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Molecular Marker AhyBA1 Closely Linked with Peanut Branch Angle and Application of Molecular Marker AhyBA1

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(71) Applicant(s)
Shandong Academy of Agricultural Sciences

(72) Inventor(s)
Wang, Xingjun;Zhao, Chuanzhi;Zhou, Ximeng;Zhao, Shuzhen;Li, Pengcheng;Tian, Ruizheng;Hou, Lei;Li, Guanghui;Xia, Han;Pan, Jiaowen

(74) Agent / Attorney
Alder IP Pty Ltd, Suite 202 24 Thomas Street, CHATSWOOD, NSW, 2067, AU

ABSTRACT

The present invention discloses a molecular marker AhyBA1 closely linked with a peanut branch angle and application of the molecular marker AhyBA1, which belong to the field of agricultural biotechnologies. Specific primer pairs of the molecular marker AhyBA1 include primers with nucleotide sequences as shown in SEQ ID NO:1 and SEQ ID NO:2. The molecular marker disclosed by the present invention can quickly, effectively and economically identify the peanut branch angle. Directional and rapid improvement in peanut plant type can be realized by virtue of a method of combining molecular marked assisted selecting with back-cross breeding, which has an important application value in cultivating prostrate type high-yield peanuts.

Molecular Marker *AhyBA1* Closely Linked with Peanut Branch Angle and Application of Molecular Marker *AhyBA1*

TECHNICAL FIELD

The present invention relates to the field of agricultural biotechnologies, and in particular, to a molecular marker AhyBA1 closely linked with a peanut branch angle and application of the molecular marker AhyBA1.

BACKGROUND

Peanuts are an important oil and cash crop in China, with an annual planting area of about 70 million mu and an output of about 17 million tons. The main objective of peanut breeding and culture is increasing the yield of peanuts and realizing mechanical production. Peanuts are crops that flower above the ground and fruit underground, and therefore, the branch angle directly affects gynophore soiling, expanding and the yield of pods, and is closely linked with planting density, cultivation method and mechanical harvesting. Therefore, the branch angle is of great importance in increasing the yield of the peanuts and realizing mechanization.

According to included angles between branches and a main stem as well as a proportion of length of the branches to length of the main stem, peanuts were divided into erect, prostrate and half-prostrate types (Wan Shubo, 2003). The prostrate type peanuts were high in degree of mechanization, with an average yield per mu higher than that of the erect type peanuts (Zhang Xinyou, 2018, Annual Report of Oil Crop Science Society of China). Gynophore of the prostrate type peanuts may be closer to ground, and may be higher in a soiling and podding rate. In addition, the prostrate type peanuts have the advantages of smaller sowing quantity, and can be directly turned over to sun-cure in a field

after coming out of the ground; and due to support of branches, pods can be prevented from being in contact with ground, and breeding of mycete and the like can be effectively reduced, and therefore, the branches play an important role in controlling aflatoxin contamination of peanuts. The prostrate type peanuts are an important direction in peanut breeding.

Branch angles and the like of the peanuts determine that characters of the peanut plant types are easily affected by environmental factors, and therefore, a multi-year repetitive test is needed for selecting a novel peanut material of ideal plants in the field, and breeding efficiency is very low. In addition, unfavorable linkage of a plurality of characters occurs in controlling sites, growth periods and the like of peanut plant types; in an offspring selection process, it is time-wasting and labor-wasting to screen a novel material with an ideal plant type and excellent comprehensive characters. Compared with phenotypic selection in the field, molecular maker assisted selection mainly focuses on genotype identification, can improve offspring selection accuracy and shortens a breeding period. As a result, it is of great significance in making molecular basis for peanut plant type formation clear, identifying a molecular marker closely linked with a peanut plant type and cultivating a novel peanut variety with an ideal plant type.

As present, the research on the peanut plant type mainly focuses on cultivation management, and therefore, researches on genetic and molecular mechanisms of the peanut plant type are a few. The research of Coffelt (1997) indicated that the branch angles of the peanuts were controlled by two genes, and the prostrate type peanuts were dominant relative to the erect type peanuts. To achieve better statistics of characters, Fonceka et al., (2012) divided branch

characters of peanuts into six grades according to angles between the branches and the main stem, so that six QTL sites which were associated with peanut branch habits were further located to be in a03, a07, b04, b05, b06 and b10 linkage groups. Galya et al., (2017) located QTL sites for controlling peanut branch angles within a 1.1 Mb interval of B05 chromosome. Development in the researches provides important reference for precisely locating QTL genes of peanut branch habits. Li et al., (2019) also located major QTL site for controlling the peanut branch angle on a B05 chromosome by constructing a high-density genetic linkage map. Recently, Zhang Xiaojun et al., (2019) identified a gene LBA5 related to a peanut plant type, and applied a patent of the gene (with a patent application No.:CN201910994923.6) and a related CAPS molecular marker (with a patent application No.:CN201910988619.0). The above content is the basis for disclosing a molecular mechanism for peanut branch angle formation and a plant for improving peanuts through a biotechnology. Especially, the CAPS molecular marker disclosed in the patent (with the patent application No.CN201910988619.0) is an important choice for peanut plant type molecular improvement. CAPS marker is an enzyme digestion based amplified polymorphismp sequence marking technology; in a practical operation process which is relatively complex, PCR amplification is firstly performed, then, an amplified product is purified and subjected to enzyme digestion and electrophoresis in sequence; and moreover, the endonuclease is higher in price, so that molecular detection cost is increased. Therefore, the development of a marker for conveniently, quickly and accurately identifying a peanut branch angle has an important application value.

SUMMARY

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The objective of the present invention is to provide a molecular marker AhyBA1 closely linked with a peanut branch angle and application of the molecular marker AhyBA1, which aim to solve the problems in the prior art. The molecular marker can be used for peanut molecular breeding and quality improvement thereof, and is beneficial for quickly obtaining a novel seed resource.

To achieve the objective, the present invention provides a following scheme:

The present invention provides a molecular marker AhyBA1 closely linked with a peanut branch angle, where specific primer pairs of the molecular marker AhyBA1 include:

a primer with a nucleotide sequence as shown in SEQ ID No.1; and

a primer with a nucleotide sequence as shown in SEQ ID No.2.

SEQ ID No.1：5'- TAATACATAAAATAATGAGTAAATATAATAAAA-3';

SEQ ID No.2：5'- CCCTCATCCATTCTTACTGTCAT -3'.

The present invention further provides a method for identifying a peanut branch angle by a molecular marker AhyBA1 closely linked with the peanut branch angle, including the following steps:

(1) extracting DNA of peanut seeds or leaves;

(2) performing PCR amplification on the extracted DNA by adopting the specific primer pairs; and

(3) performing non-denaturing polyacrylamide gel electrophoresis detection on an amplification product obtained in step (2), and judging that the offspring of a to-be-detected peanut material is a prostrate type variety if characteristic bands with sizes of 174 bp appear.

Further, in the step (2), the total volume for PCR amplification is 25μL, including: 1μL of a DNA template with a concentration of 20-30ng/μL, 0.5 μL of specific primer pairs with concentration of 0.5 pmol/μL, 0.5 μL of 10mM dNTP mix, 2.5 μL of 10×Taq Buffer, 2.0 μL of 25mM $MgCl_2$, 0.25 μL of Taq enzyme with a concentration of 5U/μL, and the balance of water.

Further, in the step (2), PCR amplification reaction conditions are as follows: pre-denaturing for 4 min at 95 ℃,30s at 94 ℃, 30s at 58 ℃, 25s at 72 ℃, 35 cycles in total, where PCR amplification reaction lasts for 5 min at 72 ℃.

Further, in the step (3), 8% un-denaturing polyacrylamide gel electrophoresis is adopted.

The present invention further provides application of the molecular marker AhyBA1 closely linked with the peanut branch angle, where the molecular marker AhyBA1 is located within an 157.42-157.58Mb area of a chromosome 15, and is used for peanut breeding and/or peanut quality improvement.

Further, the molecular marker AhyBA1 is used for accurately screening a plant type of the peanuts.

Further, the molecular marker AhyBA1 is used for auxiliary screening and cultivating of a novel prostrate type peanut variety.

The present invention discloses the following technical effects:

The present invention provides a molecular marker AhyBA1 capable of identifying prostrate type peanuts, where plant types of the peanuts are controlled by active genes, and are easily affected by influences of an environment. As a result, there is great blindness in screening and culturing prostrate or erect peanuts with naked eyes, and observation only can be performed after peanuts emerge. The marker provided by the present invention

can be used for determining the plant type of the plant by cutting a few seed leaves from seeds and detecting DNA of the seed leaves, so that breeding efficiency is improved. Moreover, peanuts are allotetraploid, A and B subgenomic allelomorphic genes, and the markers are difficult to distinguish A05 and B05 chromosomes. The molecular markers of the present invention are obtained after many scientific experiments and explorations. The results obtained are reliable and believable.

The molecular marker provided by the present invention is a simple PCR marker with simple technical requirements. Compared with a CAPS molecular marker, the molecular marker disclosed by the present invention can realize identification by PCR amplification and electrophoresis without steps such as enzyme digestion, purification and recovery, is low in operation requirements on apparatuses which are conventional experimental apparatuses, and has characteristics of being quick, efficient and low in cost.

The molecular marker provided by the present invention is convenient, quick, accurate, low in cost, can effectively identify the branch angle of the peanuts, so that on one hand, fine location, separation and cloning of genes are facilitated, on the other hand, an important application value is achieved in molecular breeding and quality improvement of peanuts.

BRIEF DESCRIPTION OF THE FIGURES

In order to explain the technical solutions in the embodiments of the present invention or the prior art more clearly, the drawings used in the embodiments will be briefly introduced below. Obviously, the drawings in the following description are some embodiments of the present invention. For a person of ordinary skill in the art, other drawings can be obtained based on

these drawings without paying creative labor.

FIG. 1 shows fine location of major genes of a peanut branch angle;

FIG. 2 shows identification on a molecular marker *AhyBA1* in an offspring material; and

FIG. 3 shows a breeding scheme for quickly cultivating a novel prostrate type peanut variety in molecular marker assisted recurrent selection.

DESCRIPTION OF THE INVENTION

A detailed description to various embodiments in the present invention is made herein, which is not to be considered as limiting the present invention, but rather be understood as a a more detailed description of certain aspects, features, and embodiments of the present invention.

It should be understood that the terms used in the present invention are merely used to describe particular embodiments, and are not intended to limit the present invention. In addition, for the numerical range in the present invention, it should be understood that each intermediate value between the upper limit and the lower limit of the range is also specifically disclosed. Each smaller range between any stated value or intermediate value within the stated range and any other stated value or intermediate value within the stated range is also included in the present invention. The upper and lower limits of these smaller ranges can be independently included or excluded from the range.

Unless otherwise specified, all technical and scientific terms used herein have the same meaning as commonly understood by a person skilled in the art in the field of the present invention. Although the present invention only describes preferred methods and materials, any methods and materials similar or equivalent to those described herein can also be used in the practice or

testing of the present invention. All documents mentioned in this specification are incorporated by reference to disclose and describe methods and/or materials related to the documents. In case of conflict with any incorporated document, the contents of this manual shall prevail.

Without departing from the scope or spirit of the present invention, various improvements and changes can be made to the specific embodiments of the present specification, which is obvious to a person skilled in the art. Other embodiments derived from the description of the present invention will be obvious to a skilled person. The specification and examples of this application of the present invention are only exemplary.

As used herein, "comprising", "including", "having", "containing", etc., are all open terms, which means including but not limited to.

Embodiment 1: location of key genes of a peanut branch angle of peanuts and design of a molecular marker AhyBA1 closely linked with the peanut branch angle

To locate the key genes of the peanut branch angle of the peanuts, the inventor utilized a prostrate type peanut variety Tifrunner as a female parent to hybridize with the erect type peanut variety “fu peanut” (male parent). According to phenotypic analysis, the branches of the prostrate type peanut variety Tifrunner grew close to the ground, and included angles between the branches and the main stem ranged from 80 degrees to 90 degrees; and branches of the erect type peanut variety “fu peanut” were completely separated from ground, and included angles between the branches and the main stem ranged from 30 degrees to 40 degrees. The included angle between the F_1 generation and the main stem ranged from 50 degrees to 70 degrees. 70 hybrid F_1 seeds of

Tifrunner × fu peanut were harvested in total, and F_1 plant was detected by the molecular marker, so that 25 true hybrid F_1 generations were found. After self-breeding, F_2 generation obtained 332 F_2 single plants in total. According to statistical results of the branch angle of F_2 single plant, 30 erect type plants and 30 prostrate type plants were separately selected to construct an extreme pool for whole genome re-sequencing with the parent plants. QTL of the branch angle was located within a Chr15:150-160Mb interval through BSA-seq (bulked segregant analysis, BSA). To further reduce a location interval, 30 InDel molecular markers and 30 KASP molecular makers were designed within the interval, and the QTL of the branch angle was finally controlled to be located within the Chr15:150-160Mb interval, with a construction rate of 29.84% and a LOD value of 21.49 (FIG. 1). Within a candidate region, the molecular marker AhyBA1 was found to have variability in the erect type and prostrate type varieties by screening through sequence analysis and comparison, and was very stable (FIG. 2).

Embodiment 2: quickly culturing a high-yield prostrate type peanut variety by molecular marker *AhyBA1*

Erect type peanuts (recurrent parent) were improved into prostrate type peanuts at about a third year through a method of selecting and combining back-cross breeding, and 97% or more of genetic background of the recurrent parent was kept, so that orientated improvement of the plant type could be realized on the premise of keeping most of the original excellent character of the erect type peanut variety.

By taking the erect type peanut haihua 9 as the recurrent parent, and taking the prostrate type peanut Tifrunner as donor parent, specific steps for quick

improvement of peanut plant type were as follows:

(1) hybridization

The erect type peanut haihua 9 was taken as a female parent (recurrent parent), and prostrate type peanut variety Tifrunner was taken as a male parent for hybridization. The hybridzation method was as follows: castration was started several days after female parent haihua 9 bloomed, generally after 16:00 every day. The base of a bud was held by a thumb and a middle finger of a left hand, and calyx, vexillum and wings were pulled away slightly by pliers held in a right hand; the pulled-way petals were pressed by a forefinger and a thumb of a left hand, and bent portions of dragonbone petals were slightly pressed by the pliers to expose flower buds; anther of eight stamens were removed completely once or by many times through the pliers, so that stigmas of the anther were not damaged, and the dragonbone petals were pushed to the original positions with fingers. Castrated flowers were artificially pollinated at 5:00-9:00 in next morning. Flowers of male parent zhonghua 9 were firstly collected before being pollinated, and pollen of the male parent flowers was squeezed out with the pilers; during pollination, the castrated flowers were supported with the index finger and the middle finger of the left hand, and the dragonbone petals were slightly squeezed by the thumb of the right hand or the pilers to expose pistil stigma, and tip ends of the pilers were dipped with pollen for being coated on the stigma.

(2) identification of true or false hybrid F_1 generation

True or false of the harvested hybrid F_1 generation was identified by the molecular marker *AhyBA1*. The method was as follows:

Sampling: all pods of the female parent plant were harvested, dried in air,

and then the harvested seeds were numbered. Part of seed skin was removed by an operating knife, and part (about 30 mg) of cotyledon tissue was cut off and put into a 1.5 mL centrifugal tube, and magnetic beads were placed in. The rest of peanut seeds were put into a refrigeration house to store, and were planted into a large field after being detected. According to the experiment, a budding rate was not affected after part of the tissues of the peanuts were cut off.

DNA extraction: DNA of the to-be-detected peanut seeds was extracted specifically as follows:

(1) 1.5 mL centrifugal tube with the peanut tissue was quickly cooled with liquid nitrogen and then was ground;

(2) a CTAB extracting solution (2% CTAB, 1.4mol/L NaCl, 20mmo/L EDTA (pH8.0),100mmol/L Tris-HCl (pH8.0) and 2% pvp-40) was preheated in a 65 ℃ water bath;

(3) mass of a sample tissue was estimated, 700 μL of preheated CTAB extracting solution was added into every 200 mg of sample to quickly mix uniformly, and was uniformly mixed in a 65 ℃ warm bath for 2-5 times for 10-30 min;

(4) phenol, chloroform and isoamyl alcohol were uniformly mixed in a volume ratio of 12:12:1 to obtain a mixture;

(5) the mixture was centrifuged for 10 min at a rate of 12000 rpm at the room temperature;

(6) supernatant was transferred to a novel centrifugal tube;

(7) chloroform and isoamylalcohol ware used as steps (4) to (6) in a volume ratio of 24:1;

(8) 0.7 time the volume of isopropanol pre-cooled at (-) 20℃ was added, inverted and uniformly mixed, and placed for 10 min at the room temperature;

(9) the mixture was centrifuged for 15 min at a rate of rpm of 12000 at the room temperature;

(10) the supernatant was poured out, and precipitates were washed for 2-3 times with 500 μl of 70% alcohol pre-cooled at (-) 20℃;

(11) after being dried, DNA of the precipitates was dissolved by 50 μl of deionized water or TE, and was put at (-) 20℃ for later use; and

(12) 5 μl of dissolved DNA was sucked and 45 μl of deionized water was added to uniformly mix to prepare genome DNA of peanuts for later use.

PCR reaction and electrophoresis analysis: specific primer pairs (as shown in SEQ ID No.1 and SEQ ID No.2) of the molecular marker *AhyBA1* were utilized to perform molecular marker detection on parent plants and all F_1 hybrid seeds; and according to electrophories results, the F_1 hybrid seeds containing parent and female specific bands were true hybrid seeds.

A PCR amplification reaction system was as follows:

Total volume for PCR amplification was 20 μL, including:

1μL of a DNA template with a concentration of 20-30ng/μL,

0.5 μL of *AhyBA1* specific primer pairs with concentration of 0.5 pmol/μL,

0.5 μL of 10mM dNTP mix,

2.0 μL of 10×Taq Buffer,

2.0 μL of 25mM $MgCl_2$,

0.20 μL of Taq enzyme with a concentration of 5U/μL,

and Add water to 20μL.

PCR amplification reaction conditions were as follows: pre-denaturing for

4 min at 95 ℃, 30s at 94 ℃,30s at 58 ℃, 25s at 72 ℃, 35 cycles in total, where PCR amplification reaction lasted for 5 min at 72 ℃.

8% non-denaturizing polyacrylamide gel (Acr:Bis = 39:1) electrophoresis was adopted for PCR amplification product detection.

A method for preparing 8% non-denaturizing polyacrylamide gel was as follows:

3 μL of an indicator, namely a sampling buffer solution (containing 50mM of Tris-HCl with a pH value of 8.0, 50 mM of EDTA, 0.25% of bromophenol blue, 0.25% of xylene blue and 50% of glycerol) was added into 10 μL of an amplification product;

an electrophoresis buffer system was 1×TBE (90mM Tris-borate pH 8.3, 2mM EDTA), and 120V electrophoresis was performed for about 4h.

30 ml of 8% non-denaturizing polyacrylamide gel was prepared as shown in table 1:

Table 1 Preparation of 30ml of 8%non-denaturizing polyacrylamide gel

40% acrylamide （Acr : Bis = 39 : 1）	6 ml
5×TBE	6 ml
H_2O	18 ml
20% ammonium persulfate	240 μl
TEMED	24 μl
Total volume	30 ml

A silver staining detection method was as follows:

a, 500 ml of 0.1% silver nitrate solution was dyed for 15-20 min;

b, deionized water was quickly rinsed for 15 sec;

c, a developing solution (1000 ml of deionized water+20g NaOH+0.5g Na_2CO_3, 1.5 ml of presently-used formaldehyde) was used for developing and was shaken continuously until DNA bands were clear;

d, rinsing was performed with tap water; and

e, scanning and photographing were performed.

(3) back-crossing and offspring screening

A breeding solution of molecular marker assisted recurrent selection was adopted twice every year, the whole period being about three years (FIG. 3). Firstly, haihua 9 was taken as a female patent (recurrent parent), true hybrid F_1 was taken as a male patent for hybridization as the method above, and harvested BC_1F_1 was detected by the molecular marker *AhyBA1*, the offspring with male parent specific bands was kept, and DNA extraction and molecular marker detection method were the same as the method above. Back-crossing and screening were continuously performed for 4 times to obtain BC_4F_1 generation, and self-crossing was performed to select pure generation for seed selection and variety register.

The plant type of the peanuts was quantitative character which was easily affected by the environment. As a result, there is great blindness in screening and culturing prostrate peanuts with naked eyes.peanuts emerge. The breeding efficiency can be improved by a method of combining marker provided by the present invention with cross-breeding recurrent selection, and germplasm innovation of the prostrate type peanuts can be realized within about 3 years (FIG. 3). Compared with the convention method, the efficiency is higher.

The embodiments described above are only intended to describe the preferred embodiments of the present invention, and are not intended to limit the scope of the present invention, and various modifications and improvements made to the technical solutions of the present invention by a person skilled in the art without departing from the spirit of the present invention

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are intended to fall within the scope as defined by the claims of the present invention.

CLAIMS

1. A molecular marker AhyBA1 closely linked with a peanut branch angle, wherein specific primer pairs of the molecular marker AhyBA1comprise:

a primer with a nucleotide sequence as shown in SEQ ID No.1; and

a primer with a nucleotide sequence as shown in SEQ ID No.2.

2. A method for identifying a peanut branch angle by the molecular marker AhyBA1 closely linked with the peanut branch angle according to claim 1, comprising the following steps:

(1) extracting DNA of peanut seeds or leaves;

(2) performing PCR amplification on the extracted DNA by adopting the specific primer pairs; and

(3) performing non-denaturing polyacrylamide gel electrophoresis detection on an amplification product obtained in step (2), and judging that the offspring of a to-be-detected peanut material is a prostrate type variety if characteristic bands with sizes of 174 bp appear.

3. The method according to claim 2, wherein in the step (2), the total volume for PCR amplification is 25μL, comprising: 1μL of a DNA template with a concentration of 20-30ng/μL, 0.5 μL of specific primer pairs with concentration of 0.5 pmol/μL, 0.5 μL of 10mM dNTP mix, 2.5 μL of 10×Taq Buffer, 2.0 μL of 25mM MgCl2, 0.25 μL of Taq enzyme with a concentration of 5U/μL, and add water to 25μL.

4. The method according to claim 2, wherein in the step (2), PCR amplification reaction conditions are as follows: pre-denaturing for 4 min at 95 ℃, 30s at 94 ℃, 30s at 58 ℃, 25s at 72 ℃, 35 cycles in total, and PCR amplification reaction lasts for 5 min at 72 ℃.

5. The method according to claim 2, wherein in the step (3), 8% undenaturing polyacrylamide gel electrophoresis is adopted.

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FIGURE OF THE SPECIFICATION

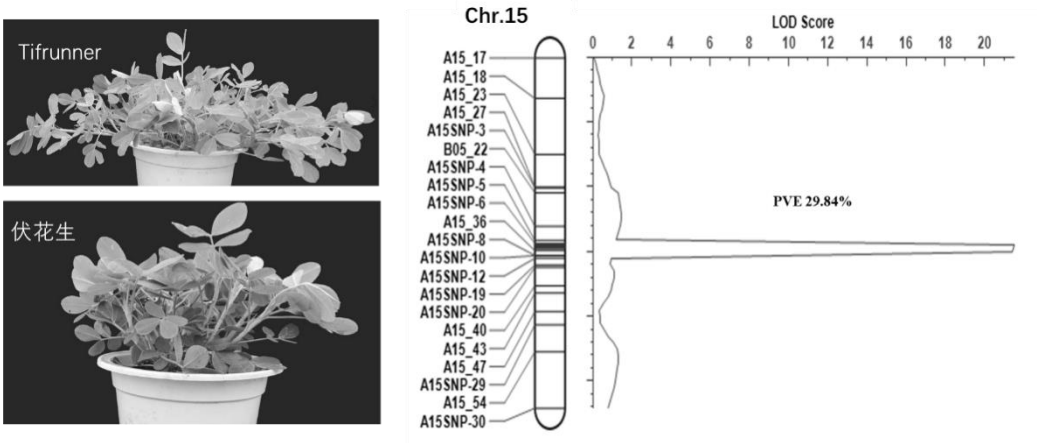

FIG. 1

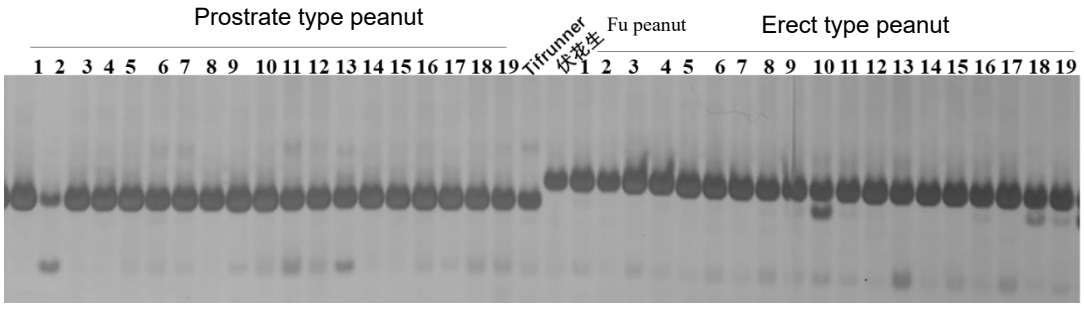

FIG. 2

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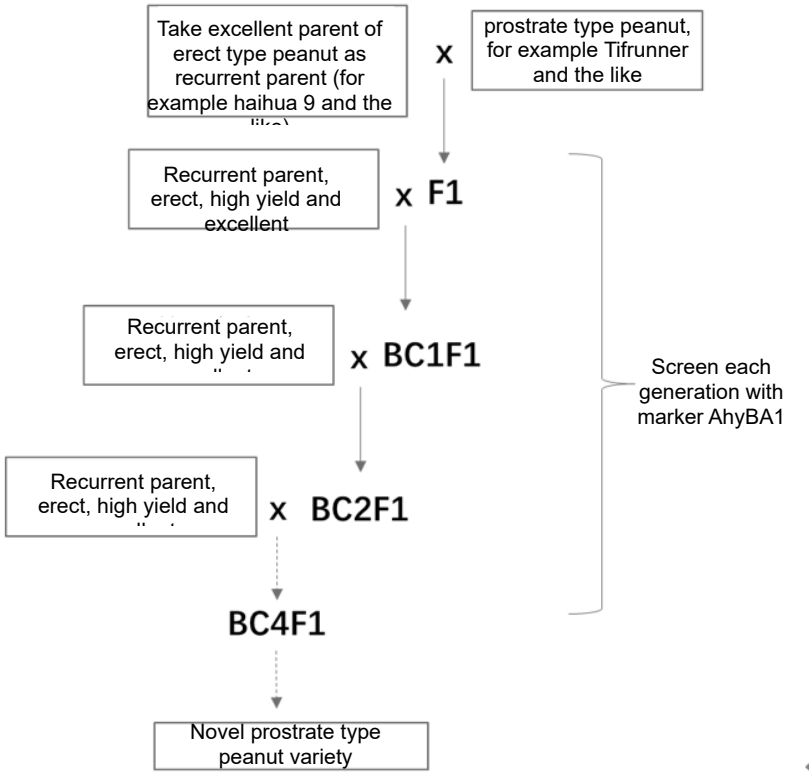

FIG. 3

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<110> Shandong Academy of Agricultural Sciences

<120> Molecular Marker AhyBA1 Closely Linked with Peanut Branch Angle and Application of Molecular Marker AhyBA1

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