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NICOTIANA-SPECIFIC MOLECULAR MARKER FOR DISCRIMINATING TOBACCO FROM NON-TOBACCO AND APPLICATION THEREOF

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ABSTRACT

The present invention belongs to the field of molecular biotechnology. More specifically, it relates to a Nicotiana-specific molecular marker for discriminating tobacco from non-tobacco and application thereof. The numbers of the Nicotiana-specific molecular marker comprise Ntsp027 and Ntsp151, and the nucleotide sequences of the PCR amplified products thereof are shown in SEQ ID No .1 and SEQ ID No .2, respectively. The application is to use the Nicotiana-specific molecular markers as described above to perform PCR amplification on the genomic DNA of the tobacco raw material involved to detect whether there is a specific nucleotide sequence of the PCR amplified product, and to accurately determine whether tobacco materials are present in the tobacco raw material involved. The Nicotiana-specific molecular marker of the present invention realizes the rapid and effective identification and detection of tobacco and non-tobacco, improves the scientificity, accuracy and authority of the identification and detection of the tobacco raw material involved, reduces judicial risks, and provides strong judicial evidence for the anti-counterfeiting and anti-smuggling in the tobacco industry monopoly.

NICOTIANA-SPECIFIC MOLECULAR MARKER FOR DISCRIMINATING TOBACCO FROM NON-TOBACCO AND APPLICATION THEREOF

FIELD

[0001] The invention belongs to the field of molecular biotechnology. More specifically, it relates to a Nicotiana-specific molecular marker for discriminating tobacco from non-tobacco and application thereof.

BACKGROUND

[0002] Tobacco is a herbaceous economic crop comprising a special alkaloid-nicotine in the Nicotiana, Solanaceae, and is also the basis of a tobacco raw material (tobacco leaf) in the tobacco industry. With the in-depth development of Chinese style cigarettes in China, the basic status of the tobacco raw material has become increasingly prominent. Although its development is protected by the monopoly law, driven by huge profits, a large number of anti-counterfeiting and anti-smuggling cases involving tobacco raw materials (i.e., whether tobacco materials are detected in the raw materials involved) are still emerging, which not only restricts the healthy and sustainable development of the tobacco industry, but also poses new challenges to the current identification and inspection of tobacco raw materials involved (Price Certification Center of the National Development and Reform Commission. Notice of the Price Certification Center of the National Development and Reform Commission on Issuing the "Operational Specifications for the Price Appraisal of Articles Involving Tobacco Cases").

[0003] At present, there are many research reports on the identification and detection of tobacco resources (variety) at the molecular level, such as the use of RFLP, RAPD, SSR, ISSR, DArT, SNP and other markers, or a combination thereof to carry out genetic diversity analysis and fingerprint map construction on tobacco resources (variety). In addition,, there are also a few reports on the rapid identification of cured tobacco leaf varieties using SSR, SCAR and RAPD markers in case the sample to be tested is known to be a tobacco materials (Jiuzhe Sun, Jinchu Yang, Dongying Su, Erbin Wang, Junting Wang, Shunfeng Zhang, Meng Li, Lin Ma, Rapid identification of cured tobacco leaf varieties based on SSR markers, Tobacco Science and Technology, 2019, 52(3): 26-32; and Jiuzhe Sun, Junting Wang, Dongying Su, Jinchu Yang, Erbin Wang, Shixi Wu, Meng Li, Lin Ma, Discrimination of tobacco cultivars using SCAR and RAPD markers, Czech Journal of

Genetics and Plant Breeding, 2020, 56(4): 170–173). However, there is no report on the identification and detection of tobacco raw materials involved at the molecular level. The domestic tobacco industry mainly adopts the sensory inspection method and chemical inspection method for the identification and detection of the tobacco raw materials involved. However, both methods have obvious defects. The sensory inspection method mainly relies on the inspectors' sensory feelings and inspection experience of the tobacco raw materials involved, and is extremely vulnerable to the subjective feelings and experience of the inspectors, so there is a high probability of misjudgment. The main principle of the chemical inspection method is to determine whether the sample is a tobacco monopoly product by determining whether the tobacco raw material to be tested comprises nicotine. However, nicotine is not unique to tobacco and can also be detected in many non-tobacco (especially Solanaceae) plants. Therefore, there is a high risk of misjudgment in the identification and detection of tobacco products involved based on the chemical inspection method for detecting the content of nicotine and N-nitrosamine in the test substance, and there have been reports of such cases of losing the lawsuit (Yong Chen, Bingjing Zhang, Dichen Chen. The smoking cessation product was identified as cigarettes, and the general manager was sentenced to 5 years in the first instance, and the case has been in court three times after the retrial [N], Yangtze Evening News, 2018-11-03), and a certain amount of public opinion on the Internet and in society has been triggered. The development of genome sequencing technology and bioinformatics as well as the acquisition of a large number of plant (especially Solanaceae) genome data has laid a solid theoretical and technical foundation for the identification and inspection of tobacco raw materials involved using a Nicotiana-specific molecular marker. Therefore, it is very necessary to research and develop a method that can quickly and easily discriminating tobacco from non-tobacco to improve the scientificity, accuracy and authority of the identification and detection of tobacco raw materials involved.

SUMMARY

[0004] To this end, the present invention provides a Nicotiana-specific molecular marker for discriminating tobacco from non-tobacco and application thereof, so as to solve the problem of significant defects and deficiencies in the use of sensory inspection methods and chemical inspection methods for the tobacco raw materials involved in the current anti-counterfeiting and anti-smuggling work in the tobacco industry.

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[0005] In order to achieve the above objective, the present invention provides the following technical solutions.

[0006] An object of the present invention is to provide a Nicotiana-specific molecular marker for discriminating tobacco from non-tobacco. The numbers of the Nicotiana-specific molecular marker comprise Ntsp027 and Ntsp151, and the nucleotide sequences of the PCR amplified products thereof are shown in SEQ ID No .1 and SEQ ID No .2, respectively.

[0007] Further, the primer sequence corresponding to the molecular marker Ntsp027 is:

[0008] Ntsp027F: 5'- GTTGTTCGCTTCCCTGATGT -3'; and

[0009] Ntsp027R: 5'-AACCAAAGCAAGCGAAATGT -3'.

[0010] Further, the primer sequence corresponding to the molecular marker Ntsp151 is:

[0011] Ntsp151F: 5'-ATTTGGCTTTGGCTATGGAA -3'; and

[0012] Ntsp151R: 5'-CGGAGACAAGAGACCCAAGT -3'.

[0013] Another object of the present invention is to provide an application of a Nicotiana-specific molecular marker for discriminating tobacco from non-tobacco, comprising the steps of:

[0014] S1. extracting the genomic DNA of a sample to be tested;

[0015] S2. performing a PCR amplification using the DNA extracted in step S1 as a template and Ntsp027 or/and Ntsp151 as primers; and

[0016] S3. analysing the PCR amplification result in step S2 by electrophoresis:

[0017] when performing the PCR amplification on the test material using Ntsp027 as a primer, if the PCR amplified product comprises a 303 bp PCR amplified nucleotide fragment, the sample to be tested is a tobacco sample;

[0018] when performing the PCR amplification on the test material using Ntsp151 as a primer, if the PCR amplified product comprises a 300 bp PCR amplified nucleotide fragment, the sample to be tested is a tobacco sample; and

[0019] if the PCR amplified product comprises either a 303 bp PCR amplified nucleotide fragment or a 300 bp PCR amplified nucleotide fragment, the sample to be tested is a non-tobacco sample.

[0020] Further, in step S1, the CTAB method or a plant tissue DNA extraction kit is used for extracting the DNA.

[0021] Further, in step S2, the annealing temperatures of the primers Ntsp027 and Ntsp151 in the PCR amplification are both 60°C.

[0022] Further, in step S3, the 303 bp PCR amplified nucleotide fragment is the nucleotide sequence shown in SEQ ID No .1 in the sequence listing; and the 300 bp PCR amplified nucleotide fragment is the nucleotide sequence shown in SEQ ID No .2 in the sequence listing.

[0023] The present invention has the following advantages.

[0024] The present invention provides a Nicotiana-specific molecular marker for discriminating tobacco from non-tobacco and application thereof, which can quickly identify and detect whether the tobacco raw materials involved are tobacco products at the molecular level. The molecular marker, primer, and method provided by the present invention realizes the rapid and effective identification and detection of tobacco and non-tobacco. Furthermore, the method is simple and easy to implement and has strong operability. It provides an objective, efficient and simple molecular-level inspection technology for the tobacco industry's anti-counterfeiting and anti-smuggling work, thereby improving the scientificity, accuracy and authority of the identification and detection of the tobacco raw material involved, reducing judicial risks, and providing strong judicial evidence for the anti-counterfeiting and anti-smuggling in the tobacco industry monopoly.

BRIEF DESCRIPTION OF DRAWINGS

[0025] In order to more clearly illustrate the embodiments of the present invention or the technical solutions in the prior art, the drawings required to be used in the description of the embodiments or the prior art will be briefly introduced below. Obviously, the drawings in the following description are only exemplary. Other implementation drawings can be derived from the provided drawings to those of ordinary skill in the art without creative work.

[0026] The content depicted in the specification is only used to cooperate with the content disclosed in the specification for the understanding and reading of those familiar with this technology, and is not used to limit the limited conditions under which the present invention can be implemented, and thus does not have technical substantive significance. Any structural

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modification, proportion change or size adjustment should still fall within the scope of the technical content disclosed in the present invention, without affecting the effects and objectives that can be achieved by the present invention.

[0027] Figure 1 shows the PCR amplification results of the primer Ntsp027 in 108 test materials in Example 3.

[0028] Figure 2 shows the PCR amplification results of the primer Ntsp151 in 108 test materials in Example 3.

DETAILED DESCRIPTION

[0029] Specific examples are given below to illustrate the embodiments of the present invention. Those familiar with this technology can easily understand the other advantages and effects of the present invention from the content disclosed in this specification. Obviously, the described examples are part, rather than all, of the examples of the present invention. Based on the examples of the present invention, all other examples obtained by those of ordinary skill in the art without creative work shall fall within the protection scope of the present invention. If specific technology or conditions are not indicated in the examples, it shall be carried out in accordance with the technology or conditions described in the literature in the art or in accordance with the instructions for the product. The reagents or instruments used, if the manufacturer is not indicated, are all conventional products that are commercially available.

[0030] The invention discloses two pairs of primers and a Nicotiana-specific molecular marker for identifying and detecting whether tobacco materials are present in the tobacco raw materials involved. By using the two pairs of primers disclosed in the present invention, PCR is performed using the genomic DNA of the tobacco raw material involved as a template, which can scientifically, efficiently, accurately and quickly identify and detect whether the raw material to be tested comprises a tobacco sample. It should be noted that, those skilled in the art can understand that the molecular marker of the present invention can be obtained by chemical synthesis in addition to the PCR amplification as described above.

[0031] Example 1

[0032] Example 1 provides a Nicotiana-specific molecular marker for discriminating tobacco from non-tobacco. The numbers of the Nicotiana-specific molecular marker comprise Ntsp027 and Ntsp151, and the nucleotide sequences of the PCR amplified products thereof are shown in SEQ

ID No .1 and SEQ ID No .2, respectively.

[0033] In this example, the primer sequence corresponding to the molecular marker Ntsp027 is:

[0034] Ntsp027F: 5'-GTTGTTCGCTTCCCTGATGT -3', as shown in SEQ ID No .3; and

[0035] Ntsp027R: 5'-AACCAAAGCAAGCGAAATGT -3', as shown in SEQ ID No .4.

[0036] In this example, the primer sequence corresponding to the molecular marker Ntsp151 is:

[0037] Ntsp151F: 5'-ATTTGGCTTTGGCTATGGAA -3', as shown in SEQ ID No .5; and

[0038] Ntsp151R: 5'-CGGAGACAAGAGACCCAAGT-3', as shown in SEQ ID No .6.

[0039] Example 2

[0040] Example 2 provides an application of a Nicotiana-specific molecular marker for discriminating tobacco from non-tobacco, comprising the steps of:

[0041] S1. extracting the genomic DNA of a sample to be tested using the CTAB method or a plant tissue DNA extraction kit;

[0042] S2. performing a PCR amplification using the DNA extracted in step S1 as a template and Ntsp027 or Ntsp151 as primers; wherein, the annealing temperatures of the primers Ntsp027 and Ntsp151 are both 60°C;

[0043] S3. analysing the PCR amplification result in step S2 by electrophoresis:

[0044] when performing the PCR amplification on the test material using Ntsp027 as a primer, if the PCR amplified product comprises a 303 bp PCR amplified nucleotide fragment, the sample to be tested is a tobacco sample;

[0045] when performing the PCR amplification on the test material using Ntsp151 as a primer, if the PCR amplified product comprises a 300 bp PCR amplified nucleotide fragment, the sample to be tested is a tobacco sample; and

[0046] if the PCR amplified product comprises either a 303 bp PCR amplified nucleotide fragment or a 300 bp PCR amplified nucleotide fragment, the sample to be tested is a non-tobacco sample.

[0047] Wherein, in step S3, the 303 bp PCR amplified nucleotide fragment is the nucleotide sequence shown in SEQ ID No .1 in the sequence listing; and the 300 bp PCR amplified nucleotide fragment is the nucleotide sequence shown in SEQ ID No .2 in the sequence listing.

[0048] Example 3

[0049] In this example, bioinformatics was used in conjunction with the relevant gene sequence data of the nicotine metabolic pathway, tobacco genome data (including unpublished data) and other Solanaceae genome data in public databases to develop the marker. Experiments were performed on 91 tobacco materials and 17 non-tobacco materials to obtain the Nicotiana-specific molecular marker that can identify and detect the presence of tobacco materials in the tobacco raw materials involved.

[0050] 1. Experimental materials

[0051] A total of 108 plant materials were tested, of which 91 were tobacco materials (see Table 1) and 17 were non-tobacco materials (see Table 2). Among 91 tobacco materials, 83 were wild tobacco materials (species) belonging to 11 groups of 3 tobacco subgenus, and 8 were cultivated tobacco materials of 4 different types. Among 17 non-tobacco materials, 1 was cruciferous material, 2 were Leguminosae materials, 2 were Camellia materials, 4 were Gramineae materials, and 8 were materials of 7 genera of Solanaceae. The 108 materials were selected with full consideration of factors such as whether the plant materials comprised nicotine, the main plant materials commonly used in the tobacco raw materials involved, and the experimental verification of the Nicotiana-specificity of the developed marker. In addition, in order to comply with the drying state of the tobacco raw materials involved, the DNA extraction and purification of the materials as described above were divided into two parts. That is, the fresh leaf tissue pieces were divided into two parts, one is the fresh leaf without any treatment, and the other was sterilized by high temperature in the oven to a drying state similar to that of the cured tobacco leaf. See Table 1 and Table 2 for specific material information.

[0052] Table 1 Detailed information of 91 Nicotiana materials

Sub-genus	Section	Species	PI code	Accessions
		N.benavedesii	PI 555471	M001
		N.cordifolia	PI 555493	M002
		N.glauca	PI 555504	M003
		N.glauca	PI 307908	M004
Rustica	*Paniculatae*	*N.knightiana*	PI 555527	M005
		N.paniculata	PI 555545	M006
		N.paniculata	PI 555550	M007
		N.raimondii	PI 555549	M008
		N.solanifolia	PI 555558	M009

	Rusticae	*N.rustica*	PI 243561	M010
Tabacum	*Tomentosae*	*N.glutinosa*	PI 555507	M011
		N.glutinosa	PI 555505	M012
		N.otophora	PI 555542	M013
		N.otophora	PI 235553	M014
		N.setchellii	PI 555557	M015
		N.tomemtosa	PI 574525	M016
		N.tomemtosiformis	PI 555572	M017
Petunioides	*Undulatae*	*N.undulata*	PI 555574	M018
		N.undulata	PI 555575	M019
		N.wigandioides	PI 302471	M020
	Alatae	*N.alata*	PI 42334	M021
		N.bonariensis	PI 555489	M022
		N.forgetiana	PI 555501	M023
		N.langsdorffii	PI 42337	M024
		N.langsdorffii	PI 555529	M025
		N.longiflora	PI 555531	M026
		N.longiflora	PI 555532	M027
		N.longiflora	PI 555533	M028
		N.plumbaginifolia	PI 555548	M029
		N.plumbaginifolia	PI 302476	M030
		N.plumbaginifolia	PI 302478	M031
		N.sylvestris	PI 555569	M032
		N.sylvestris	PI 555570	M033
		N.sylvestris	PI 555571	M034
	Nudicaulisae	*N.nudicaulis*	PI 555540	M035
	Repandae	*N.repanda*	PI 555552	M036
		N.repanda	PI 555551	M037
		N.stocktonii	PI 555538	M038
		N.stocktonii	PI 555539	M039
		N.stocktonii	PI 555560	M040
	Noctiflorae	*N.noctiflora*	PI 417918	M041
		N.noctiflora	PI 475832	M042
		N.petunioides	PI 555547	M043
	Acuminatae	*N.acuminata*	PI 555477	M044
		N.attenuata	PI 555476	M045
		N.corymbosa	PI 114824	M046
		N.pauciflora	PI 555546	M047
	Bigelovianae	*N.bigelovii*	PI 555485	M048
		N.clavelandii	PI 555491	M049
	Suaveolensae	*N.africana*	PI 555472	M050
		N.amplxicaulis	PI 271989	M051
		N.amplxicaulis	PI 555682	M052
		N.benthamiana	PI 555478	M053
		N.cavicola	PI 271990	M054
		N. debneyi	PI 503320	M055

N.excelsior	PI 224063	M056
N.excelsior	PI 555685	M057
N.goodspeedii	PI 241012	M058
N.gossei	PI 230953	M059
N.linearis	PI 555530	M060
N.miersii	PI 555537	M061
N.maritima	PI 555535	M062
N.megalosiphon	PI 555536	M063
N.megalosiphon	PI 555688	M064
N.occidentalis	PI 271991	M065
N.occidentalis	PI 555687	M066
N.occidentalis	PI 555541	M067
N.occidentalis	PI 555690	M068
N.rosulata	PI 244635	M069
N.rosulata	PI 244624	M070
N.rosulata	PI 244628	M071
N.rotundifolia	PI 555553	M072
N.rotundifolia	PI 555691	M073
N.suaveolens	PI 555500	M074
N.suaveolens	PI 555565	M075
N.suaveolens	PI 230960	M076
N.umbratica	PI 271993	M077
N.velutina	PI 244638	M078
N.velutina	PI 244630	M079
N.velutina	PI 244631	M080
N.acuminata	PI 555469	M081
N.acuminata	PI 42347	M082
N.benthamiana	PI 555684	M083

Names	Types	Accessions
K326	Flue-cured	M084
Honghua dajinyuan HD	Flue-cured	M085
TN90	Burley	M086
Burley 21	Burley	M087
Basma Xanthi	Oriental	M088
Sumsun NN	Oriental	M089
Beinhart1000-1	Cigar	M090
Florida301	Cigar	M091

Note: The numbers of 83 wild tobacco species were counted based on PI numbers, that is, there were wild tobacco species with the same name and different PI numbers.

[0053] Table 2 Information of 17 non-tobacco materials

Family	Genus	Name	Accessions
Leguminosae	*Arachis* L.	*Arachis hypogaea* L.	M092
	Vicia L.	*Vicia faba* L.	M093
Poaceae	*Zea* L.	*Zea mays* L.	M094

	Triticum L.	*Triticum aestivum* L.	M095
	Hordeum L.	*Hordeum vulgare* L.	M096
	Oryza L.	*Oryza sativa* L.	M097
	Capsicum L.	*Capsicum annuum* L.	M098
	Solanum L.	*Solanum melongena* L.	M099
	Solanum L.	*Solanum tuberosum* L.	M100
Solanaceae	*Lycopersicon* L.	*Solanum lycopersicum* L.	M101
	Petunia Juss.	*Petunia hybrida* Vil.	M102
	Datura L.	*Datura stramonium* L.	M103
	Lycium L.	*Lycium chinense* Mill.	M104
	Cestrum L.	Cestrum purpureum	M105
Brassicaceae	*Brassica* L.	*Brassica napus* L.	M106
Theaceae	*Camellia* L.	*Camellia sinensis* (L.) O.Kuntze	M107
Mirb.	*Camellia* L.	*Camellia sinensis* var. assamica	M108

[0054] A set of primer sequences (SEQ ID No .3-SEQ ID No .6) and molecular marker sequences (SEQ ID No .1 and SEQ ID No .2) disclosed in the present invention were obtained by the following technical solutions. Firstly, 209 pairs of nicotine-related primers were developed based on a total of 46 genetic information known in the public database related to the synthesis, transport, conversion and metabolism of tobacco nicotine; secondly, the primers obtained were compared with known Solanaceae plant genome data and unpublished tobacco genome data in the tobacco industry (de novo sequencing genome data such as: Honghua Dajinyuan, Yunyan 87, Beinhart1000-1, Yunsha No. 1 and Huanghuayan G366, etc.; and 369 tobacco core resources' 10-15X genome resequencing data) to screen and obtain candidate Nicotiana-specific primers that can be completely aligned and unique in all tobacco genome data, but cannot be aligned in the genomes of other Solanaceae plants except tobacco; and finally, 108 test materials (Nicotiana materials: 83 wild species derived from 11 groups of 3 tobacco subgenus, and 8 cultivated varieties of 4 types commonly used in the samples involved; nicotine-containing Solanaceae (except Nicotiana) materials: 8 representative materials of 7 genera including Capsicum, Solanum, Lycopersicum, Petunia, Datura, Lycium and Cestrum; and nicotine-free plant materials: a total of 9 materials of 8 genera and 4 families that are common (mainly-planted) crops in daily life and commonly used in the samples involved) were used to verify the candidate Nicotiana-specific primers, and 2 Nicotiana-specific markers were finally obtained.

[0055] 2. Tobacco genomic DNA extraction

[0056] The conventional CTAB method or plant tissue DNA extraction kit was used to extract the genomic DNA of the sample to be tested. As for the method, the existing literature or the

instructions in the kit can be referred to.

[0057] 3. PCR amplification and electrophoresis detection

[0058] The PCR amplification system was a conventional system, for which the related published literature can be referred to. In the PCR amplification program, the annealing temperatures of the above two pairs of primers are both 60°C. For the specific PCR amplification program information, the relevant literature can be referred to. In addition, electrophoresis detection also uses conventional methods, for which the related published literature can be referred to.

[0059] 4. Experimental verification and application of the Nicotiana-specific molecular marker

[0060] The above two pairs of primers were used to respectively amplify the genomic DNA of 108 test materials to be tested, detect the PCR amplified product sequence, and accurately identify whether there are tobacco samples in the 108 materials to be tested according to the presence or absence of the product sequence. That is, when performing the PCR amplification on the 108 test materials using Ntsp027 as a primer, if there is a PCR amplified nucleotide fragment with a size of 303 bp, the sample to be tested is a tobacco sample; in the same way, when performing the PCR amplification on the 108 test materials using Ntsp151 as a primer, if there is a PCR-amplified nucleotide fragment with a size of 300 bp, the sample to be tested is a tobacco sample; and if there is no PCR-amplified nucleotide fragment with a size of 303 bp or 300 bp, the sample to be tested is a non-tobacco sample. See Figure 1 and Figure 2 for details. Among them, Figure 1 shows the PCR amplification results of the primer Ntsp027 in 108 test materials. No. M001-M091 are Nicotiana materials, of which M001-M083 are wild tobacco materials belonging to 11 groups of 3 tobacco subgenus, and M084-M091 are cultivated tobacco materials of 4 different types; and No. M092-M108 are non-Nicotiana materials, of which M098-M105 are materials of 7 genera of Solanaceae. The rightmost lane represents a 500 bp DNA marker, and from bottom to top are 300 bp and 400 bp respectively. Figure 2 shows the PCR amplification results of the primer Ntsp151 in 108 test materials. No. M001-M091 are Nicotiana materials, of which M001-M083 are wild tobacco materials belonging to 11 groups of 3 tobacco subgenus, and M084-M091 are cultivated tobacco materials of 4 different types; and No. M092-M108 are non-Nicotiana materials, of which M098-M105 are materials of 7 genera of Solanaceae. The rightmost lane represents a 300 bp DNA marker.

[0061] The sequences of the molecular markers and primers of the present invention are as

follows:

SEQ ID No .1

GTTGTTCGCTTCCCTGATGTGTTGAAAAACCGGTTGGAATCTCTGCAATCGGCTTT
TGATCTCGCGGTTCATTCCCAGGGCTATGGGGCCCACTACCAAGGTGTTTATCCCG
TGAAATGCAATCAAGACAGGTTCGTGGTGGAAGATATCGTGAAATTCGGGTCGCC
ATTCCGGTTCGGGTTGGAAGCCGGGTCTAAACCCGAGCTCCTGTTAGCCATGAGC
TGTCTCTGCAAGGGCAGTGCTGAGGGCCTTCTCGTTTGCAATGGTTTCAAGGACG
CTGAGTACATTTCGCTTGCTTTGGTT

SEQ ID No .2

ATTTGGCTTTGGCTATGGAATTTATCACATCTATATTCCTTTTTCTTGGCACATTTTCT
GATGATGAAGTGGATGAAGAAGAGGATGGCTCGGGTGAGCGCGTACGACTACGCC
TCGCTGTTACTCTAGCCGGCTTAGGCATGTTTGAGCCAATTAAGTGGGGAAAATTG
AGCCGAGCTCTCGAGCCGCGGATTTTGAAAGCGGCTTGATCGTAAGCCAACGCTG
CATCCTCTGCGGTCTCGTAAGTTCCTAACCACAGCCTTGCACCTTCACCTTTCCTAC
TTGGGTCTCTTGTCTCCG

SEQ ID No .3

GTTGTTCGCTTCCCTGATGT

SEQ ID No .4

AACCAAAGCAAGCGAAATGT

SEQ ID No .5

ATTTGGCTTTGGCTATGGAA

SEQ ID No .6

CGGAGACAAGAGACCCAAGT

[0062] Although the present invention has been described in detail with general illustrations and specific embodiments above, it is obvious to those skilled in the art that some modifications or improvements can be made on the basis of the present invention. Therefore, these modifications or improvements made without departing from the spirit of the present invention all fall within the scope claimed in the present invention.

CLAIMS

1. A Nicotiana-specific molecular marker for discriminating tobacco from non-tobacco, wherein: the numbers of the Nicotiana-specific molecular marker comprise Ntsp027 and Ntsp151, and the nucleotide sequences of the PCR amplified products thereof are shown in SEQ ID No .1 and SEQ ID No .2, respectively.

2. The Nicotiana-specific molecular marker for discriminating tobacco from non-tobacco according to claim 1, wherein the primer sequence corresponding to the molecular marker Ntsp027 is:

Ntsp027F: 5'- GTTGTTCGCTTCCCTGATGT -3'; and

Ntsp027R: 5'-AACCAAAGCAAGCGAAATGT -3'.

3. The Nicotiana-specific molecular marker for discriminating tobacco from non-tobacco according to claim 1, wherein the primer sequence corresponding to the molecular marker Ntsp151 is:

Ntsp151F: 5'-ATTTGGCTTTGGCTATGGAA -3'; and

Ntsp151R: 5'-CGGAGACAAGAGACCCAAGT -3'.

4. An application of a Nicotiana-specific molecular marker for discriminating tobacco from non-tobacco, comprising the steps of:

S1. extracting the genomic DNA of a sample to be tested;

S2. performing a PCR amplification using the DNA extracted in step S1 as a template and Ntsp027 or/and Ntsp151 as primers; and

S3. analysing the PCR amplification result in step S2 by electrophoresis:

when performing the PCR amplification on the test material using Ntsp027 as a primer, if the PCR amplified product comprises a 303 bp PCR amplified nucleotide fragment, the sample to be tested is a tobacco sample;

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when performing the PCR amplification on the test material using Ntsp151 as a primer, if the PCR amplified product comprises a 300 bp PCR amplified nucleotide fragment, the sample to be tested is a tobacco sample; and

if the PCR amplified product comprises either a 303 bp PCR amplified nucleotide fragment or a 300 bp PCR amplified nucleotide fragment, the sample to be tested is a non-tobacco sample.

5. The application of a Nicotiana-specific molecular marker for discriminating tobacco from non-tobacco according to claim 4, wherein, in step S1, the CTAB method or a plant tissue DNA extraction kit is used for extracting the DNA.

6. The application of a Nicotiana-specific molecular marker for discriminating tobacco from non-tobacco according to claim 4, wherein, in step S2, the annealing temperatures of the primers Ntsp027 and Ntsp151 in the PCR amplification are both 60°C.

7. The application of a Nicotiana-specific molecular marker for discriminating tobacco from non-tobacco according to claim 4, wherein, in step S3, the 303 bp PCR amplified nucleotide fragment is the nucleotide sequence shown in SEQ ID No .1 in the sequence listing; and the 300 bp PCR amplified nucleotide fragment is the nucleotide sequence shown in SEQ ID No .2 in the sequence listing.

DRAWINGS

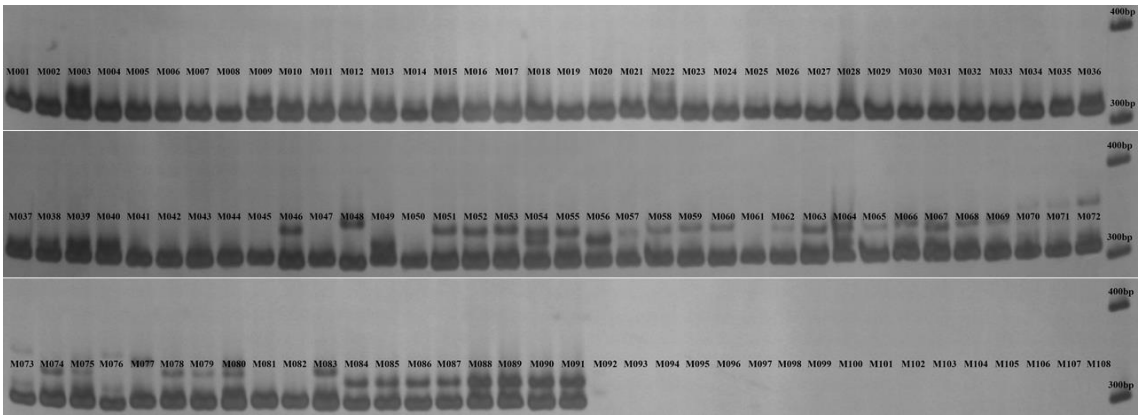

Figure 1

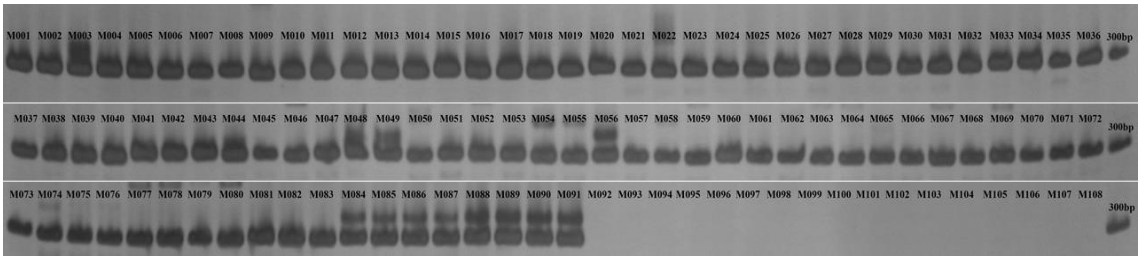

Figure 2

SEQUENCE LISTING

<110> Yunnan Tobacco Quality Supervision and testing station; Yunnan Academy of Tobacco Agricultural Sciences

<120> A Nicotiana-specific molecular marker for discriminating tobacco from non-tobacco and application thereof

<130> 2021

<160> 6

<170> PatentIn version 3.5

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