than about 0.10 % dry weight. For example, the nornicotine content in such plants can be about 1.2, 1.0, 0.7, 0.5, 0.4, 0.2, 0.1, 0.09, 0.085, 0.08, 0.075, 0.07, 0.065, 0.06, 0.055, 0.05, 0.045, 0.04, 0.035, 0.025, 0.01, 0.009, 0.0075, 0.005, 0.0025, 0.001, 0.0009, 0.00075, 0.0005, 0.00025, or 0.0001 % dry weight, or undetectable, or any range therein. In another aspect, the nornicotine content can be less than about 1.2, 1.0, 0.9, 0.8, 0.7, 0.5, 0.4, 0.2, 0.1, 0.075, 0.05, 0.025, 0.01, 0.009, 0.0075, 0.005, 0.0025, 0.001, 0.0009, 0.00075, 0.0005, 0.00025, or 0.0001 % dry weight, or any range therein. In another aspect, the nornicotine content in such plants can be in a range from about 1.2% to about 1.0%, about 0.7% to about 0.5%, about 0.4 % to about 0.2%, about 0.1% to about 0.075%,

about 0.05% to about 0.025%, about 0.01% to about 0.0075%, about 0.005% to about 0.0025%, about 0.001% to about 0.00075%, about 0.0005% to about 0.00025%, or about 0.0005% to about 0.0001 % dry weight. In some aspects, in a plant of the present disclosure, the nornicotine is a relatively small percentage of total alkaloids in the plant compared to a commercial seedlot of TND950 (phph), Narrow Leaf Madole (phph), Narrow Leaf Madole, CMS TND950, CMS Narrow Leaf Madole, CMS KY171 or hybrid cultivars PD7305 LC, PD7309 LC, PD7312 LC, PD7318 LC, or PD7319 LC. In some aspects, the nornicotine in a plant of the present disclosure can be about 2% to about 1%, less than 3%, about 2%, about 1.5%, about 1%, or 0.75% of total alkaloids. Tobacco products having a reduced amount of nitrosamine content can be manufactured using tobacco plant material from plants and plant parts of the present disclosure. Thus, in some embodiments, a tobacco product manufactured using tobacco plant material from plants and plant parts of the present disclosure can comprise a reduced amount of nornicotine of less than about 3 mg/g. For example, the nornicotine content in such a product can be 3.0 mg/g, 2.5 mg/g, 2.0 mg/g, 1.5 mg/g, 1.0 mg/g, 750 µg/g, 500 µg/g, 250 µg/g, 100 µg/g, 75 µg/g, 50 µg/g, 25 µg/g, 10 µg/g, 5 µg/g, 1 µg/g, 750 ng/g, 500 ng/g, 250 ng/g, 100 ng/g, 75 ng/g, 50 ng/g, 25 ng/g, 10 ng/g, 5 ng/g, 1 ng/g, 750 pg/g, 500 pg/g, 250 pg/g, 100 pg/g, 75 pg/g, 50 pg/g, 25 pg/g, 10 pg/g, 7.0 pg/g, 5.0 pg/g, 4.0 pg/g, 2.0 pg/g, 1.0 pg/g, 0.5 pg/g, 0.4 pg/g, 0.2 pg/g, 0.1 pg/g, 0.05 pg/g, 0.01 pg/g, and the like, or undetectable, or any range therein.. The tobacco product typically has a reduced amount of NNN of less than about 10 pg/g. For example, the NNN content in such a product can be about 10 pg/g, 7.0 pg/g, 5.0 pg/g, 4.0 pg/g, 2.0 pg/g, 1.0 pg/g, 0.5 pg/g, 0.4 pg/g, 0.2 pg/g, 0.1 pg/g, 0.05 pg/g, 0.01 pg/g, and the like, or undetectable, or any range therein. The percentage of secondary alkaloids relative to total alkaloid content contained in a plant of the present disclosure may not be statistically different than from a commercial seedlot of TND950 (phph), Narrow Leaf Madole (phph), Narrow Leaf Madole, CMS TND950, CMS Narrow Leaf Madole, CMS KY171 or hybrid cultivars PD7305 LC, PD7309 LC, PD7312 LC, PD7318 LC, or PD7319 LC.

Differences between two inbred tobacco varieties or two hybrid tobacco varieties can be evaluated using statistical approaches. Statistical analysis includes the calculation of mean values, determination of the statistical significance of the sources of variation, and the calculation of the appropriate variance components. Methods for determining statistical significance are known in the art. Statistical software is available, for example, the PROC GLM function of SAS. Significance is generally presented as a “p-value.” A statistically significant p-value is less than 0.10. In a preferred aspect, the p-value is less than, or equal

to, 0.05. In another aspect, the p-value is 0.04 or less, 0.03 or less, or 0.02 or less. In yet another aspect, a statistically significant value is less than 0.01. In yet another aspect, it can be less than 0.009, less than 0.008, less than 0.007, less than 0.006, less than 0.005, less than 0.004, less than 0.003, less than 0.002, or less than 0.001.

5 Tobacco plants of the present disclosure that are homozygous for the *cyp82e4* *W329Stop*, the *cyp82e5v2 W422Stop*, and *cyp82e10 P381S* alleles have a reversion rate that is statistically significantly lower than corresponding control low-converter plants having wild type nicotine demethylase CYP82E4, E5, and E10 genes. In addition, homozygous CYP82E4, CYP82E5, and CYP82E10 triple mutant tobacco plants have a percent conversion
10 to nornicotine of less than about 2.0%, *e.g.*, undetectable to about 2.0%, 1.9%, 1.8%, 1.7%, 1.6%, 1.5%, 1.4%, 1.3%, 1.2%, 1.1%, 1.0%, 0.9%, 0.8%, or any range therein. In some aspects, the triple mutant tobacco plants have a percent conversion to nornicotine in a range from, for example, about 1.0% to 2.0%, 0.8% to 1.8%, 0.8% to 2.0%, or 1.0% to 2.0%.

Nicotine and nornicotine can be measured in ethylene-treated leaves using methods
15 known in the art (*e.g.*, gas chromatography). Percent nicotine demethylation in a sample is calculated by dividing the level of nornicotine by the combined level of nicotine and nornicotine as measured in the sample, and multiplying by 100. Percent nicotine demethylation in a sample from a plant of the present disclosure is about 50, 40, 30, 20, or 10 percent of a sample from an individual plant grown from a commercial seedlot of TND950
20 (phph), Narrow Leaf Madole (phph), Narrow Leaf Madole, CMS TND950, CMS Narrow Leaf Madole, CMS KY171 or hybrid cultivars PD7305 LC, PD7309 LC, PD7312 LC, PD7318 LC, or PD7319 LC.

In an aspect, the tobacco plants of the present disclosure have a USDA quality index of about 73, about 72, about 71, about 70, about 69, about 68, about 67 or about 66 or any
25 range therein. In an aspect, the tobacco plants of the present disclosure have a USDA quality index of about 65. In another aspect, the quality index may be at least about 55, 60, 62.5 or greater, or any range therein. In another aspect, tobacco plants of the present disclosure can have a quality index in the range of about 60 to about 65, about 60 to about 70, about 62.5 to about 65, about 62.5 to about 70, or about 65 to about 70.

30 A plant of the present disclosure, including TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, CMS KY171 SRC or hybrid cultivars PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC, can have any yield potential, including high (*e.g.*, over

3000 lbs/A), moderately high (*e.g.*, 2200-3000 lbs/A), and moderate (*e.g.*, less than 2000 lbs/A) yield potential.

In another aspect, the present disclosure also provides for a plant grown from the seed of a TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, CMS KY171 SRC or hybrid cultivar PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC plant in which alkaloids obtained from tobacco plants grown for the seed have decreased nornicotine, as well as plant parts and tissue cultures from such plants, representative sample seeds of these cultivars having been deposited with the ATCC. Seeds of hybrid cultivar PD7305 SRC are obtainable by crossing plants of cultivars CMS TND950 SRC and TND950 (phph) SRC and collecting seeds. Seeds of hybrid cultivar PD7309 SRC are obtainable by crossing plants of cultivars CMS Narrow Leaf Madole SRC and Narrow Leaf Madole (phph) SRC and collecting seeds. Seeds of hybrid cultivar PD7312 SRC are obtainable by crossing plants of cultivars CMS KY171 SRC and Narrow Leaf Madole SRC and collecting seeds. Seeds of hybrid cultivar PD7318 SRC are obtainable by crossing plants of cultivars CMS KY171 SRC and Narrow Leaf Madole (phph) SRC and collecting seeds. Seeds of hybrid cultivar PD7319 SRC are obtainable by crossing plants of cultivars CMS TND950 SRC and Narrow Leaf Madole (phph) SRC and collecting seeds.

An aspect of the present disclosure provides for parts of the cultivar TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, CMS KY171 SRC or hybrid cultivar PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC. A part of a cultivar can comprise any plant part and includes, but is not limited to, leaves, pollen, embryos, cotyledons, hypocotyls, roots, root tips, anthers, flowers, ovules, shoots, stems, stalks, pith and capsules, tissue culture comprising tissue, callus, cells or protoplasts of the cultivars TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, CMS KY171 SRC or hybrid cultivars PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC. In another aspect, the present disclosure provides for parts from hybrids of cultivars TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, CMS KY171 SRC or hybrid cultivars PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC derived tobacco plants. In yet another aspect, the present disclosure provides for parts from genetically modified (*e.g.*, by conventional breeding or genetic engineering techniques) forms of the foregoing plants and tissue culture.

Additional aspects of the present disclosure provide products comprising tobacco from the plants of the present disclosure, and parts thereof. Other aspects of the disclosure provide cured plant parts, which include, but are not limited to, a leaf, pollen, ovule, embryo, cotyledon, hypocotyl, meristematic cell, protoplast, root, root tip, pistil, anther, flower, shoot, stem, pod, petiole, and the like, and combinations thereof.

Thus, in some aspects, the present disclosure provides a cured tobacco comprising the leaves of the tobacco plant designated TND950 (phph) SRC. In another aspect, the present disclosure provides a cured tobacco comprising the leaves of the tobacco plant designated Narrow Leaf Madole (phph) SRC. In another aspect, the present disclosure provides a cured tobacco comprising the leaves of the tobacco plant designated Narrow Leaf Madole SRC. In another aspect, the present disclosure provides a cured tobacco comprising the leaves of the tobacco plant designated CMS TND950 SRC. In another aspect, the present disclosure provides a cured tobacco comprising the leaves of the tobacco plant designated CMS Narrow Leaf Madole SRC. In another aspect, the present disclosure provides a cured tobacco comprising the leaves of the tobacco plant designated CMS KY171 SRC. In yet another aspect, the present disclosure provides a cured tobacco comprising the leaves of the hybrid tobacco plant designated PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC. Seeds of hybrid cultivar PD7305 SRC are obtainable by crossing plants of cultivars CMS TND950 SRC and TND950 (phph) SRC and collecting seeds. Seeds of hybrid cultivar PD7309 SRC are obtainable by crossing plants of cultivars CMS Narrow Leaf Madole SRC and Narrow Leaf Madole (phph) SRC and collecting seeds. Seeds of hybrid cultivar PD7312 SRC are obtainable by crossing plants of cultivars CMS KY171 SRC and Narrow Leaf Madole SRC and collecting seeds. Seeds of hybrid cultivar PD7318 SRC are obtainable by crossing plants of cultivars CMS KY171 SRC and Narrow Leaf Madole (phph) SRC and collecting seeds. Seeds of hybrid cultivar PD7319 SRC are obtainable by crossing plants of cultivars CMS TND950 SRC and Narrow Leaf Madole (phph) SRC and collecting seeds.

In an aspect, the present disclosure provides a cured tobacco comprising the stems of the tobacco plant designated TND950 (phph) SRC. In another aspect, the present disclosure provides a cured tobacco comprising the stems of the tobacco plant designated Narrow Leaf Madole (phph) SRC. In an aspect, the present disclosure provides a cured tobacco comprising the stems of the tobacco plant designated Narrow Leaf Madole SRC. In another aspect, the present disclosure provides a cured tobacco comprising the leaves of the tobacco plant designated CMS TND950 SRC. In another aspect, the present disclosure provides a

cured tobacco comprising the stems of the tobacco plant designated CMS Narrow Leaf Madole SRC. In another aspect, the present disclosure provides a cured tobacco comprising the stems of the tobacco plant designated CMS KY171 SRC. In yet another aspect, the present disclosure provides a cured tobacco comprising the stems of the hybrid tobacco plant designated PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC.

Seeds of hybrid cultivar PD7305 SRC are obtainable by crossing plants of cultivars CMS TND950 SRC and TND950 (phph) SRC and collecting seeds. Seeds of hybrid cultivar PD7309 SRC are obtainable by crossing plants of cultivars CMS Narrow Leaf Madole SRC and Narrow Leaf Madole (phph) SRC and collecting seeds. Seeds of hybrid cultivar PD7312 SRC are obtainable by crossing plants of cultivars CMS KY171 SRC and Narrow Leaf Madole SRC and collecting seeds. Seeds of hybrid cultivar PD7318 SRC are obtainable by crossing plants of cultivars CMS KY171 SRC and Narrow Leaf Madole (phph) SRC and collecting seeds. Seeds of hybrid cultivar PD7319 SRC are obtainable by crossing plants of cultivars CMS TND950 SRC and Narrow Leaf Madole (phph) SRC and collecting seeds.

In an aspect, the present disclosure provides a cured tobacco comprising the leaves and stems of the tobacco plants designated TND950 (phph) SRC. In another aspect, the present disclosure provides a cured tobacco comprising the leaves and stems of the tobacco plants designated Narrow Leaf Madole (phph) SRC. In an aspect, the present disclosure provides a cured tobacco comprising the leaves and stems of the tobacco plants designated Narrow Leaf Madole SRC. In another aspect, the present disclosure provides a cured tobacco comprising the leaves and stems of the tobacco plant designated CMS TND950 SRC. In another aspect, the present disclosure provides a cured tobacco comprising the leaves and stems of the tobacco plant designated CMS Narrow Leaf Madole SRC. In another aspect, the present disclosure provides a cured tobacco comprising the leaves and stems of the tobacco plant designated CMS KY171 SRC. In yet another aspect, the present disclosure provides a cured tobacco comprising the leaves and stems of the hybrid tobacco plant designated PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC. Seeds of hybrid cultivar PD7305 SRC are obtainable by crossing plants of cultivars CMS TND950 SRC and TND950 (phph) SRC and collecting seeds. Seeds of hybrid cultivar PD7309 SRC are obtainable by crossing plants of cultivars CMS Narrow Leaf Madole SRC and Narrow Leaf Madole (phph) SRC and collecting seeds. Seeds of hybrid cultivar PD7312 SRC are obtainable by crossing plants of cultivars CMS KY171 SRC and Narrow Leaf Madole SRC and collecting seeds. Seeds of hybrid cultivar PD7318 SRC are obtainable by crossing plants of cultivars CMS KY171 SRC and Narrow Leaf Madole (phph) SRC and collecting seeds.

Seeds of hybrid cultivar PD7319 SRC are obtainable by crossing plants of cultivars CMS TND950 SRC and Narrow Leaf Madole (phph) SRC and collecting seeds.

The present disclosure also provides a container of TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, CMS KY171 SRC, or hybrid PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC seeds or other seeds of the present disclosure in which alkaloids obtained from tobacco plants grown from greater than about 50% of the seeds have decreased nornicotine. In another aspect, alkaloids obtained from TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, CMS KY171 SRC, or hybrid PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC plants or other plants of the present disclosure grown from greater than about 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% of the seeds in the container have decreased nornicotine, representative samples of seeds of these cultivars having been deposited with the ATCC. Seeds of hybrid cultivar PD7305 SRC are obtainable by crossing plants of cultivars CMS TND950 SRC and TND950 (phph) SRC and collecting seeds. Seeds of hybrid cultivar PD7309 SRC are obtainable by crossing plants of cultivars CMS Narrow Leaf Madole SRC and Narrow Leaf Madole (phph) SRC and collecting seeds. Seeds of hybrid cultivar PD7312 SRC are obtainable by crossing plants of cultivars CMS KY171 SRC and Narrow Leaf Madole SRC and collecting seeds. Seeds of hybrid cultivar PD7318 SRC are obtainable by crossing plants of cultivars CMS KY171 SRC and Narrow Leaf Madole (phph) SRC and collecting seeds. Seeds of hybrid cultivar PD7319 SRC are obtainable by crossing plants of cultivars CMS TND950 SRC and Narrow Leaf Madole (phph) SRC and collecting seeds.

The container of TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, CMS KY171 SRC, or hybrid PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC seeds or other seeds of the present disclosure may contain any number, weight or volume of seeds. For example, a container can contain at least, or greater than, about 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000 or more seeds. Alternatively, the container can contain at least, or greater than, about 1 ounce, 5 ounces, 10 ounces, 1 pound, 2 pounds, 3 pounds, 4 pounds, 5 pounds or more seeds. Representative samples of seeds of these cultivars having been deposited with the ATCC. Seeds of hybrid cultivar PD7305 SRC are obtainable by crossing plants of cultivars CMS TND950 SRC and TND950 (phph) SRC and collecting seeds. Seeds of hybrid cultivar PD7309 SRC are obtainable by

crossing plants of cultivars CMS Narrow Leaf Madole SRC and Narrow Leaf Madole (phph) SRC and collecting seeds. Seeds of hybrid cultivar PD7312 SRC are obtainable by crossing plants of cultivars CMS KY171 SRC and Narrow Leaf Madole SRC and collecting seeds. Seeds of hybrid cultivar PD7318 SRC are obtainable by crossing plants of cultivars CMS
5 KY171 SRC and Narrow Leaf Madole (phph) SRC and collecting seeds. Seeds of hybrid cultivar PD7319 SRC are obtainable by crossing plants of cultivars CMS TND950 SRC and Narrow Leaf Madole (phph) SRC and collecting seeds.

Containers of TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, CMS KY171 SRC, or
10 hybrid PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC seeds or other seeds of the present disclosure may be any container available in the art. By way of a non-limiting example, a container may be a box, a bag, a packet, a pouch, a tape roll, a pail, a foil, or a tube. Representative samples of seeds of these cultivars having been deposited with the ATCC, for example. Seeds of hybrid cultivar PD7305 SRC are obtainable by crossing
15 plants of cultivars CMS TND950 SRC and TND950 (phph) SRC and collecting seeds. Seeds of hybrid cultivar PD7309 SRC are obtainable by crossing plants of cultivars CMS Narrow Leaf Madole SRC and Narrow Leaf Madole (phph) SRC and collecting seeds. Seeds of hybrid cultivar PD7312 SRC are obtainable by crossing plants of cultivars CMS KY171 SRC and Narrow Leaf Madole SRC and collecting seeds. Seeds of hybrid cultivar PD7318 SRC
20 are obtainable by crossing plants of cultivars CMS KY171 SRC and Narrow Leaf Madole (phph) SRC and collecting seeds. Seeds of hybrid cultivar PD7319 SRC are obtainable by crossing plants of cultivars CMS TND950 SRC and Narrow Leaf Madole (phph) SRC and collecting seeds.

In another aspect, the present disclosure also provides a container of TND950 (phph)
25 SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, CMS KY171 SRC, or hybrid PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC in which greater than about 50% of TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, CMS KY171 SRC, or hybrid PD7305 SRC, PD7309
30 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC or other seeds of the present disclosure have decreased normicotine. Representative samples of seeds of these cultivars having been deposited with the ATCC. Seeds of hybrid cultivar PD7305 SRC are obtainable by crossing plants of cultivars CMS TND950 SRC and TND950 (phph) SRC and collecting seeds. Seeds of hybrid cultivar PD7309 SRC are obtainable by crossing plants of cultivars CMS Narrow

Leaf Madole SRC and Narrow Leaf Madole (phph) SRC and collecting seeds. Seeds of hybrid cultivar PD7312 SRC are obtainable by crossing plants of cultivars CMS KY171 SRC and Narrow Leaf Madole SRC and collecting seeds. Seeds of hybrid cultivar PD7318 SRC are obtainable by crossing plants of cultivars CMS KY171 SRC and Narrow Leaf Madole (phph) SRC and collecting seeds. Seeds of hybrid cultivar PD7319 SRC are obtainable by crossing plants of cultivars CMS TND950 SRC and Narrow Leaf Madole (phph) SRC and collecting seeds.

In one aspect, the present disclosure provides a seed of a TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, CMS KY171 SRC, or hybrid PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC plant or other plant of the present disclosure in which a plant grown from a seed is male sterile. Representative samples of seeds of these cultivars having been deposited with the ATCC. Seeds of hybrid cultivar PD7305 SRC are obtainable by crossing plants of cultivars CMS TND950 SRC and TND950 (phph) SRC and collecting seeds. Seeds of hybrid cultivar PD7309 SRC are obtainable by crossing plants of cultivars CMS Narrow Leaf Madole SRC and Narrow Leaf Madole (phph) SRC and collecting seeds. Seeds of hybrid cultivar PD7312 SRC are obtainable by crossing plants of cultivars CMS KY171 SRC and Narrow Leaf Madole SRC and collecting seeds. Seeds of hybrid cultivar PD7318 SRC are obtainable by crossing plants of cultivars CMS KY171 SRC and Narrow Leaf Madole (phph) SRC and collecting seeds. Seeds of hybrid cultivar PD7319 SRC are obtainable by crossing plants of cultivars CMS TND950 SRC and Narrow Leaf Madole (phph) SRC and collecting seeds.

Tobacco material obtained from the tobacco lines, varieties or hybrids of the present disclosure can be used to make tobacco products including, without limitation, cigarette products (*e.g.*, cigarettes and bidi cigarettes), cigar products (*e.g.*, cigar wrapping tobacco and cigarillos), pipe tobacco products, smokeless cigarette products, smokeless tobacco products (*e.g.*, moist snuff, dry snuff, and chewing tobacco), films, chewables, tabs, shaped parts, gels, consumable units, insoluble matrices, hollow shapes and the like. *See, e.g.*, U.S. Patent Publication No. US 2006/0191548, which is herein incorporated by reference in its entirety.

Tobacco products derived from plants of the present disclosure also include cigarettes and other smoking articles, particularly those smoking articles including filter elements, wherein the rod of smokeable material includes cured tobacco within a tobacco blend. In an aspect, a tobacco product can include but is not limited to pipe tobacco, cigar tobacco,

cigarette tobacco, chewing tobacco, leaf tobacco, shredded tobacco, and/or cut tobacco or any combination thereof.

In an aspect, a tobacco product of the instant disclosure is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, and cut tobacco. In another aspect, a tobacco product of the instant disclosure is a smokeless tobacco product. In a further aspect, a tobacco product of the instant disclosure is selected from the group consisting of loose leaf chewing tobacco, plug chewing tobacco, moist snuff, and nasal snuff. In yet another aspect, a tobacco product of the instant disclosure is selected from the group consisting of an electronically heated cigarette, an e-cigarette, an electronic vaping device.

In an aspect, the tobacco product of the present disclosure can be a blended tobacco product. In other aspects of the disclosure, the tobacco product of the present disclosure can be a reduced nicotine tobacco product. In still other aspects, the tobacco product of the present disclosure can be a blended tobacco product with reduced nicotine content. Thus, the tobacco product of the present disclosure can be a blended reduced nicotine tobacco product. Tobacco product material comprises a blend of tobacco materials from the present disclosure, wherein the blend comprises at least about 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 95 percent by weight of a cured tobacco, or any range therein, based on the dry weight of the tobacco material. US 2008/0245377 is herein incorporated by reference for blend mixtures in its entirety.

In an aspect, tobacco products having a reduced amount of nitrosamine content can be manufactured using tobacco plant material from plants and plant parts of the present disclosure. Thus, in some aspects, a tobacco product manufactured using tobacco plant material from plants and plant parts of the present disclosure can comprise a reduced amount of nornicotine of less than about 3 mg/g. For example, the nornicotine content in such a product can be 3.0 mg/g, 2.5 mg/g, 2.0 mg/g, 1.5 mg/g, 1.0 mg/g, 750 µg/g, 500 µg/g, 250 µg/g, 100 µg/g, 75 µg/g, 50 µg/g, 25 µg/g, 10 µg/g, 5 µg/g, 1 µg/g, 750 ng/g, 500 ng/g, 250 ng/g, 100 ng/g, 75 ng/g, 50 ng/g, 25 ng/g, 10 ng/g, 5 ng/g, 1 ng/g, 750 pg/g, 500 pg/g, 250 pg/g, 100 pg/g, 75 pg/g, 50 pg/g, 25 pg/g, 10 pg/g, 7.0 pg/g, 5.0 pg/g, 4.0 pg/g, 2.0 pg/g, 1.0 pg/g, 0.5 pg/g, 0.4 pg/g, 0.2 pg/g, 0.1 pg/g, 0.05 pg/g, 0.01 pg/g, or undetectable, or any range therein. The tobacco product typically has a reduced amount of NNN of less than about 10 pg/g. For example, the NNN content in such a product can be about 10 pg/g, 7.0 pg/g, 5.0 pg/g, 4.0 pg/g, 2.0 pg/g, 1.0 pg/g, 0.5 pg/g, 0.4 pg/g, 0.2 pg/g, 0.1 pg/g, 0.05 pg/g, 0.01 pg/g,

or undetectable, or any range therein. The percentage of secondary alkaloids relative to total alkaloid content contained in a plant of the present disclosure may not be statistically different than from a commercial seedlot of TND950 (phph), Narrow Leaf Madole (phph), Narrow Leaf Madole, CMS TND950, CMS Narrow Leaf Madole, CMS KY171 or hybrid cultivars PD7305 LC, PD7309 LC, PD7312 LC, PD7318 LC, or PD7319 LC.

A tobacco plant of the present disclosure designated TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, CMS KY171 SRC, or hybrid PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC, carrying the *cyp82e4* W329Stop, the *cyp82e5v2* W422Stop, and *cyp82e10* P381S alleles can be used in a plant breeding program to create useful lines, cultivars, varieties, progeny, inbreds, and hybrids. Thus, in some aspects, an F₁, F₂, F₃, or later generation tobacco plant containing the *cyp82e4* W329Stop, the *cyp82e5v2* W422Stop, and *cyp82e10* P381S alleles is crossed with a second *Nicotiana* plant, and progeny of the cross are identified in which the *cyp82e4* W329Stop, the *cyp82e5v2* W422Stop, and *cyp82e10* P381S alleles are present. It will be appreciated that the second *Nicotiana* plant can be TN90 or any other *Nicotiana* species or line, optionally with an additional desirable trait, such as herbicide resistance.

In still other aspects, methods of the present disclosure further include self-pollinating or pollinating a male sterile pollen acceptor with a pollen donor capable of being used in production of a progeny plant of the present disclosure, such as a male sterile hybrid of the present disclosure. Either the male sterile pollen acceptor plant or the pollen donor plant has at least one mutant allele, two, or even three mutant alleles at a nicotine demethylase locus, such as the *cyp82e4* W329Stop, *cyp82e5v2* W422Stop, and *cyp82e10* P381S alleles. In an aspect, all three alleles at each nicotine demethylase locus are mutant alleles, making the plant homozygous for *cyp82e4* W329Stop, the *cyp82e5v2* W422Stop, and *cyp82e10* P381S.

Breeding can be carried out via any known procedures. DNA fingerprinting, SNP or similar technologies may be used in a marker-assisted selection (MAS) breeding program to transfer or breed mutant alleles of a nicotine demethylase gene into other tobaccos. For example, a breeder can create segregating populations from hybridizations of a genotype containing *cyp82e4* W329Stop, *cyp82e5v2* W422Stop, and *cyp82e10* P381S alleles with an agronomically desirable genotype. Plants in the F₂ or backcross generations can be screened using a marker developed from *cyp82e4* W329Stop, the *cyp82e5v2* W422Stop, or *cyp82e10* P381S alleles or a fragment thereof, using one of the techniques known in the art or disclosed herein. Plants identified as possessing one or more *cyp82e4* W329Stop, the *cyp82e5v2*

W422Stop, and *cyp82e10* P381S alleles can be backcrossed or self-pollinated to create a second population to be screened. Depending on the expected inheritance pattern or the MAS technology used, it may be necessary to self-pollinate the selected plants before each cycle of backcrossing to aid identification of the desired individual plants. Backcrossing or other breeding procedure can be repeated until the desired phenotype of the recurrent parent is recovered. A recurrent parent in the present disclosure can be TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, or CMS KY171 SRC. Other breeding techniques can be found, for example, in Wernsman, E.A., and Rufty, R.C., 1987. Chapter Seventeen. Tobacco. pages 669-698 In: Cultivar Development. Crop Species. W.H. Fehr (ed.), MacMillan Publishing Go., Inc., New York, N.Y., incorporated herein by reference in their entireties.

Nicotiana species which exhibit breeding compatibility with *Nicotiana tabacum* include *Nicotiana amplexicaulis*, PI 271989; *Nicotiana benthamiana* PI 555478; *Nicotiana bigelovii* PI 555485; *Nicotiana debneyi*; *Nicotiana excelsior* PI 224063; *Nicotiana glutinosa* PI 555507; *Nicotiana goodspeedii* PI 241012; *Nicotiana gossei* PI 230953; *Nicotiana hesperis* PI 271991; *Nicotiana knightiana* PI 555527; *Nicotiana maritima* PI 555535; *Nicotiana megalosiphon* PI 555536; *Nicotiana nudicaulis* PI 555540; *Nicotiana paniculata* PI 555545; *Nicotiana plumbaginifolia* PI 555548; *Nicotiana repanda* PI 555552; *Nicotiana rustica*; *Nicotiana suaveolens* PI 230960; *Nicotiana sylvestris* PI 555569; *Nicotiana tomentosa* PI 266379; *Nicotiana tomentosiformis*; and *Nicotiana trigonophylla* PI 555572. See also, Compendium of Tobacco Diseases published by American Phytopathology Society, or The Genus *Nicotiana* Illustrated, published by Japan Tobacco Inc, hereby incorporated by reference in their entireties.

The result of a plant breeding program using the mutant tobacco plants described herein includes useful lines, cultivars, varieties, progeny, inbreds, and hybrids. As used herein, the term “cultivar” or “variety” refers to a population of plants that share constant characteristics which separate them from other plants of the same species. A cultivar or variety is often, although not always, sold commercially. While possessing one or more distinctive traits, a cultivar or variety is further characterized by a very small overall variation between individuals within that cultivar or variety. A “pure line” variety may be created by several generations of self-pollination and selection, or vegetative propagation from a single parent using tissue or cell culture techniques. A cultivar or variety can be essentially derived from another cultivar, line, or variety. As defined by the International Convention for the Protection of New Varieties of Plants (Dec. 2, 1961, as revised at Geneva on Nov. 10, 1972,

on Oct. 23, 1978, and on Mar. 19, 1991), a cultivar or variety is “essentially derived” from an initial cultivar or variety if: a) it is predominantly derived from the initial cultivar or variety, or from a cultivar or variety that is predominantly derived from the initial cultivar or variety, while retaining the expression of the essential characteristics that result from the genotype or combination of genotypes of the initial cultivar or variety; b) it is clearly distinguishable from the initial cultivar or variety; and c) except for the differences which result from the act of derivation, it conforms to the initial cultivar or variety in the expression of the essential characteristics that result from the genotype or combination of genotypes of the initial cultivar or variety. Essentially derived varieties can be obtained, for example, by the selection of a natural or induced mutant, a somaclonal variant, a variant individual from plants of the initial cultivar or variety, backcrossing, or transformation. A “line” as distinguished from a cultivar or variety most often denotes a group of plants used non-commercially, for example in plant research. A line typically displays little overall variation between individuals for one or more traits of interest, although there may be some variation between individuals for other traits.

Hybrid tobacco varieties can be produced by preventing self-pollination of female parent plants (*i.e.*, seed parents) of a first variety, permitting pollen from male parent plants of a second variety to fertilize the female parent plants, and allowing F₁ hybrid seeds to form on the female plants. Self-pollination of female plants can be prevented by emasculating the flowers at an early stage of flower development. Alternatively, pollen formation can be prevented on the female parent plants using a form of male sterility. For example, male sterility can be produced by cytoplasmic male sterility (CMS), or transgenic male sterility wherein a transgene inhibits microsporogenesis and/or pollen formation, or self-incompatibility. Female parent plants containing CMS are particularly useful. In aspects in which the female parent plants are CMS, pollen may be harvested from male fertile plants and applied manually to the stigmas of CMS female parent plants, and the resulting F₁ seed is harvested.

Plants can be used to form single-cross tobacco F₁ hybrids. In such an aspect, the plants of the parent varieties can be grown as substantially homogeneous adjoining populations to facilitate natural cross-pollination from the male parent plants to the female parent plants. The F₁ seed formed on the female parent plants is selectively harvested by conventional means. One also can grow the two parent plant varieties in bulk and harvest a blend of F₁ hybrid seed formed on the female parent and seed formed upon the male parent as the result of self-pollination. Alternatively, three-way crosses can be carried out wherein a

single-cross F_1 hybrid is used as a female parent and is crossed with a different male parent. As another alternative, double-cross hybrids can be created wherein the F_1 progeny of two different single-crosses are themselves crossed. Self-incompatibility can be used to particular advantage to prevent self-pollination of female parents when forming a double-cross hybrid.

Successful crosses yield F_1 plants that are fertile, have *cyp82e4* W329Stop, *cyp82e5v2* W422Stop, and *cyp82e10* P381S alleles, and can be backcrossed with one of the parents, such as TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, CMS KY171 SRC, PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC, if desired. In some aspects, a plant population in the F_2 generation is screened for *cyp82e4* W329Stop, the *cyp82e5v2* W422Stop, and *cyp82e10* P381S alleles. Selected plants can be crossed with one of the parents and the first backcross (BC1) generation plants are self-pollinated to produce a BC1 F_2 population that is again screened for variant nicotine demethylase gene expression (e.g., the null version of the nicotine demethylase gene). The process of backcrossing, self-pollination, and screening is repeated, for example, at least four times, until the final screening produces a plant that is fertile and reasonably similar to the recurrent parent. This plant, if desired, is self-pollinated and the progeny are subsequently screened again to confirm that the plant exhibits the same low nicotine conversion phenotype as TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, CMS KY171 SRC, PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC. Breeder's seed of the selected plant is produced using standard methods including, for example, field testing, confirmation of the null condition for nicotine demethylase, chemical analyses of cured leaf to determine the level of alkaloids and/or chemical analyses of cured leaf to determine the ratio of nornicotine to nicotine+nornicotine.

In one aspect, a F_1 progeny is the result of a cross between TND950 SRC and CMS TND950 SRC to generate F_1 progeny that are male sterile. In another aspect, a F_1 progeny is the result of a cross between Narrow Leaf Madole SRC and CMS Narrow Leaf Madole SRC to generate F_1 progeny that are male sterile. In another aspect, a F_1 progeny is the result of a cross between Narrow Leaf Madole (phph) SRC and CMS Narrow Leaf Madole (phph) SRC to generate F_1 progeny that are male sterile. In another aspect, a F_1 progeny is the result of a cross between KY171 SRC and CMS KY171 SRC to generate F_1 progeny that are male sterile. In another aspect, a F_1 progeny is the result of a cross between TND950 (phph) SRC and CMS TND950 (phph) SRC to generate F_1 progeny that are male sterile. Male sterile

tobacco plants may be produced by any method known in the art. Methods of producing male sterile tobacco are described in Wernsman, E. A., and Rufty, R. C. 1987. Chapter Seventeen. Tobacco. Pages 669-698 In: Cultivar Development. Crop Species. W. H. Fehr (ed.), MacMillan Publishing Co., Inc., New York, N.Y. 761 pp.

5 The present disclosure further provides methods of producing a tobacco plant by crossing one of cultivars TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, CMS KY171 SRC, PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC with itself or a different tobacco line. The disclosure further relates to methods for producing other tobacco
10 cultivars or breeding lines derived from cultivars TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, CMS KY171 SRC, PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC by crossing a plant of cultivars TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole
15 SRC, CMS KY171 SRC, PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC with a second tobacco plant and growing the progeny seed to yield a TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, CMS KY171 SRC, PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC -derived tobacco plant. An additional aspect of
20 the present disclosure provides a method for producing a tobacco plant that contains in its genetic material one or more transgenes, comprising crossing cultivars of the present disclosure with a second cultivar containing one or more transgenes wherein progeny are produced, so that the genetic material of the progeny that result from the cross comprise the transgene(s) optionally operably linked to one or more regulatory elements. In one aspect,
25 the second cultivar may be a plant derived from cultivars TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, CMS KY171 SRC, PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC transformed with one or more transgenes.

 The disclosure further provides for the vegetative propagation of a plant of cultivars
30 TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, CMS KY171 SRC, or hybrid PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC, hybrids and progeny thereof. In one aspect, the disclosure provides for a method of vegetatively propagating a plant of a tobacco cultivar comprising collecting tissue capable of being propagated from a plant of

cultivars TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, CMS KY171 SRC, or hybrid PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC, cultivating the tissue to obtain a proliferated shoot and rooting the proliferated shoots to obtain a rooted

5 plantlet. In another aspect, the plant tissue may be collected from an F₁ hybrid of a plant of cultivars TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, CMS KY171 SRC, or hybrid PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC. In an aspect, the plant tissue may be collected from an F₂, F₃, F₄ or later progeny plant obtained by breeding a
10 plant of cultivars TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, CMS KY171 SRC, or hybrid PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC.

A plant comprising a mutation in a nicotine demethylase gene can be identified by selecting or screening the mutagenized plant material, or progeny thereof. Such screening
15 and selection methodologies are known to those having ordinary skill in the art. Examples of screening and selection methodologies include, but are not limited to, Southern analysis, PCR amplification for detection of a polynucleotide, Northern blots, RNase protection, primer-extension, RT-PCR amplification for detecting RNA transcripts, enzymatic assays for detecting enzyme or ribozyme activity of polypeptides and polynucleotides, and protein gel
20 electrophoresis, Western blots, immunoprecipitation, and enzyme-linked immunoassays to detect polypeptides. Other techniques such as *in situ* hybridization, enzyme staining, and immunostaining also can be used to detect the presence or expression of polypeptides and/or polynucleotides. Methods for performing all of the referenced techniques are known.

It is understood that a tobacco plant of the present disclosure, including TND950
25 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, CMS KY171 SRC, or hybrid PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC, can be transformed by a genetic construct (nucleic acid construct) or transgene using any technique known in the art. Without limitation, an example of a desired trait can include herbicide resistance, pest resistance,
30 disease resistance, high yield, high grade index, curability, curing quality, mechanical harvestability, holding ability, leaf quality, height, plant maturation (*e.g.*, early maturing, early to medium maturing, medium maturing, medium to late maturing, or late maturing), stalk size (*e.g.*, small, medium, or large stalk), or leaf number per plant (*e.g.*, small (*e.g.*, 5-10 leaves), medium (*e.g.*, 11-15 leaves), or large (*e.g.*, 16-21) number of leaves), or any

combination. Any plant of the present disclosure can be used as a basis for tissue culture, regeneration, transformed, or a combination of any of these. In an aspect, a plant of the present disclosure derived by tissue culture, transformation, or both has all, or essentially all, of the morphological and physiological characteristics of cultivar TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, CMS KY171 SRC, or hybrid PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, or PD7319 SRC.

In one aspect, a method is provided comprising curing the leaves in high-temperature fire-curing conditions, where tobacco comprising SRC technology comprises at least 50% less nornicotine and at least 50% less NNN as compared to tobacco grown comprising LC technology when both are cured under high-temperature conditions. In one aspect, a high-temperature fire-curing condition comprises a maximum curing temperature of at least 160°F. In another aspect, curing of SRC and LC tobacco is performed under normal fire-curing conditions. In a further aspect, tobacco comprising SRC technology comprises at least 50% less nornicotine and at least 50% less NNN as compared to tobacco grown comprising LC technology when both are cured under normal fire-curing conditions. In one aspect, a normal fire-curing condition comprises a maximum curing temperature of less than 145°F. In another aspect, a method further comprises growing tobacco comprising SRC technology and harvesting the leaves. In an aspect of a method provided herein, KY171 SRC and KY171 LC are grown.

In an aspect, KY171 SRC comprises at least 60% less nornicotine as compared to KY171 LC when cured under normal fire-curing conditions. In an aspect, KY171 SRC comprises at least 70% less nornicotine as compared to KY171 LC when cured under normal fire-curing conditions. In a further aspect, KY171 SRC comprises at least 75% less nornicotine as compared to KY171 LC when cured under normal fire-curing conditions. In another aspect, KY171 SRC comprises at least 55% less NNN as compared to KY171 LC when cured under normal fire-curing conditions. In another aspect, KY171 SRC comprises at least 60% less NNN as compared to KY171 LC when cured under normal fire-curing conditions.

In an aspect, KY171 SRC comprises at least 55% less nornicotine as compared to KY171 LC when cured under high-temperature fire-curing conditions. In a further aspect, KY171 SRC comprises at least 60% less nornicotine as compared to KY171 LC when cured under high-temperature fire-curing conditions. In another aspect, KY171 SRC comprises at least 70% less nornicotine as compared to KY171 LC when cured under high-temperature

fire-curing conditions. In an aspect, KY171 SRC comprises at least 55% less NNN as compared to KY171 LC when cured under high-temperature fire-curing conditions. In a further aspect, KY171 SRC comprises at least 60% less NNN as compared to KY171 LC when cured under high-temperature fire-curing conditions.

5 Having now generally described the disclosure, the same will be more readily understood through reference to the following examples that are provided by way of illustration, and are not intended to be limiting of the present disclosure, unless specified.

EXAMPLES

Example 1

10 **Breeding of homozygous *cyp82e4* W329Stop, the *cyp82e5v2* W422Stop, and *cyp82e10* P381S mutant plants into the KY171 Dark Tobacco Cultivar**

KY171 SRC is a backcross-derived version of dark tobacco cultivar KY171 carrying introduced mutations in three genes (*CYP82E4* (SEQ ID NO: 5), *CYP82E5* (SEQ ID NO: 8), and *CYP82E10* (SEQ ID NO: 9)) previously documented to encode for nicotine demethylase enzymes (Lewis *et al.*, 2010). The introduced mutations in *CYP82E4* (*cyp82e4* W329Stop (SEQ ID NO: 1)) and *CYP82E5* (*cyp82e5v2* W422Stop (SEQ ID NO: 2)) encode for premature stop codons which render the genes non-functional. The introduced mutation in *CYP82E10* (*cyp82e10* P381S (SEQ ID NO: 11)) does not encode for a premature stop codon, but does render the gene product non-functional for converting nicotine to nornicotine (Lewis *et al.* 2010). When in homozygous condition, the three mutations result in tobacco plants with (1) reduced genetic capacity to demethylate nicotine to form nornicotine, and (2) a corresponding diminished potential to accumulate *N*-nitrosonornicotine (NNN). The mutation in *CYP82E4* (*cyp82e4* W329Stop (SEQ ID NO: 1)) also provides phenotypic stability for the “nicotine conversion” trait and eliminates the requirement to utilize the labor-intensive “LC” screening method for reducing levels of nornicotine in tobacco cultivars.

The original tobacco cultivar KY171 is a fertile inbred line. CMS KY171 is a cytoplasmic male-sterile version of KY171. To develop KY171 SRC, an individual plant of KY171 is pollinated with a plant of the genetic background DH98-325-6 carrying mutations in each of the three nicotine demethylase genes. F₁ individuals originating from this cross and heterozygous for each mutation are backcrossed to the recurrent parent, KY171, to produce BC₁F₁ progeny. BC₁F₁ progeny are screened using genotyping methodologies to identify individuals heterozygous for all three mutations. A single triple heterozygous BC₁F₁

plant is backcrossed to the recurrent parent to produce BC₂F₁ progeny. The process of backcrossing and identification of individuals heterozygous for all three mutations is repeated through the BC₃F₁, BC₄F₁, BC₅F₁, BC₆F₁, and BC₇F₁ stages. At the BC₇F₁ stage, individuals heterozygous for all three mutations from each pedigree are self-pollinated to produce BC₇F₂ seed. A large number of BC₇F₂ progeny from each pedigree are genotyped to identify individuals homozygous for all three mutations. A single BC₇F₂ plant homozygous for all three mutations is self-pollinated to produce a BC₇F₃ family (KY171 SRC) in which the wild-type *CYP82E4*, *CYP82E5v2*, and *CYP82E10* alleles are replaced by the mutant alleles (*cyp82e4* W329Stop, *cyp82e5v2* W422Stop, and *cyp82e10* P381S).

The male-sterile (CMS) version of KY171 SRC (CMS KY171 SRC) is produced by crossing a plant of CMS KY171 as a female with pollen of KY171 SRC to produce male-sterile plants heterozygous for all three mutations. A single male-sterile plant resulting from this cross is then subsequently backcrossed as a female to KY171 SRC to produce progeny that are segregating for individuals homozygous for all three mutations. Triple homozygous individuals are identified by DNA genotyping to produce the CMS KY171 SRC line.

Because the line is male-sterile, it is maintained via pollination with KY171 SRC.

Commercial KY171 SRC is produced by pollinating plants of CMS KY171 SRC with pollen of KY171 SRC.

Example 2

Breeding of homozygous *cyp82e4* W329Stop, the *cyp82e5v2* W422Stop, and *cyp82e10* P381S mutant plants into the TND90 Dark Tobacco Cultivar

TND90 SRC is a backcross-derived version of dark tobacco cultivar TND90 carrying introduced mutations in three genes (*CYP82E4* (SEQ ID NO: 5), *CYP82E5* (SEQ ID NO: 8), and *CYP82E10* (SEQ ID NO: 9)) previously documented to encode for nicotine demethylase enzymes (Lewis *et al.*, 2010). The introduced mutations in *CYP82E4* (*cyp82e4* W329Stop (SEQ ID NO: 1)) and *CYP82E5* (*cyp82e5v2* W422Stop (SEQ ID NO: 2)) encode for premature stop codons which render the genes non-functional. The introduced mutation in *CYP82E10* (*cyp82e10* P381S (SEQ ID NO: 11)) does not encode for a premature stop codon, but does render the gene product non-functional for converting nicotine to nornicotine (Lewis *et al.* 2010). When in homozygous condition, the three mutations result in tobacco plants with (1) reduced genetic capacity to demethylate nicotine to form nornicotine, and (2) a corresponding diminished potential to accumulate *N*-nitrosonornicotine (NNN). The mutation in *CYP82E4* (*cyp82e4* W329Stop (SEQ ID NO: 1)) also provides phenotypic

stability for the “nicotine conversion” trait and eliminates the requirement to utilize the labor-intensive “LC” screening method for reducing levels of nicotine in tobacco cultivars.

The original tobacco cultivars TND90, is a fertile inbred line. CMS TND90 is a cytoplasmic male-sterile version of TND90. To develop TND90 SRC, an individual plant of TND90 is pollinated with a plant of the genetic background DH98-325-6 carrying mutations in each of the three nicotine demethylase genes. F₁ individuals originating from this cross and heterozygous for each mutation are backcrossed to the recurrent parent, TND90, to produce BC₁F₁ progeny. BC₁F₁ progeny are screened using genotyping methodologies to identify individuals heterozygous for all three mutations. A single triple heterozygous BC₁F₁ plant is backcrossed to the recurrent parent to produce BC₂F₁ progeny. The process of backcrossing and identification of individuals heterozygous for all three mutations is repeated through the BC₃F₁, BC₄F₁, BC₅F₁, BC₆F₁, and BC₇F₁ stages. At the BC₇F₁ stage, individuals heterozygous for all three mutations from each pedigree are self-pollinated to produce BC₇F₂ seed. A large number of BC₇F₂ progeny from each pedigree are genotyped to identify individuals homozygous for all three mutations. A single BC₇F₂ plant homozygous for all three mutations is self-pollinated to produce a BC₇F₃ family (TND90 SRC) in which the wild-type *CYP82E4*, *CYP82E5v2*, and *CYP82E10* alleles are replaced by the mutant alleles (*cyp82e4* W329Stop, *cyp82e5v2* W422Stop, and *cyp82e10* P381S).

The male-sterile (CMS) version of TND90 SRC (CMS TND90 SRC) is produced by crossing a plant of CMS TND90 as a female with pollen of TND90 SRC to produce male-sterile plants heterozygous for all three mutations. A single male-sterile plant resulting from this cross is then subsequently backcrossed as a female to TND90 SRC to produce progeny that are segregating for individuals homozygous for all three mutations. Triple homozygous individuals are identified by DNA genotyping to produce the CMS TND90 SRC line.

Because the line is male-sterile, it is maintained via pollination with TND90 SRC.

Commercial TND90 SRC is produced by pollinating plants of CMS TND90 SRC with pollen of TND90 SRC.

Example 3

Breeding of homozygous *cyp82e4* W329Stop, the *cyp82e5v2* W422Stop, and *cyp82e10* P381S mutant plants into the TND90 (phph) Dark Tobacco Cultivar

TND90 (phph) SRC is a backcross-derived version of dark tobacco cultivar TND90 (phph) carrying introduced mutations in three genes (*CYP82E4* (SEQ ID NO: 5), *CYP82E5* (SEQ ID NO: 8), and *CYP82E10* (SEQ ID NO: 9)) previously documented to encode for

nicotine demethylase enzymes (Lewis *et al.*, 2010). The introduced mutations in *CYP82E4* (*cyp82e4* W329Stop (SEQ ID NO: 1)) and *CYP82E5* (*cyp82e5v2* W422Stop (SEQ ID NO: 2)) encode for premature stop codons which render the genes non-functional. The introduced mutation in *CYP82E10* (*cyp82e10* P381S (SEQ ID NO: 11)) does not encode for a premature stop codon, but does render the gene product non-functional for converting nicotine to nornicotine (Lewis *et al.* 2010). When in homozygous condition, the three mutations result in tobacco plants with (1) reduced genetic capacity to demethylate nicotine to form nornicotine, and (2) a corresponding diminished potential to accumulate *N*-nitrosonornicotine (NNN). The mutation in *CYP82E4* (*cyp82e4* W329Stop (SEQ ID NO: 1)) also provides phenotypic stability for the “nicotine conversion” trait and eliminates the requirement to utilize the labor-intensive “LC” screening method for reducing levels of nornicotine in tobacco cultivars.

The original tobacco cultivar TND90 (phph) is a fertile inbred line. CMS TND90 (phph) is a cytoplasmic male-sterile version of TND90 (phph). To develop TND90 (phph) SRC, an individual plant of TND90 (phph) is pollinated with a plant of the genetic background DH98-325-6 carrying mutations in each of the three nicotine demethylase genes. F₁ individuals originating from this cross and heterozygous for each mutation are backcrossed to the recurrent parent, TND90 (phph), to produce BC₁F₁ progeny. BC₁F₁ progeny are screened using genotyping methodologies to identify individuals heterozygous for all three mutations. A single triple heterozygous BC₁F₁ plant is backcrossed to the recurrent parent to produce BC₂F₁ progeny. The process of backcrossing and identification of individuals heterozygous for all three mutations is repeated through the BC₃F₁, BC₄F₁, BC₅F₁, BC₆F₁, and BC₇F₁ stages. At the BC₇F₁ stage, individuals heterozygous for all three mutations from each pedigree are self-pollinated to produce BC₇F₂ seed. A large number of BC₇F₂ progeny from each pedigree are genotyped to identify individuals homozygous for all three mutations. A single BC₇F₂ plant homozygous for all three mutations is self-pollinated to produce a BC₇F₃ family (TND90 (phph) SRC) in which the wild-type *CYP82E4*, *CYP82E5v2*, and *CYP82E10* alleles are replaced by the mutant alleles (*cyp82e4* W329Stop, *cyp82e5v2* W422Stop, and *cyp82e10* P381S).

The male-sterile (CMS) version of TND90 (phph) SRC (CMS TND90 (phph) SRC) is produced by crossing a plant of CMS TND90 (phph) as a female with pollen of TND90 (phph) SRC to produce male-sterile plants heterozygous for all three mutations. A single male-sterile plant resulting from this cross is then subsequently backcrossed as a female to TND90 (phph) SRC to produce progeny that are segregating for individuals homozygous for

all three mutations. Triple homozygous individuals are identified by DNA genotyping to produce the TND90 (phph) SRC line. Because the line is male-sterile, it is maintained via pollination with TND90 (phph) SRC.

Commercial TND90 (phph) SRC is produced by pollinating plants of CMS TND90 (phph) SRC with pollen of TND90 (phph) SRC.

Example 4

Breeding of homozygous *cyp82e4* W329Stop, the *cyp82e5v2* W422Stop, and *cyp82e10* P381S mutant plants into the Narrow Leaf Madole Dark Tobacco Cultivar

Narrow Leaf Madole SRC is a backcross-derived version of dark tobacco cultivar Narrow Leaf Madole carrying introduced mutations in three genes (*CYP82E4* (SEQ ID NO: 5), *CYP82E5* (SEQ ID NO: 8), and *CYP82E10* (SEQ ID NO: 9)) previously documented to encode for nicotine demethylase enzymes (Lewis *et al.*, 2010). The introduced mutations in *CYP82E4* (*cyp82e4* W329Stop (SEQ ID NO: 1)) and *CYP82E5* (*cyp82e5v2* W422Stop (SEQ ID NO: 2)) encode for premature stop codons which render the genes non-functional. The introduced mutation in *CYP82E10* (*cyp82e10* P381S (SEQ ID NO: 11)) does not encode for a premature stop codon, but does render the gene product non-functional for converting nicotine to nornicotine (Lewis *et al.* 2010). When in homozygous condition, the three mutations result in tobacco plants with (1) reduced genetic capacity to demethylate nicotine to form nornicotine, and (2) a corresponding diminished potential to accumulate *N*-nitrosonornicotine (NNN). The mutation in *CYP82E4* (*cyp82e4* W329Stop (SEQ ID NO: 1)) also provides phenotypic stability for the “nicotine conversion” trait and eliminates the requirement to utilize the labor-intensive “LC” screening method for reducing levels of nornicotine in tobacco cultivars.

The original tobacco cultivar Narrow Leaf Madole is a fertile inbred line. CMS Narrow Leaf Madole is a cytoplasmic male-sterile version of Narrow Leaf Madole. To develop Narrow Leaf Madole SRC, an individual plant of Narrow Leaf Madole is pollinated with a plant of the genetic background DH98-325-6 carrying mutations in each of the three nicotine demethylase genes. F₁ individuals originating from this cross and heterozygous for each mutation are backcrossed to the recurrent parent, Narrow Leaf Madole, to produce BC₁F₁ progeny. BC₁F₁ progeny are screened using genotyping methodologies to identify individuals heterozygous for all three mutations. A single triple heterozygous BC₁F₁ plant is backcrossed to the recurrent parent to produce BC₂F₁ progeny. The process of backcrossing and identification of individuals heterozygous for all three mutations is repeated through the

BC₃F₁, BC₄F₁, BC₅F₁, BC₆F₁, and BC₇F₁ stages. At the BC₇F₁ stage, individuals heterozygous for all three mutations from each pedigree are self-pollinated to produce BC₇F₂ seed. A large number of BC₇F₂ progeny from each pedigree are genotyped to identify individuals homozygous for all three mutations. A single BC₇F₂ plant homozygous for all three mutations is self-pollinated to produce a BC₇F₃ family (Narrow Leaf Madole SRC) in which the wild-type *CYP82E4*, *CYP82E5v2*, and *CYP82E10* alleles of NC1209-23 are replaced by the mutant alleles (*cyp82e4* W329Stop, *cyp82e5v2* W422Stop, and *cyp82e10* P381S).

The male-sterile (CMS) version of Narrow Leaf Madole SRC (CMS Narrow Leaf Madole SRC) is produced by crossing a plant of CMS Narrow Leaf Madole as a female with pollen of Narrow Leaf Madole SRC to produce male-sterile plants heterozygous for all three mutations. A single male-sterile plant resulting from this cross is then subsequently backcrossed as a female to Narrow Leaf Madole SRC to produce progeny that are segregating for individuals homozygous for all three mutations. Triple homozygous individuals are identified by DNA genotyping to produce the CMS Narrow Leaf Madole SRC line. Because the line is male-sterile, it is maintained via pollination with Narrow Leaf Madole SRC.

Commercial Narrow Leaf Madole SRC is produced by pollinating plants of CMS Narrow Leaf Madole SRC with pollen of Narrow Leaf Madole SRC.

Example 5

Assessing nornicotine and NNN amounts in fire-cured KY171 SRC Dark Tobacco

To assess the differences in nornicotine and NNN in KY171 SRC, both KY171 SRC and KY171 LC are grown using standard growth conditions and fire-cured according to recommended fire-curing practices (See, e.g., 2017-2018 *Burley and Dark Tobacco Production Guide* (B. Pearce ed., (2017))). 36 sticks of both KY171 SRC and KY171 LC are cured for a total of 6 replications per variety.

The level of the alkaloid nornicotine is determined with Gas Chromatography followed by Mass Spectrometry (GC-MS). For example, one gram of cured leaf tissue is mixed with 10 mls of 2N NaOH, followed by incubation at room temperature for fifteen minutes. Nornicotine is then extracted by addition of 50 mls of 0.04% quinolone (w/v) dissolved in methyl-tert-butyl ether followed by rotation on a linear shaker for three hours. After phase separation, nornicotine levels are determined using an Agilent 6890 Gas

Chromatograph and an Agilent 5973N Mass Spectrometer. Under normal curing conditions, KY171 SRC tobacco shows a 78% reduction in nornicotine levels (Figure 1).

NNN is measured by crushing 750 mg of freeze-dried leaves and mixing with 30 mls of 10 mM ammonium acetate. After incubation in a shaker for 30 minutes, approximately 4 mls of sample is transferred into disposable culture tubes containing 0.25 ml of concentrated ammonium hydroxide. The sample is vortexed briefly and 1.5 mls is added to a prewashed and conditioned extraction cartridge with a flow rate of 1 to 2 drops per second. Analytes are eluted from the sample using 1.5 mls of 70:30 methanol with 0.1% acetic acid. Samples are analyzed using liquid chromatography with tandem mass spectrometry (LC/MS/MS). Under normal curing conditions, KY171 SRC tobacco shows a 64% reduction in NNN levels compared to KY171 LC (Figure 2).

The amounts of NNN and nornicotine are also measured for both KY171 SRC and KY171 LC tobacco cured under high- temperature stress conditions. To create high-temperature stress conditions, tobacco that is being cured is subjected to higher temperature fires. Similar to recommended fire-curing practices, four fires are burned during the curing period; however in the stressed barn, the fires are brought to a much higher temperature (Figure 3). Under the high temperature stressed curing conditions, nornicotine is again reduced by 70% in KY171 SRC tobacco compared to KY171 LC (Figure 1) and NNN is reduced by 61% in KY171 SRC tobacco compared to KY171 LC (Figure 2). SRC technology in KY171 acts to reduce the amount of both nornicotine and NNN compared to KY171 LC Dark Tobacco lines under both recommended and high-temperature stress curing conditions.

Example 6

Breeding of homozygous *cyp82e4* W329Stop, the *cyp82e5v2* W422Stop, and *cyp82e10* P381S mutant plants into the Narrow Leaf Madole (phph) Dark Tobacco Cultivar

Narrow Leaf Madole (phph) SRC is a backcross-derived version of dark tobacco cultivar Narrow Leaf Madole (phph) carrying introduced mutations in three genes (*CYP82E4* (SEQ ID NO: 5), *CYP82E5* (SEQ ID NO: 8), and *CYP82E10* (SEQ ID NO: 9)) previously documented to encode for nicotine demethylase enzymes (Lewis *et al.*, 2010). The introduced mutations in *CYP82E4* (*cyp82e4* W329Stop (SEQ ID NO: 1)) and *CYP82E5* (*cyp82e5v2* W422Stop (SEQ ID NO: 2)) encode for premature stop codons which render the genes non-functional. The introduced mutation in *CYP82E10* (*cyp82e10* P381S (SEQ ID

NO: 11)) does not encode for a premature stop codon, but does render the gene product non-functional for converting nicotine to nornicotine (Lewis *et al.* 2010). When in homozygous condition, the three mutations result in tobacco plants with (1) reduced genetic capacity to demethylate nicotine to form nornicotine, and (2) a corresponding diminished potential to accumulate *N*-nitrosornicotine (NNN). The mutation in *CYP82E4* (*cyp82e4* W329Stop (SEQ ID NO: 1)) also provides phenotypic stability for the “nicotine conversion” trait and eliminates the requirement to utilize the labor-intensive “LC” screening method for reducing levels of nornicotine in tobacco cultivars.

The original tobacco cultivar Narrow Leaf Madole (phph) is a fertile inbred line. CMS Narrow Leaf Madole (phph) is a cytoplasmic male-sterile version of Narrow Leaf Madole (phph). To develop Narrow Leaf Madole (phph) SRC, an individual plant of Narrow Leaf Madole (phph) is pollinated with a plant of the genetic background DH98-325-6 carrying mutations in each of the three nicotine demethylase genes. F₁ individuals originating from this cross and heterozygous for each mutation are backcrossed to the recurrent parent, Narrow Leaf Madole (phph), to produce BC₁F₁ progeny. BC₁F₁ progeny are screened using genotyping methodologies to identify individuals heterozygous for all three mutations. A single triple heterozygous BC₁F₁ plant is backcrossed to the recurrent parent to produce BC₂F₁ progeny. The process of backcrossing and identification of individuals heterozygous for all three mutations is repeated through the BC₃F₁, BC₄F₁, BC₅F₁, BC₆F₁, and BC₇F₁ stages. At the BC₇F₁ stage, individuals heterozygous for all three mutations from each pedigree are self-pollinated to produce BC₇F₂ seed. A large number of BC₇F₂ progeny from each pedigree are genotyped to identify individuals homozygous for all three mutations. A single BC₇F₂ plant homozygous for all three mutations is self-pollinated to produce a BC₇F₃ family (Narrow Leaf Madole (phph) SRC) in which the wild-type *CYP82E4*, *CYP82E5v2*, and *CYP82E10* alleles are replaced by the mutant alleles (*cyp82e4* W329Stop, *cyp82e5v2* W422Stop, and *cyp82e10* P381S).

The male-sterile (CMS) version of Narrow Leaf Madole (phph) SRC (CMS Narrow Leaf Madole (phph) SRC) is produced by crossing a plant of CMS Narrow Leaf Madole (phph) as a female with pollen of Narrow Leaf Madole (phph) SRC to produce male-sterile plants heterozygous for all three mutations. A single male-sterile plant resulting from this cross is then subsequently backcrossed as a female to Narrow Leaf Madole (phph) SRC to produce progeny that are segregating for individuals homozygous for all three mutations. Triple homozygous individuals are identified by DNA genotyping to produce the CMS

Narrow Leaf Madole (phph) SRC line. Because the line is male-sterile, it is maintained via pollination with Narrow Leaf Madole (phph) SRC.

Commercial Narrow Leaf Madole (phph) SRC is produced by pollinating plants of CMS Narrow Leaf Madole (phph) SRC with pollen of Narrow Leaf Madole (phph) SRC.

5 **Table 1: Breeding of Hybrid SRC lines**

Hybrid	Female parent	Male Parent
PD7305 SRC	CMS TND950 SRC	TND950 (phph) SRC
PD7309 SRC	CMS Narrow Leaf Madole SRC	Narrow Leaf Madole (phph) SRC
PD7312 SRC	CMS KY171 SRC	Narrow Leaf Madole SRC
PD7318 SRC	CMS KY171 SRC	Narrow Leaf Madole (phph) SRC
PD7319 SRC	CMS TND950 SRC	Narrow Leaf Madole (phph) SRC

Example 7

Breeding of homozygous *cyp82e4* W329Stop, the *cyp82e5v2* W422Stop, and *cyp82e10* P381S mutant plants into the PD7305 LC Dark Tobacco Cultivar

10 The original tobacco cultivar PD7305 LC is a hybrid generated by pollinating plants of a male-sterile breeding line CMS TND950 with pollen produced by fertile breeding line TND950 (phph). Hybrid cultivar PD7305 SRC is a hybrid generated by pollinating plants of a male-sterile breeding line CMS TND950 SRC with pollen produced by fertile breeding line TND950 (phph) SRC (Table 1).

15 Each SRC line carries introduced deleterious mutations in three genes (*CYP82E4* (SEQ ID NO: 5), *CYP82E5* (SEQ ID NO: 8), and *CYP82E10* (SEQ ID NO: 9)) previously documented to encode for nicotine demethylase enzymes (Lewis *et al.*, *Phytochemistry*, 71 (2010), 1988-1998). The introduced mutations in *CYP82E4* (*cyp82e4* W329Stop (SEQ ID NO: 1)) and *CYP82E5* (*cyp82e5v2* W422Stop (SEQ ID NO: 2)) encode for premature stop
20 codons which render the genes non-functional. The introduced mutation in *CYP82E10* (*cyp82e10* P381S (SEQ ID NO: 11)) does not encode for a premature stop codon, but does render the gene product non-functional for converting nicotine to nornicotine (*Id.*). When in homozygous condition, the three mutations result in tobacco plants with (1) reduced genetic capacity to demethylate nicotine to form nornicotine, and (2) a corresponding diminished
25 potential to accumulate *N*-nitrosonornicotine (NNN). The mutation in *CYP82E4* (*cyp82e4* W329Stop (SEQ ID NO: 1)) also provides phenotypic stability for the “nicotine conversion” trait and eliminates the requirement to utilize the labor-intensive “LC” screening method for reducing levels of nornicotine in tobacco cultivars (*see e.g.*, Jack et al. 2007. Implications of

reducing nornicotine accumulation in burley tobacco: appendix A – the LC protocol. *Rec. Adv. Tob. Sci.* 33: 58-79).

BC₁F₁ progenies are screened using genotyping methodologies to identify individuals heterozygous for all three mutations. A single BC₁F₁ plant from each pedigree is backcrossed to the recurrent parent, to produce BC₂F₁ progenies. The process of backcrossing and identification of individuals heterozygous for all three mutations is repeated through the BC₃F₁, BC₄F₁, BC₅F₁, BC₆F₁, and BC₇F₁ stages. At the BC₇F₁ stage, individuals heterozygous for all three mutations from each pedigree are self-pollinated to produce BC₇F₂ seed. A large number of BC₇F₂ progeny from each pedigree are genotyped to identify individuals homozygous for all three mutations. BC₇F₂ plants homozygous for all three mutations are self-pollinated to produce BC₇F₃ families (SRC lines) in which the wild-type *CYP82E4*, *CYP82E5v2*, and *CYP82E10* alleles are replaced by the mutant alleles (*cyp82e4* W329Stop, *cyp82e5v2* W422Stop, and *cyp82e10* P381S).

The female parental line of PD7305 LC, CMS TND950, is cytoplasmic male sterile which causes pollen to not be produced. To develop CMS TND950 SRC dark tobacco, a plant of CMS TND950 was crossed with TND950 SRC dark tobacco to produce male-sterile plants heterozygous for all three mutations. A single male-sterile plant resulting from this cross was then subsequently backcrossed as a female to TND950 SRC dark tobacco to produce progeny that were segregating for individuals homozygous for all three mutations. Triple homozygous individuals were identified by DNA genotyping to produce the CMS TND950 SRC dark tobacco line. Because the line is male-sterile, it is maintained via pollination with TND950 SRC dark tobacco.

Example 8

Breeding of homozygous *cyp82e4* W329Stop, the *cyp82e5v2* W422Stop, and *cyp82e10* P381S mutant plants into the PD7309 LC Dark Tobacco Cultivar

The original tobacco cultivar PD7309 LC is a hybrid generated by pollinating plants of a male-sterile breeding line CMS Narrow Leaf Madole with pollen produced by fertile breeding line Narrow Leaf Madole (phph). Hybrid cultivar PD7309 SRC is a hybrid generated by pollinating plants of a male-sterile breeding line CMS Narrow Leaf Madole SRC with pollen produced by fertile breeding line Narrow Leaf Madole (phph) SRC (Table 1).

Each SRC line carries introduced deleterious mutations in three genes (*CYP82E4* (SEQ ID NO: 5), *CYP82E5* (SEQ ID NO: 8), and *CYP82E10* (SEQ ID NO: 9)) previously

documented to encode for nicotine demethylase enzymes (Lewis *et al.*, *Phytochemistry*, 71 (2010), 1988-1998). The introduced mutations in *CYP82E4* (*cyp82e4* W329Stop (SEQ ID NO: 1)) and *CYP82E5* (*cyp82e5v2* W422Stop (SEQ ID NO: 2)) encode for premature stop codons which render the genes non-functional. The introduced mutation in *CYP82E10* (*cyp82e10* P381S (SEQ ID NO: 11)) does not encode for a premature stop codon, but does render the gene product non-functional for converting nicotine to nornicotine (*Id.*). When in homozygous condition, the three mutations result in tobacco plants with (1) reduced genetic capacity to demethylate nicotine to form nornicotine, and (2) a corresponding diminished potential to accumulate *N*-nitrosonornicotine (NNN). The mutation in *CYP82E4* (*cyp82e4* W329Stop (SEQ ID NO: 1)) also provides phenotypic stability for the “nicotine conversion” trait and eliminates the requirement to utilize the labor-intensive “LC” screening method for reducing levels of nornicotine in tobacco cultivars (*see e.g.*, Jack et al. 2007. Implications of reducing nornicotine accumulation in burley tobacco: appendix A – the LC protocol. *Rec. Adv. Tob. Sci.* 33: 58-79).

BC₁F₁ progenies are screened using genotyping methodologies to identify individuals heterozygous for all three mutations. A single BC₁F₁ plant from each pedigree is backcrossed to the recurrent parent, to produce BC₂F₁ progenies. The process of backcrossing and identification of individuals heterozygous for all three mutations is repeated through the BC₃F₁, BC₄F₁, BC₅F₁, BC₆F₁, and BC₇F₁ stages. At the BC₇F₁ stage, individuals heterozygous for all three mutations from each pedigree are self-pollinated to produce BC₇F₂ seed. A large number of BC₇F₂ progeny from each pedigree are genotyped to identify individuals homozygous for all three mutations. BC₇F₂ plants homozygous for all three mutations are self-pollinated to produce BC₇F₃ families (SRC lines) in which the wild-type *CYP82E4*, *CYP82E5v2*, and *CYP82E10* alleles are replaced by the mutant alleles (*cyp82e4* W329Stop, *cyp82e5v2* W422Stop, and *cyp82e10* P381S).

The female parental line of PD7309 LC, CMS Narrow Leaf Madole, is cytoplasmic male sterile which causes pollen to not be produced. To develop CMS Narrow Leaf Madole SRC dark tobacco, a plant of CMS Narrow Leaf Madole was crossed with Narrow Leaf Madole SRC dark tobacco to produce male-sterile plants heterozygous for all three mutations. A single male-sterile plant resulting from this cross was then subsequently backcrossed as a female to Narrow Leaf Madole SRC dark tobacco to produce progeny that were segregating for individuals homozygous for all three mutations. Triple homozygous individuals were identified by DNA genotyping to produce the CMS Narrow Leaf Madole

SRC dark tobacco line. Because the line is male-sterile, it is maintained via pollination with Narrow Leaf Madole SRC dark tobacco.

Example 9

Breeding of homozygous *cyp82e4* W329Stop, the *cyp82e5v2* W422Stop, and *cyp82e10* P381S mutant plants into the PD7312 LC LC Dark Tobacco Cultivar

The original tobacco cultivar PD7312 LC is a hybrid generated by pollinating plants of a male-sterile breeding line CMS KY171 with pollen produced by fertile breeding line Narrow Leaf Madole. Hybrid cultivar PD7312 SRC is a hybrid generated by pollinating plants of a male-sterile breeding line CMS KY171 SRC with pollen produced by fertile breeding line Narrow Leaf Madole SRC (Table 1).

Each SRC line carries introduced deleterious mutations in three genes (*CYP82E4* (SEQ ID NO: 5), *CYP82E5* (SEQ ID NO: 8), and *CYP82E10* (SEQ ID NO: 9)) previously documented to encode for nicotine demethylase enzymes (Lewis *et al.*, *Phytochemistry*, 71 (2010), 1988-1998). The introduced mutations in *CYP82E4* (*cyp82e4* W329Stop (SEQ ID NO: 1)) and *CYP82E5* (*cyp82e5v2* W422Stop (SEQ ID NO: 2)) encode for premature stop codons which render the genes non-functional. The introduced mutation in *CYP82E10* (*cyp82e10* P381S (SEQ ID NO: 11)) does not encode for a premature stop codon, but does render the gene product non-functional for converting nicotine to nornicotine (*Id.*). When in homozygous condition, the three mutations result in tobacco plants with (1) reduced genetic capacity to demethylate nicotine to form nornicotine, and (2) a corresponding diminished potential to accumulate *N*-nitrosonornicotine (NNN). The mutation in *CYP82E4* (*cyp82e4* W329Stop (SEQ ID NO: 1)) also provides phenotypic stability for the “nicotine conversion” trait and eliminates the requirement to utilize the labor-intensive “LC” screening method for reducing levels of nornicotine in tobacco cultivars (*see e.g.*, Jack et al. 2007. Implications of reducing nornicotine accumulation in burley tobacco: appendix A – the LC protocol. *Rec. Adv. Tob. Sci.* 33: 58-79).

BC₁F₁ progenies are screened using genotyping methodologies to identify individuals heterozygous for all three mutations. A single BC₁F₁ plant from each pedigree is backcrossed to the recurrent parent, to produce BC₂F₁ progenies. The process of backcrossing and identification of individuals heterozygous for all three mutations is repeated through the BC₃F₁, BC₄F₁, BC₅F₁, BC₆F₁, and BC₇F₁ stages. At the BC₇F₁ stage, individuals heterozygous for all three mutations from each pedigree are self-pollinated to produce BC₇F₂ seed. A large number of BC₇F₂ progeny from each pedigree are genotyped to identify

individuals homozygous for all three mutations. BC₇F₂ plants homozygous for all three mutations are self-pollinated to produce BC₇F₃ families (SRC lines) in which the wild-type *CYP82E4*, *CYP82E5v2*, and *CYP82E10* alleles are replaced by the mutant alleles (*cyp82e4* W329Stop, *cyp82e5v2* W422Stop, and *cyp82e10* P381S).

5 The female parental line of PD7312 LC, CMS KY171, is cytoplasmic male sterile which causes pollen to not be produced. To develop CMS KY171 SRC dark tobacco, a plant of CMS KY171 was crossed with KY171 SRC dark tobacco to produce male-sterile plants heterozygous for all three mutations. A single male-sterile plant resulting from this cross was then subsequently backcrossed as a female to KY171 SRC dark tobacco to produce progeny
10 that were segregating for individuals homozygous for all three mutations. Triple homozygous individuals were identified by DNA genotyping to produce the CMS KY171 SRC dark tobacco line. Because the line is male-sterile, it is maintained via pollination with KY171 SRC dark tobacco.

Example 10

15 **Breeding of homozygous *cyp82e4* W329Stop, the *cyp82e5v2* W422Stop, and *cyp82e10* P381S mutant plants into the PD7318 LC Dark Tobacco Cultivar**

The original tobacco cultivar PD7318 LC is a hybrid generated by pollinating plants of a male-sterile breeding line CMS KY171 with pollen produced by fertile breeding line Narrow Leaf Madole (phph). Hybrid cultivar PD7318 SRC is a hybrid generated by
20 pollinating plants of a male-sterile breeding line CMS KY171 SRC with pollen produced by fertile breeding line Narrow Leaf Madole (phph) SRC (Table 1).

Each SRC line carries introduced deleterious mutations in three genes (*CYP82E4* (SEQ ID NO: 5), *CYP82E5* (SEQ ID NO: 8), and *CYP82E10* (SEQ ID NO: 9)) previously documented to encode for nicotine demethylase enzymes (Lewis *et al.*, *Phytochemistry*, 71
25 (2010), 1988-1998). The introduced mutations in *CYP82E4* (*cyp82e4* W329Stop (SEQ ID NO: 1)) and *CYP82E5* (*cyp82e5v2* W422Stop (SEQ ID NO: 2)) encode for premature stop codons which render the genes non-functional. The introduced mutation in *CYP82E10* (*cyp82e10* P381S (SEQ ID NO: 11)) does not encode for a premature stop codon, but does render the gene product non-functional for converting nicotine to nornicotine (*Id.*). When in
30 homozygous condition, the three mutations result in tobacco plants with (1) reduced genetic capacity to demethylate nicotine to form nornicotine, and (2) a corresponding diminished potential to accumulate *N*-nitrosornicotine (NNN). The mutation in *CYP82E4* (*cyp82e4* W329Stop (SEQ ID NO: 1)) also provides phenotypic stability for the “nicotine conversion”

trait and eliminates the requirement to utilize the labor-intensive “LC” screening method for reducing levels of nornicotine in tobacco cultivars (*see e.g.*, Jack et al. 2007. Implications of reducing nornicotine accumulation in burley tobacco: appendix A – the LC protocol. *Rec. Adv. Tob. Sci.* 33: 58-79).

5 BC₁F₁ progenies are screened using genotyping methodologies to identify individuals heterozygous for all three mutations. A single BC₁F₁ plant from each pedigree is backcrossed to the recurrent parent, to produce BC₂F₁ progenies. The process of backcrossing and identification of individuals heterozygous for all three mutations is repeated through the BC₃F₁, BC₄F₁, BC₅F₁, BC₆F₁, and BC₇F₁ stages. At the BC₇F₁ stage, individuals
 10 heterozygous for all three mutations from each pedigree are self-pollinated to produce BC₇F₂ seed. A large number of BC₇F₂ progeny from each pedigree are genotyped to identify individuals homozygous for all three mutations. BC₇F₂ plants homozygous for all three mutations are self-pollinated to produce BC₇F₃ families (SRC lines) in which the wild-type *CYP82E4*, *CYP82E5v2*, and *CYP82E10* alleles are replaced by the mutant alleles (*cyp82e4*
 15 W329Stop, *cyp82e5v2* W422Stop, and *cyp82e10* P381S).

The female parental line of PD7318 LC, CMS KY171, is cytoplasmic male sterile which causes pollen to not be produced. To develop CMS KY171 SRC dark tobacco, a plant of CMS KY171 was crossed with KY171 SRC dark tobacco to produce male-sterile plants heterozygous for all three mutations. A single male-sterile plant resulting from this cross was
 20 then subsequently backcrossed as a female to KY171 SRC dark tobacco to produce progeny that were segregating for individuals homozygous for all three mutations. Triple homozygous individuals were identified by DNA genotyping to produce the CMS KY171 SRC dark tobacco line. Because the line is male-sterile, it is maintained via pollination with KY171 SRC dark tobacco.

25 **Example 11**

Breeding of homozygous *cyp82e4* W329Stop, the *cyp82e5v2* W422Stop, and *cyp82e10* P381S mutant plants into the PD7319 LC Dark Tobacco Cultivar

The original tobacco cultivar PD7319 LC is a hybrid generated by pollinating plants of a male-sterile breeding line CMS TND950 with pollen produced by fertile breeding line
 30 Narrow Leaf Madole (phph). Hybrid cultivar PD7319 SRC is a hybrid generated by pollinating plants of a male-sterile breeding line CMS TND950 SRC with pollen produced by fertile breeding line Narrow Leaf Madole (phph) SRC (Table 1).

Each SRC line carries introduced deleterious mutations in three genes (*CYP82E4* (SEQ ID NO: 5), *CYP82E5* (SEQ ID NO: 8), and *CYP82E10* (SEQ ID NO: 9)) previously documented to encode for nicotine demethylase enzymes (Lewis *et al.*, *Phytochemistry*, 71 (2010), 1988-1998). The introduced mutations in *CYP82E4* (*cyp82e4* W329Stop (SEQ ID NO: 1)) and *CYP82E5* (*cyp82e5v2* W422Stop (SEQ ID NO: 2)) encode for premature stop codons which render the genes non-functional. The introduced mutation in *CYP82E10* (*cyp82e10* P381S (SEQ ID NO: 11)) does not encode for a premature stop codon, but does render the gene product non-functional for converting nicotine to nornicotine (*Id.*). When in homozygous condition, the three mutations result in tobacco plants with (1) reduced genetic capacity to demethylate nicotine to form nornicotine, and (2) a corresponding diminished potential to accumulate *N*-nitrosonornicotine (NNN). The mutation in *CYP82E4* (*cyp82e4* W329Stop (SEQ ID NO: 1)) also provides phenotypic stability for the “nicotine conversion” trait and eliminates the requirement to utilize the labor-intensive “LC” screening method for reducing levels of nornicotine in tobacco cultivars (*see e.g.*, Jack *et al.* 2007. Implications of reducing nornicotine accumulation in burley tobacco: appendix A – the LC protocol. *Rec. Adv. Tob. Sci.* 33: 58-79).

BC₁F₁ progenies are screened using genotyping methodologies to identify individuals heterozygous for all three mutations. A single BC₁F₁ plant from each pedigree is backcrossed to the recurrent parent, to produce BC₂F₁ progenies. The process of backcrossing and identification of individuals heterozygous for all three mutations is repeated through the BC₃F₁, BC₄F₁, BC₅F₁, BC₆F₁, and BC₇F₁ stages. At the BC₇F₁ stage, individuals heterozygous for all three mutations from each pedigree are self-pollinated to produce BC₇F₂ seed. A large number of BC₇F₂ progeny from each pedigree are genotyped to identify individuals homozygous for all three mutations. BC₇F₂ plants homozygous for all three mutations are self-pollinated to produce BC₇F₃ families (SRC lines) in which the wild-type *CYP82E4*, *CYP82E5v2*, and *CYP82E10* alleles are replaced by the mutant alleles (*cyp82e4* W329Stop, *cyp82e5v2* W422Stop, and *cyp82e10* P381S).

The female parental line of PD7319 LC, CMS TND950, is cytoplasmic male sterile which causes pollen to not be produced. To develop CMS TND950 SRC dark tobacco, a plant of CMS TND950 was crossed with TND950 SRC dark tobacco to produce male-sterile plants heterozygous for all three mutations. A single male-sterile plant resulting from this cross was then subsequently backcrossed as a female to TND950 SRC dark tobacco to produce progeny that were segregating for individuals homozygous for all three mutations.

Triple homozygous individuals were identified by DNA genotyping to produce the CMS TND950 SRC dark tobacco line. Because the line is male-sterile, it is maintained via pollination with TND950 SRC dark tobacco.

Example 12

Testing of Hybrid Cultivars PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, and PD7319 SRC

Hybrid cultivars PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, and PD7319 SRC are evaluated for cured leaf chemistry, yield, and physical quality at three locations during 2015 (Blackstone (VA), Springfield (TN) and Princeton (KY)). Hybrid cultivars PD7305 LC, PD7309 LC, PD7312 LC, PD7318 LC, and PD7319 LC are included for comparison. The experimental design at each location is a randomized complete block design with three replications. Plots are harvested and cured. Plot weights are used to determine per acre yields. Cured leaf is evaluated by a former USDA tobacco grader. Composite leaf samples are collected from each plot and analyzed for percent nicotine, nornicotine, percent nicotine conversion, parts per million of total TSNA's, and parts per million of NNN using gas chromatography equipment and techniques described in Example 5 above (Figure 4, 5, and Table 2).

SRC dark tobacco varieties exhibit a TSNA reduction of about 22% and an NNN reduction of about 74% compared to LC varieties grown in similar field plots (Figures 4 and 5). Comparisons using the Student's t test indicated that PD7309 SRC dark tobacco hybrid, PD7312 SRC dark tobacco hybrid PD7318 SRC dark tobacco hybrid, and PD7319 SRC dark tobacco hybrid had significantly ($P < 0.05$) lower levels of nornicotine, percent nicotine conversion and NNN relative to PD7309 LC, PD7312 LC, PD7318 LC, and PD7319 LC, respectively (Table 2). Each SRC and LC comparison did not show significant differences from each other for percent nicotine, yield, or cured leaf quality indices. Although we did not see a statistical difference for levels of nornicotine and percent of nicotine conversion between PD7305 LC and PD7305 SRC dark tobacco hybrid, statistical differences in the NNN levels between these two hybrids indicates the efficacy of the SRC technology in PD7305 SRC dark tobacco hybrid.

Example 13

Smokeless product prototypes comprising dark tobacco with SRC technology

Smokeless tobacco product prototypes are developed from dark fire-cured tobacco, dark air-cured tobacco, and Burley stems comprising SRC technology and from dark fire-

cured tobacco, dark air-cured tobacco, and Burley stems from LC varieties grown during the 2014 field season. SRC and LC tobacco is grown and cured using standard conditions and curing practices (*See, e.g., 2017-2018 Burley and Dark Tobacco Production Guide* (B. Pearce ed., (2017))). Tobacco included in smokeless product prototypes is aged for 2 years, processed, blended, fermented over three cures, and finished similar to the CRP2 standard moist snuff reference product.

Total TSNA and NNN amounts are determined for each blend component (dark fire-cured tobacco, dark air-cured tobacco, and burley stems) using techniques described in Example 5. Both TSNA and NNN amounts are lower in each blend component comprising SRC technology as compared to the LC variety controls grown and cured using the same conditions (Figures 6 and 7). Total TSNA and NNN amounts are also determined at the blending stage, once out of cure, and as finished products (Figures 8 and 9). Both TSNA and NNN amounts are lower at each step of the production process in tobacco comprising SRC technology as compared to the LC variety control undergoing the same processing steps. The reduction in total TSNA and NNN amounts in the finished smokeless product prototypes created using tobacco comprising SRC technology is consistent with the reductions seen in the raw materials.

Table 2. Means for field test of SRC dark tobacco hybrids in three locations in 2015.

Genotype	Yield (lbs/A)	Grade Index	Nicotine (%)	Nornicotine (%)	% Nicotine Conversion	NNN
PD7305 LC	2974	58	5.74	0.124	2.21	3.29*
PD7305 SRC	2900	55	5.78	0.019	0.33	0.52*
PD7309 LC	3030	63	6.66	0.087*	1.31*	2.07*
PD7309 SRC	2948	69	6.69	0.023*	0.33*	0.47*
PD7312 LC	3069	63	7.06	0.104*	1.38*	2.13*
PD7312 SRC	3069	63	7.55	0.024*	0.30*	0.60*
PD7318 LC	3001	67	6.49	0.075*	1.14*	1.87*
PD7318 SRC	2971	67	6.94	0.022*	0.30*	0.54*
PD7319 LC	3077	63	6.44	0.065*	0.97*	1.65*
PD7319 SRC	3034	68	6.89	0.021*	0.30*	0.58*

* Statistical difference between LC and SRC dark tobacco hybrids based on Student's t test with alpha = 0.05

5 PD7305 LC is a hybrid variety generated by pollinating plants of a male-sterile breeding line CMS TND950 with pollen produced by fertile breeding line TND950 (phph).

PD7305 SRC tobacco hybrid is a backcross-derived version of dark tobacco hybrid PD7305 LC carrying introduced deleterious mutations in three genes (*cyp82e4* W329Stop, *cyp82e5v2* W422Stop, and *cyp82e10* P381S).

10 PD7309 LC is a hybrid variety generated by pollinating plants of a male-sterile breeding line CMS Narrow Leaf Madole with pollen produced by fertile breeding line Narrow Leaf Madole (phph).

PD7309 SRC tobacco hybrid is a backcross-derived version of dark tobacco hybrid PD7309 LC carrying introduced deleterious mutations in three genes (*cyp82e4* W329Stop, *cyp82e5v2* W422Stop, and *cyp82e10* P381S).

15 PD7312 LC is a hybrid variety generated by pollinating plants of a male-sterile breeding line CMS KY171 with pollen produced by fertile breeding line Narrow Leaf Madole.

PD7312 SRC tobacco hybrid is a backcross-derived version of dark tobacco hybrid PD7312 LC carrying introduced deleterious mutations in three genes (*cyp82e4* W329Stop, *cyp82e5v2* W422Stop, and *cyp82e10* P381S).

5 PD7318 LC is a hybrid variety generated by pollinating plants of a male-sterile breeding line CMS KY171 with pollen produced by fertile breeding line Narrow Leaf Madole (phph).

PD7318 SRC tobacco hybrid is a backcross-derived version of dark tobacco hybrid PD7318 LC carrying introduced deleterious mutations in three genes (*cyp82e4* W329Stop, *cyp82e5v2* W422Stop, and *cyp82e10* P381S).

10 PD7319 LC is a hybrid variety generated by pollinating plants of a male-sterile breeding line CMS TND950 with pollen produced by fertile breeding line Narrow Leaf Madole (phph).

PD7319 SRC tobacco hybrid is a backcross-derived version of dark tobacco hybrid PD7319 LC carrying introduced deleterious mutations in three genes (*cyp82e4* W329Stop, *cyp82e5v2* W422Stop, and *cyp82e10* P381S).

15 DEPOSIT INFORMATION

A deposit of the proprietary inbred plant lines disclosed above and recited in the appended claims have been made with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110. The date of deposit for TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf
20 Madole SRC, CMS KY171 SRC was November 22, 2016. The deposits of 2500 seeds for each variety was taken from the same deposits maintained since prior to the filing date of this application. Upon issuance of a patent, all restrictions upon the deposits will be irrevocably removed, and the deposits are intended by Applicant to meet all of the requirements of 37 C.F.R. § 1.801-1.809. The ATCC has issued the following accession numbers: ATCC Accession No.
25 PTA-123665 for TND950 (phph) SRC; ATCC Accession No. PTA-123664 for Narrow Leaf Madole (phph) SRC; ATCC Accession No. PTA-123663 for Narrow Leaf Madole SRC, ATCC Accession No. PTA-123662 for CMS TND950 SRC, ATCC Accession No. PTA-123661 for CMS Narrow Leaf Madole SRC, and ATCC Accession No. PTA-123660 for CMS KY171 SRC. These deposits will be maintained in the depository for a period of 30 years, or 5 years after the
30 last request, or for the effective life of the patent, whichever is longer, and will be replaced as necessary during that period. Applicants do not waive any infringement of their rights granted under this patent or under the Plant Variety Protection Act (7 U.S.C. 2321 et seq.).

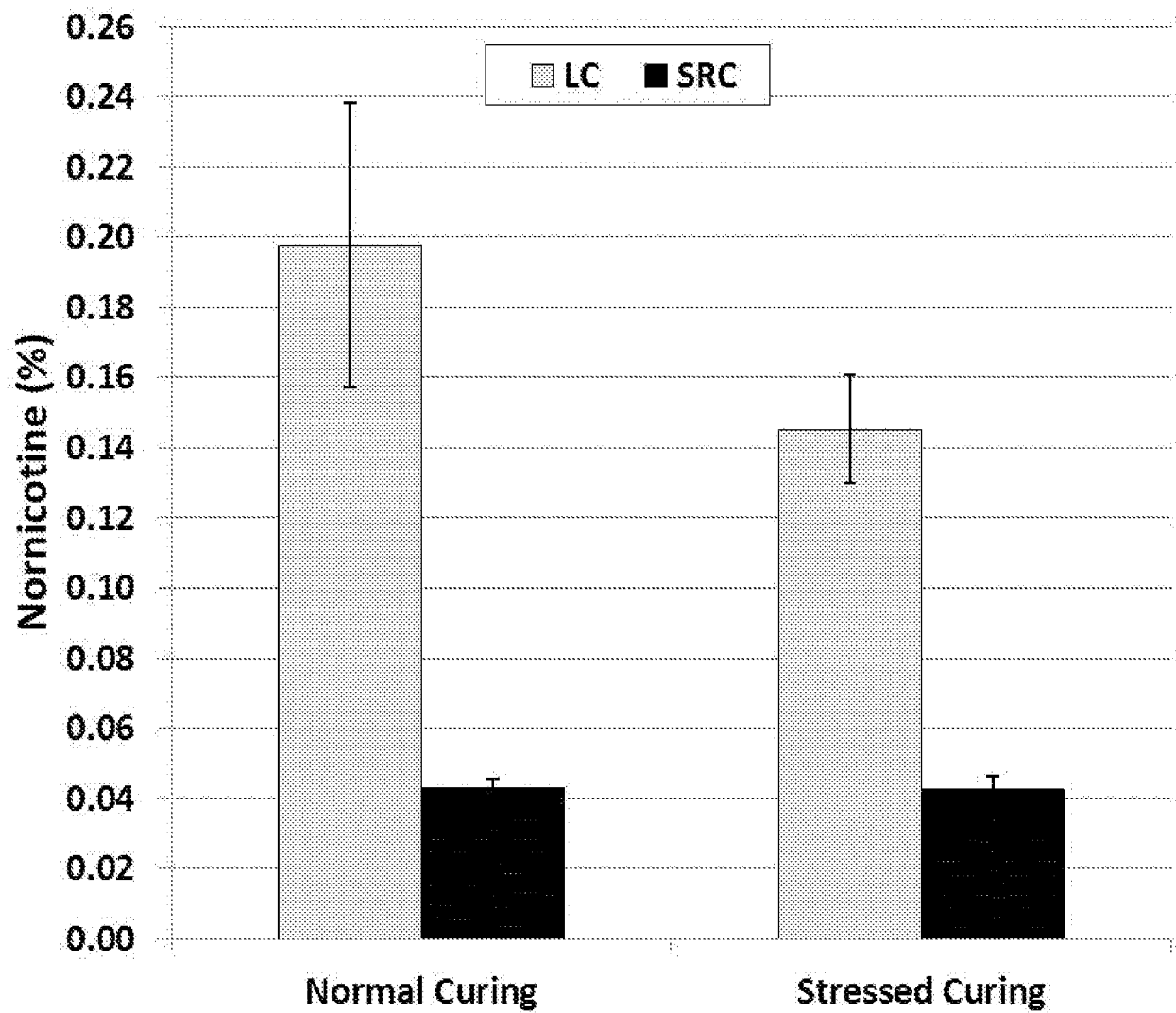
What is claimed is:

1. Cured tobacco material or a tobacco product prepared therefrom, wherein said cured tobacco material is from a tobacco plant, or part thereof, of a hybrid, a cultivar, or a progeny hybrid
5 produced from said cultivar,
said hybrid is selected from the group consisting of PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, and PD7319 SRC,
said cultivar is selected from the group consisting of TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf
10 Madole SRC, and CMS KY171 SRC; a representative sample seed of said TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, CMS KY171 SRC having been deposited with the ATCC under ATCC Accession Nos. PTA-123665, PTA-123664, PTA-123663, PTA-123662, PTA-123661, and PTA-123660, respectively.
- 15 2. The tobacco product of claim 1, wherein said tobacco product is selected from the group consisting of a pipe tobacco, a cigar tobacco, a cigarette tobacco, a chewing tobacco, a leaf tobacco, a shredded tobacco, a cut tobacco, a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, and a chewing tobacco.
- 20 3. The tobacco product of claim 1, wherein said tobacco product is selected from the group consisting of a cigarillo, a non-ventilated recess filter cigarette, a vented recess filter cigarette, a cigar, snuff, pipe tobacco, cigar tobacco, cigarette tobacco, chewing tobacco, leaf tobacco, hookah tobacco, shredded tobacco, cut tobacco, loose leaf chewing tobacco, plug chewing tobacco, moist snuff, and nasal snuff.
4. The tobacco product of claim 3, wherein said tobacco product has an amount of nornicotine
25 of no more than about 3 mg/g.
5. The tobacco product of claim 4, wherein said amount of nornicotine is less than 3.0 mg/g, 2.5 mg/g, 2.0 mg/g, 1.5 mg/g, 1.0 mg/g, 750 µg/g, 500 pg/g, 250 pg/g, 100 pg/g, 75 pg/g, 50 pg/g, 25 pg/g, 10 pg/g, 7.0 pg/g, 5.0 pg/g, 4.0 pg/g, 2.0 pg/g, 1.0 pg/g, 0.5 pg/g, 0.4 pg/g, 0.2 pg/g, 0.1 pg/g, 0.05 pg/g, 0.01 pg/g, or undetectable.
- 30 6. The tobacco product of claim 3, wherein said tobacco product has an amount of N'-nitrosonornicotine (NNN) of no more than about 10 pg/g.

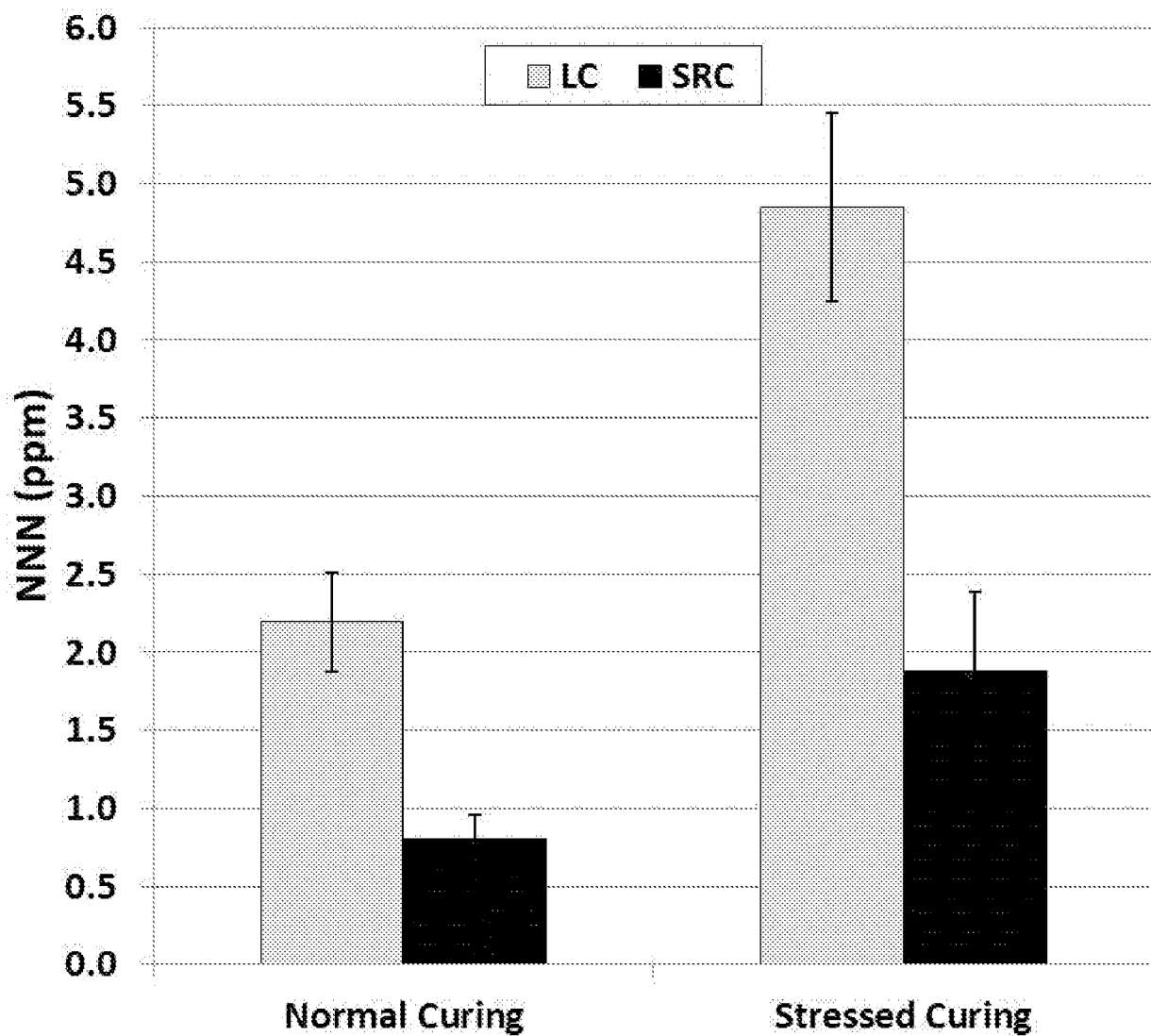
7. The tobacco product of claim 6, wherein said amount of NNN is less than 10 pg/g, 7.0 pg/g, 5.0 pg/g, 4.0 pg/g, 2.0 pg/g, 1.0 pg/g, 0.5 pg/g, 0.4 pg/g, 0.2 pg/g, 0.1 pg/g, 0.05 pg/g, 0.01 pg/g, or undetectable.
8. The tobacco product of claim 1, wherein said cured tobacco material is at least about 5% by
5 dry weight of the total tobacco material in said tobacco product.
9. The tobacco product of claim 8, wherein said cured tobacco material has a dry weight percentage of said total tobacco material selected from the group consisting of at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, and at least about
10 95%.
10. A method of producing a tobacco product, comprising:
- a. providing cured tobacco material from a tobacco plant, or part thereof, of a hybrid, a cultivar, or a hybrid produced from said cultivar; said hybrid is selected from the group consisting of PD7305 SRC, PD7309 SRC, PD7312 SRC, PD7318 SRC, and
15 PD7319 SRC, said cultivar is selected from the group consisting of TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, and CMS KY171 SRC; a representative sample seed of said TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, and CMS
20 KY171 SRC tobacco cultivars having been deposited with the ATCC under ATCC Accession Nos. PTA-123665, PTA-123664, PTA-123663, PTA-123662, PTA-123661, and PTA-123660, respectively;
 - b. preparing a tobacco product from said cured tobacco material.
11. A seed of tobacco cultivar selected from the group consisting of TND950 (phph) SRC,
25 Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, and CMS KY171 SRC; a representative sample seed of said TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, and CMS KY171 SRC tobacco cultivars having been deposited with the ATCC under ATCC Accession Nos. PTA-123665, PTA-
30 123664, PTA-123663, PTA-123662, PTA-123661, and PTA-123660, respectively.
12. A tobacco plant, or part thereof, produced from said seed of claim 11.

13. The part of the plant of claim 12, wherein said part is selected from the group consisting of a harvested leaf, a pollen, an ovule, an embryo, a cotyledon, a hypocotyl, a meristematic cell, a protoplast, a root, a root tip, a pistil, an anther, a flower, a shoot, a stem, a pod, a petiole, and combinations thereof.
- 5 14. A harvested leaf of said tobacco plant of claim 12.
15. The harvested leaf of claim 14, wherein said leaf has a reduced amount of nornicotine and/or N'-nitrosonornicotine (NNN).
16. The harvested leaf of claim 15, wherein said reduced amount of nornicotine and/or N-nitrosonornicotine (NNN) is reduced in a smoke stream produced from said leaf.
- 10 17. An F₁ progeny plant of a tobacco cultivar selected from the group consisting of TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, and CMS KY171 SRC; a representative sample seed of said TND950 (phph) SRC, Narrow Leaf Madole (phph) SRC, Narrow Leaf Madole SRC, CMS TND950 SRC, CMS Narrow Leaf Madole SRC, and CMS KY171 SRC tobacco
- 15 cultivars having been deposited with the ATCC under ATCC Accession Nos. PTA-123665, PTA-123664, PTA-123663, PTA-123662, PTA-123661, and PTA-123660, respectively.
18. The F₁ progeny plant of claim 17, wherein said F₁ plant is male sterile.

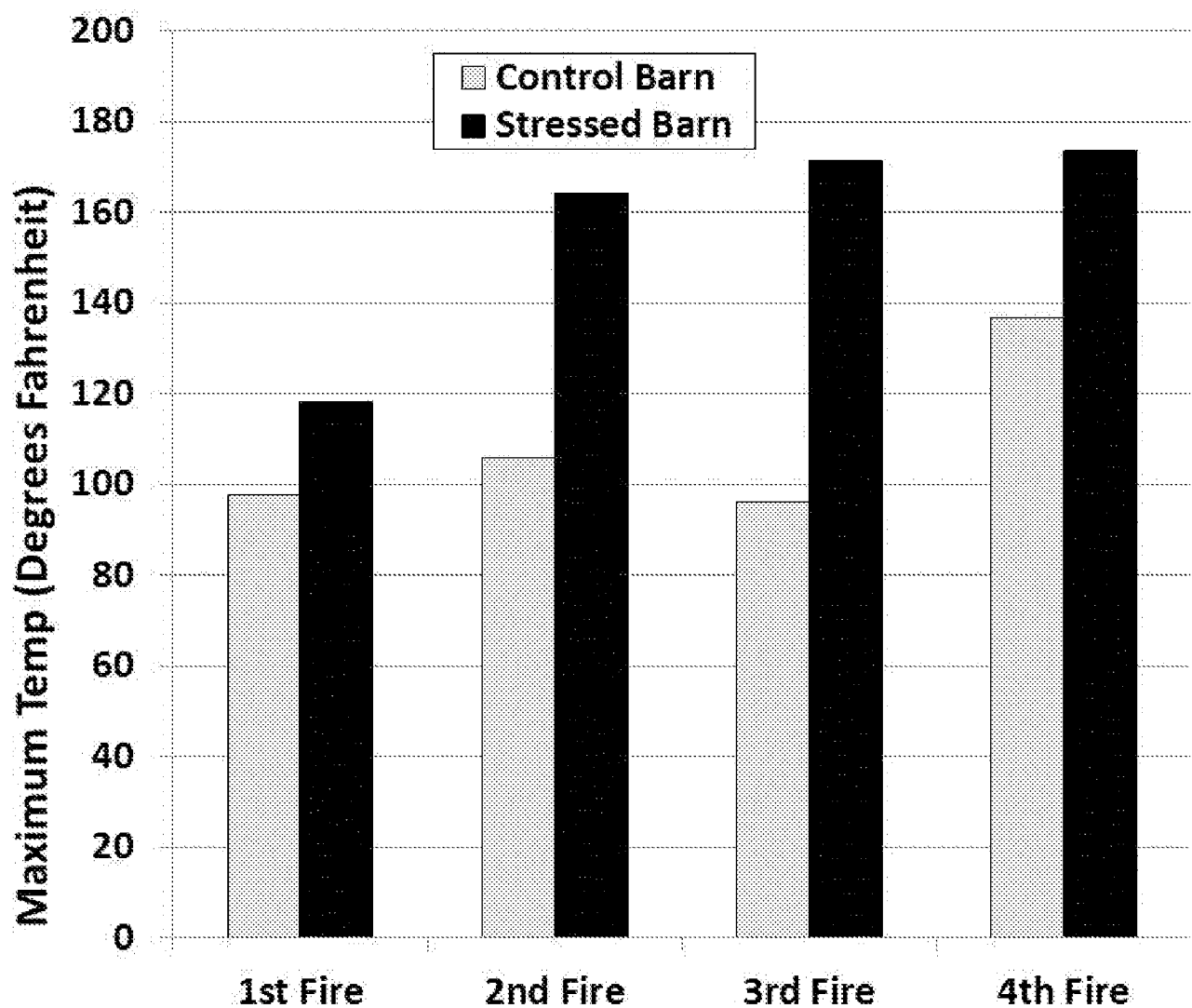
1/9

FIGURE 1

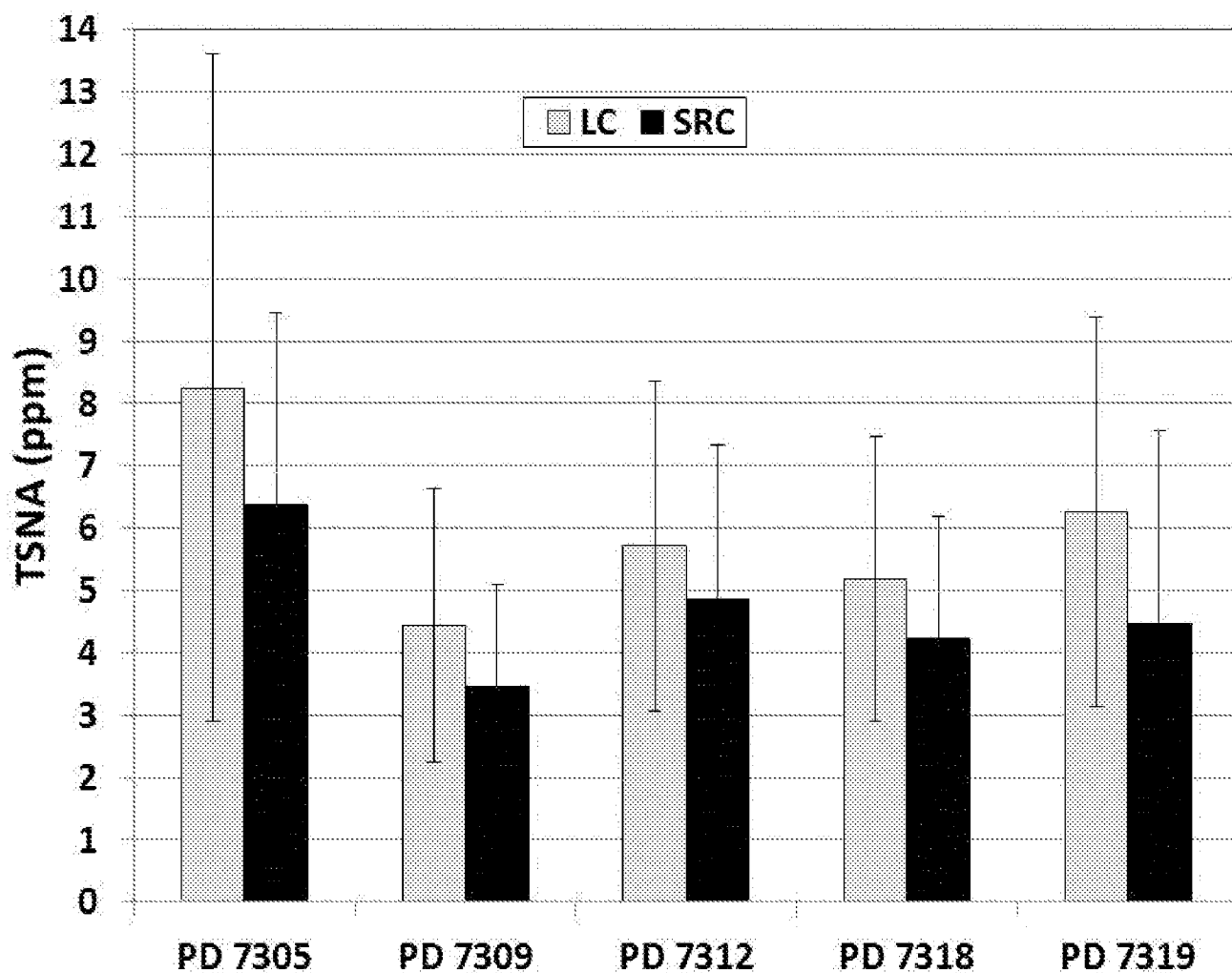
2/9

FIGURE 2

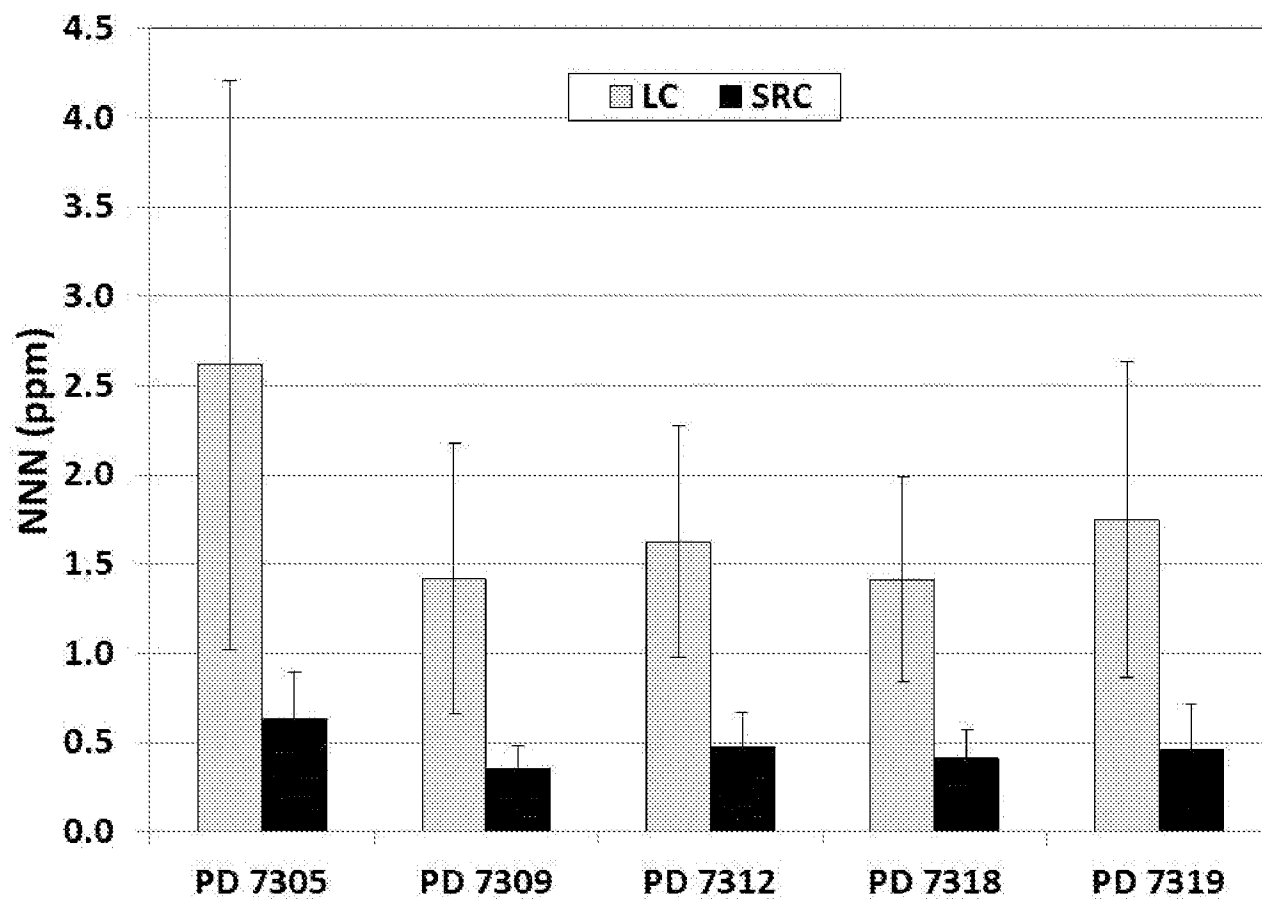
3/9

FIGURE 3

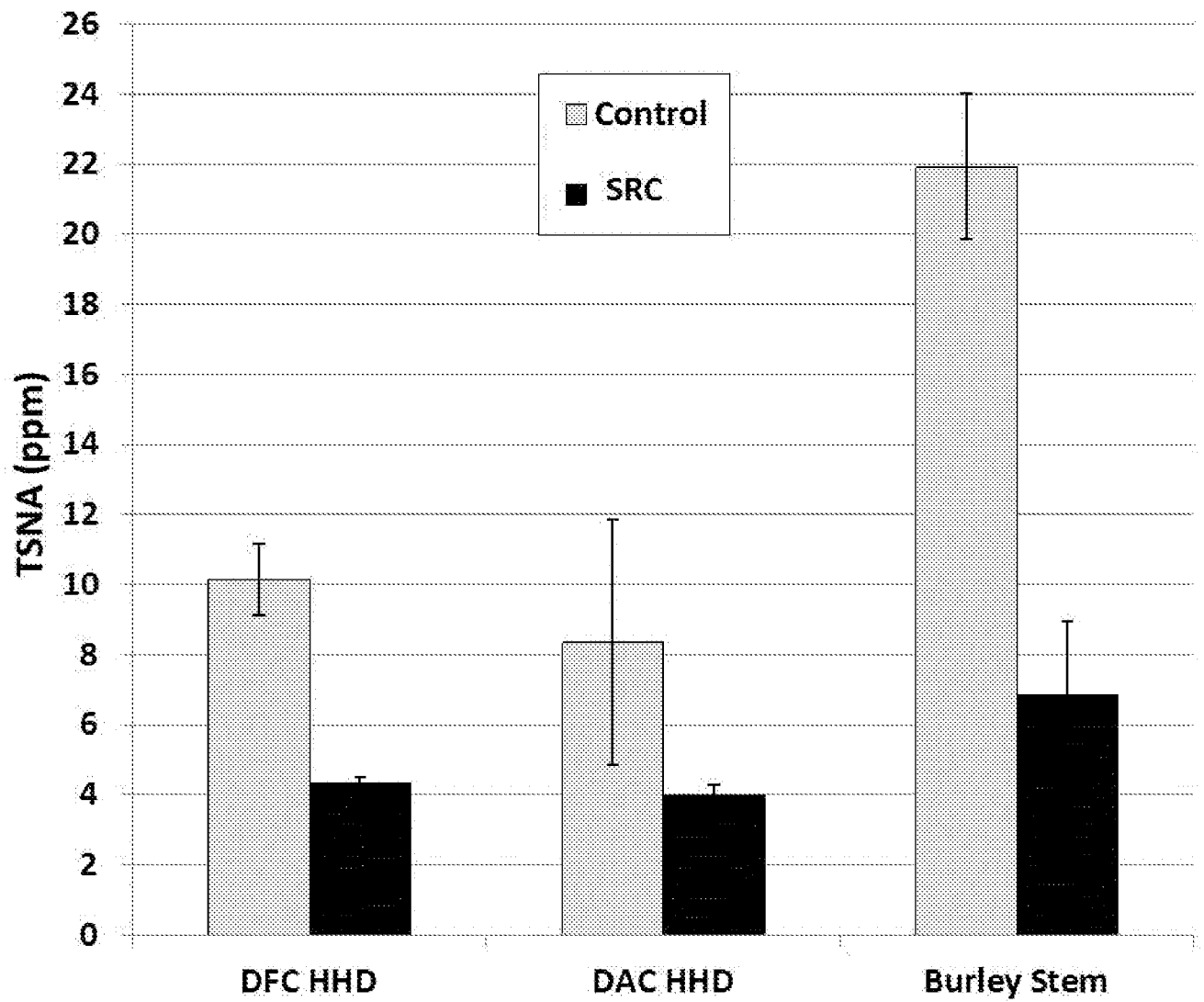
4/9

FIGURE 4

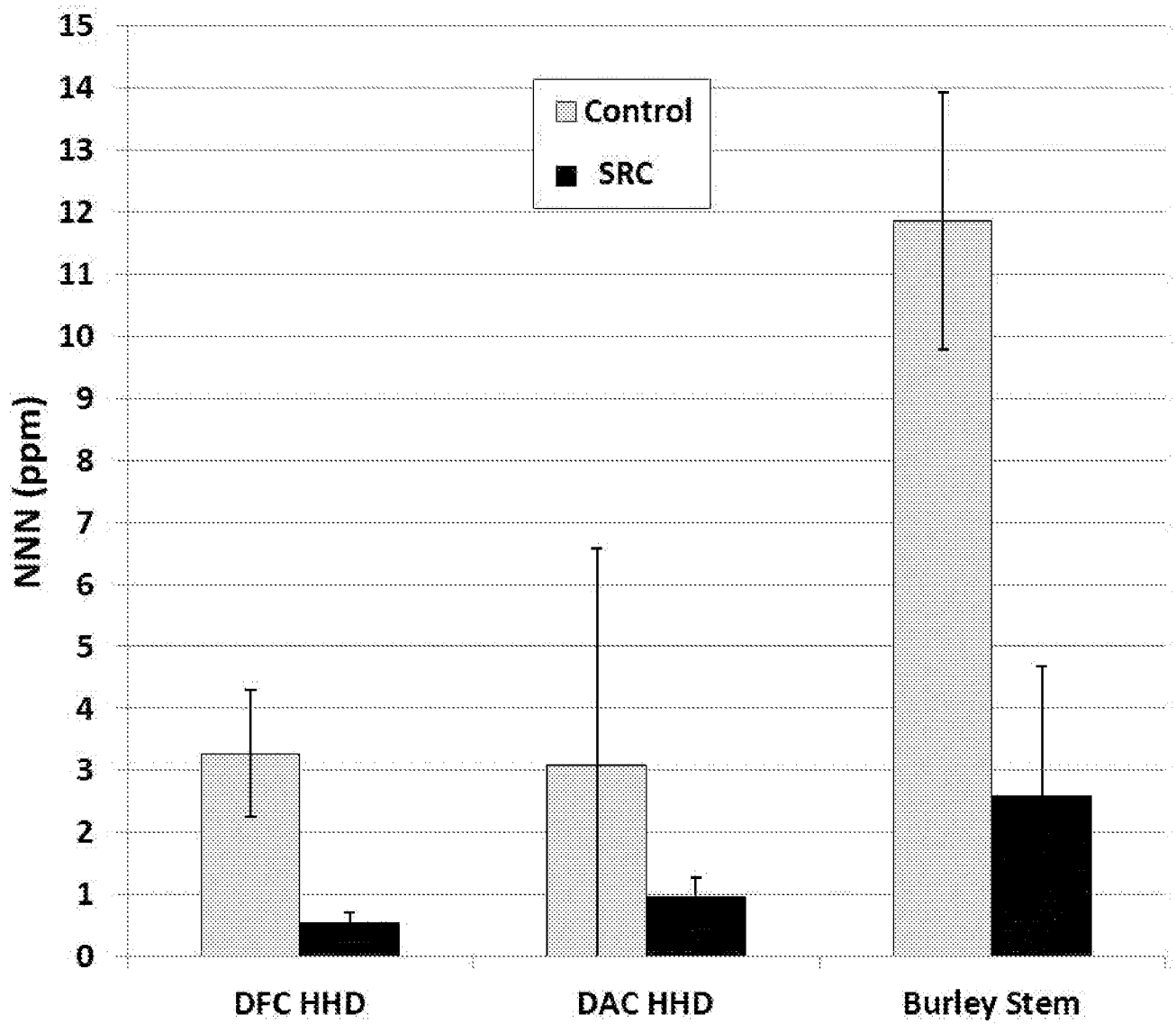
5/9

FIGURE 5

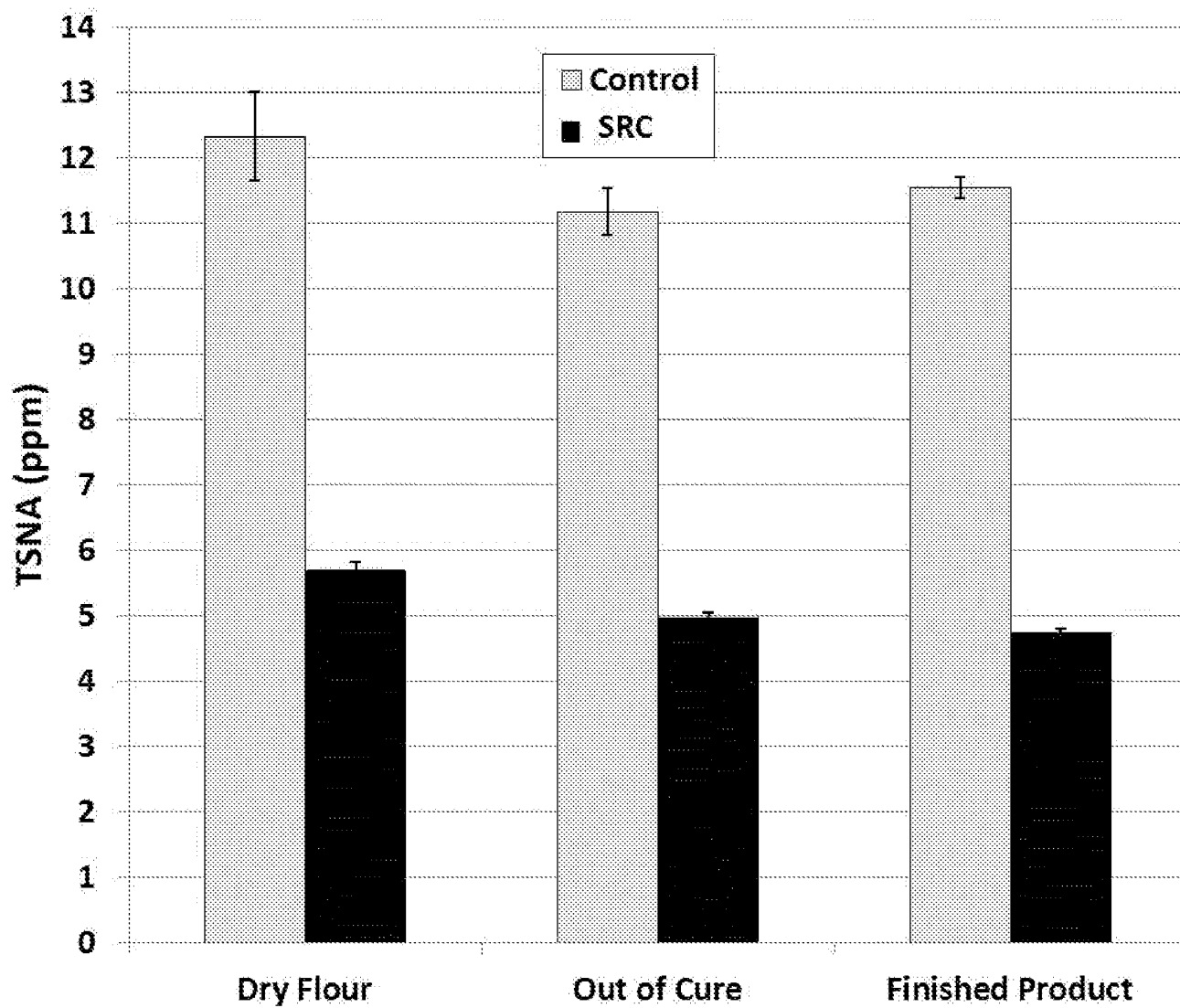
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FIGURE 6

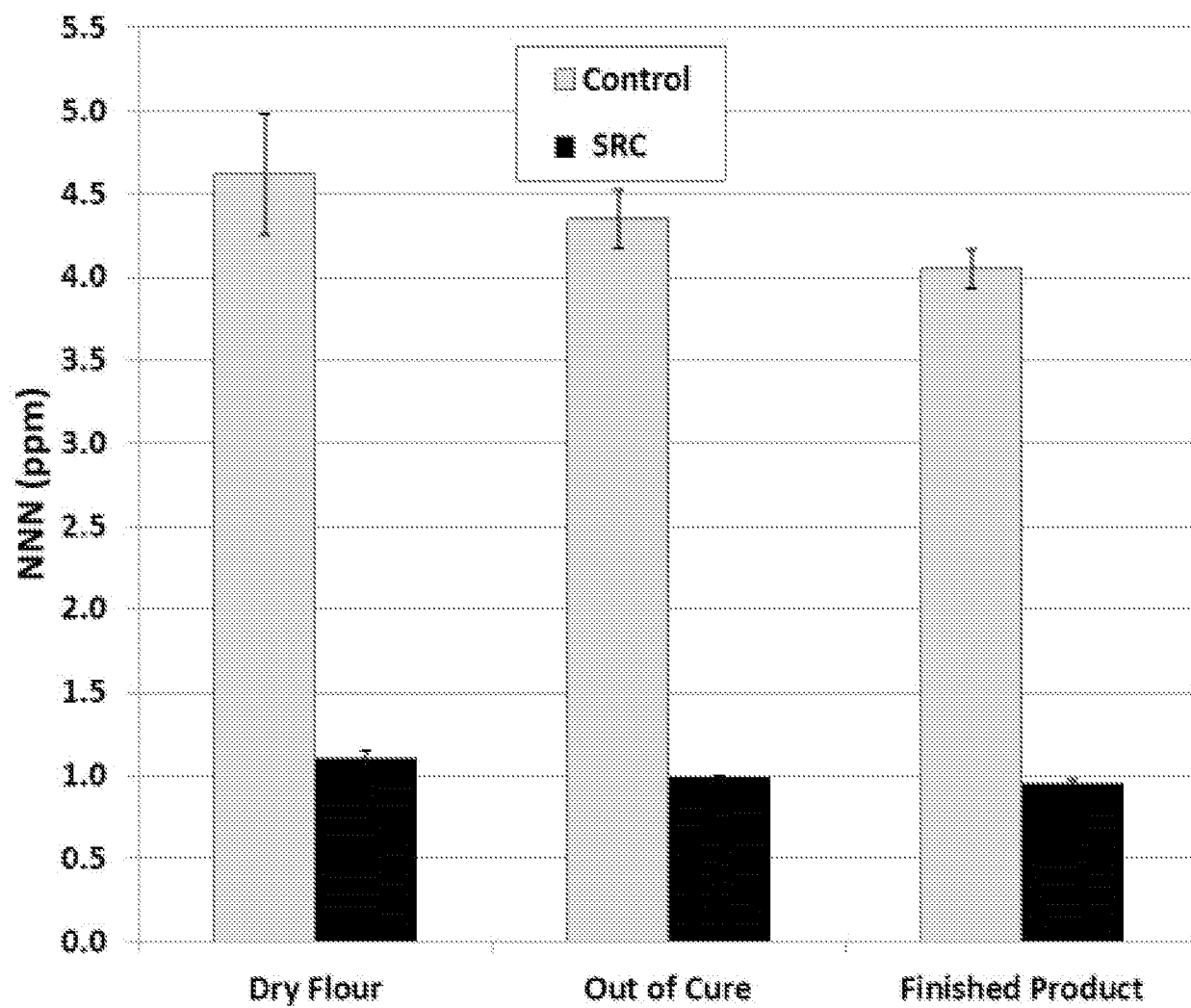
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FIGURE 7

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FIGURE 8

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FIGURE 9

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/060814

A. CLASSIFICATION OF SUBJECT MATTER
INV. A01H5/00 A24B13/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A01H A24B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2015/245584 A1 (LEWIS RAMSEY S [US] ET AL) 3 September 2015 (2015-09-03) abstract; example 3 claims 1-16	1-18

X	US 2014/251353 A1 (LEWIS RAMSEY S [US] ET AL) 11 September 2014 (2014-09-11) abstract claims 1-17 examples 1-10	1-18

	-/--	



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

15 March 2018

Date of mailing of the international search report

09/04/2018

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Keller, Yves

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/060814

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
- a. ☒ forming part of the international application as filed:
- ☒ in the form of an Annex C/ST.25 text file.
- ☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
- ☐ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
- ☐ on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/060814

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2017/060814

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EMILIE JULIO ET AL: "Reducing the content of nornicotine in tobacco via targeted mutation breeding", MOLECULAR BREEDING, KLUWER ACADEMIC PUBLISHERS, DO, vol. 21, no. 3, 17 October 2007 (2007-10-17), pages 369-381, XP019578642, ISSN: 1572-9788 the whole document -----	1-18
A	GAVILANO L B ET AL: "Genetic engineering of Nicotiana tabacum for reduced nornicotine content", JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, AMERICAN CHEMICAL SOCIETY, BOOKS AND JOURNALS DIVISION, US, vol. 54, no. 24, 11 July 2006 (2006-07-11), pages 9071-9078, XP002485237, ISSN: 0021-8561, DOI: 10.1021/JF0610458 [retrieved on 2008-07-11] the whole document -----	1-18
T	M LUSSO ET AL: "N-nitrosonornicotine reduction in dark tobacco varieties and smokeless product prototypes", RESEARCH, DEVELOPMENT & REG AFFAIRS, 601 EAST JACKSON STREET, 18 September 2017 (2017-09-18), XP55459187, the whole document -----	
T	A Bailey ET AL: "Evaluation of Dark Fire-Cured SRC (Stable Reduced Converter) Lines with ZYVERT™ Technology in Kentucky and Tennessee Field Trials", 16 January 2018 (2018-01-16), XP55459194, Retrieved from the Internet: URL:https://www.coresta.org/sites/default/files/abstracts/2018_TWC15_Bailey.pdf [retrieved on 2018-03-14] the whole document ----- -/--	

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2017/060814

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	Jeffrey R Ammann ET AL: "Variability of NNN in Tobacco and NNN Levels in Smokeless Tobacco Products", Journal of agricultural and food chemistry, 23 September 2017 (2017-09-23), page 4400, XP55459212, United States Retrieved from the Internet: URL:https://www.coresta.org/sites/default/files/abstracts/2017_IG01_Lusso.pdf [retrieved on 2018-03-14] the whole document -----	
T	W Hall ET AL: "Supping with the Devil? The role of law in promoting tobacco harm reduction using low nitrosamine smokeless tobacco products", Public Health, 1 January 2017 (2017-01-01), pages 287-291, XP55459185, Netherlands DOI: 10.1016/j.puhe.2008.12.018 Retrieved from the Internet: URL:https://www.coresta.org/sites/default/files/abstracts/2017_AP08_Lusso.pdf the whole document -----	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2017/060814

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2015245584 A1	03-09-2015	EP 3113602 A1	11-01-2017
		US 2015245584 A1	03-09-2015
		WO 2015134423 A1	11-09-2015

US 2014251353 A1	11-09-2014	EP 2964016 A1	13-01-2016
		US 2014251353 A1	11-09-2014
		US 2017164574 A1	15-06-2017
		WO 2014137802 A1	12-09-2014

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-18

Tobacco plant, seed, cured material, product, from hybrid
PD7305 SRC

1.1. claims: 1-18(partially)

Tobacco plant, seed, cured material, product, from hybrid
PD7309 SRC

1.2. claims: 1-18(partially)

Tobacco plant, seed, cured material, product, from hybrid
PD7312 SRC

1.3. claims: 1-18(partially)

Tobacco plant, seed, cured material, product, from hybrid
PD7318 SRC

1.4. claims: 1-18(partially)

Tobacco plant, seed, cured material, product, from hybrid
PD7319 SRC

1.5. claims: 1-18(partially)

Tobacco plant, seed, cured material, product, from cultivar
TND950 (phph) SRC

1.6. claims: 1-18(partially)

Tobacco plant, seed, cured material, product, from cultivar
Narrow Leaf Madole (phph) SRC

1.7. claims: 1-18(partially)

Tobacco plant, seed, cured material, product, from cultivar
Narrow leaf Madole SRC

1.8. claims: 1-18(partially)

Tobacco plant, seed, cured material, product, from cultivar
CMS TND950 SRC

1.9. claims: 1-18(partially)

Tobacco plant, seed, cured material, product, from cultivar
CMS Narrow Leaf Madole SRC

1.10. claims: 1-18(partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Tobacco plant, seed, cured material, product, from cultivar
CMS KY171 SRC
