(12) STANDARD PATENT APPLICATION (11) Application No. **AU 2022438471 A1**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
RECESSIVE PHOTOPERIOD-SENSITIVE GENIC MALE STERILE GENE Ghpsm5 AND USE THEREOF IN COTTON

(51) International Patent Classification(s)
C12N 15/29 (2006.01) ***A01H 6/60*** (2018.01)
A01H 5/00 (2018.01) ***C07K 14/415*** (2006.01)
A01H 5/02 (2018.01) ***C12N 15/82*** (2006.01)

(21) Application No: **2022438471** (22) Date of Filing: **2022.12.07**

(30) Priority Data

(31) Number	(32) Date	(33) Country
202211463130.X	**2022.11.22**	**CN**

(43) Publication Date: **2024.06.06**
(43) Publication Journal Date: **2024.06.06**

(71) Applicant(s)
INSTITUTE OF COTTON RESEARCH OF CAAS

(72) Inventor(s)
FAN, Shuli;ZHANG, Chaojun;MA, Qifeng;QIAO, Kaikai

(74) Agent / Attorney
Davies Collison Cave Pty Ltd, Level 15 1 Nicholson Street, MELBOURNE, VIC, 3000, AU

ABSTRACT OF THE DISCLOSURE

The present disclosure discloses a recessive photoperiod-sensitive genic male sterilie (RPGMS) gene *Ghpsm5* and use thereof in cotton, and relates to the technical field of genetic engineering and genetic breeding of agricultural crop. The present disclosure provides a cotton RPGMS gene *Ghpsm5*. Changing the sequence of *Ghpsm5* gene in normal cotton plants may make *Ghpsm5* unable to be expressed normally, which leads to male sterility of cotton under long sunshine conditions with a sunshine duration greater than or equal to 12.5 hours, restored male fertility under short sunshine conditions with a sunshine duration less than or equal to 12.0 hours, and failures of self-crossing and boll-setting under a condition with a sunshine duration greater than 12.0 hours but less than 12.5 hours where the cotton may still be used for hybrid seed production. Changing *Ghpsm5* has no effect on female fertility. The gene may be used for cotton breeding, and cotton RPGMS lines may be prepared by inhibiting the expression of this gene through biotechnologies.

ABSTRACT DRAWING

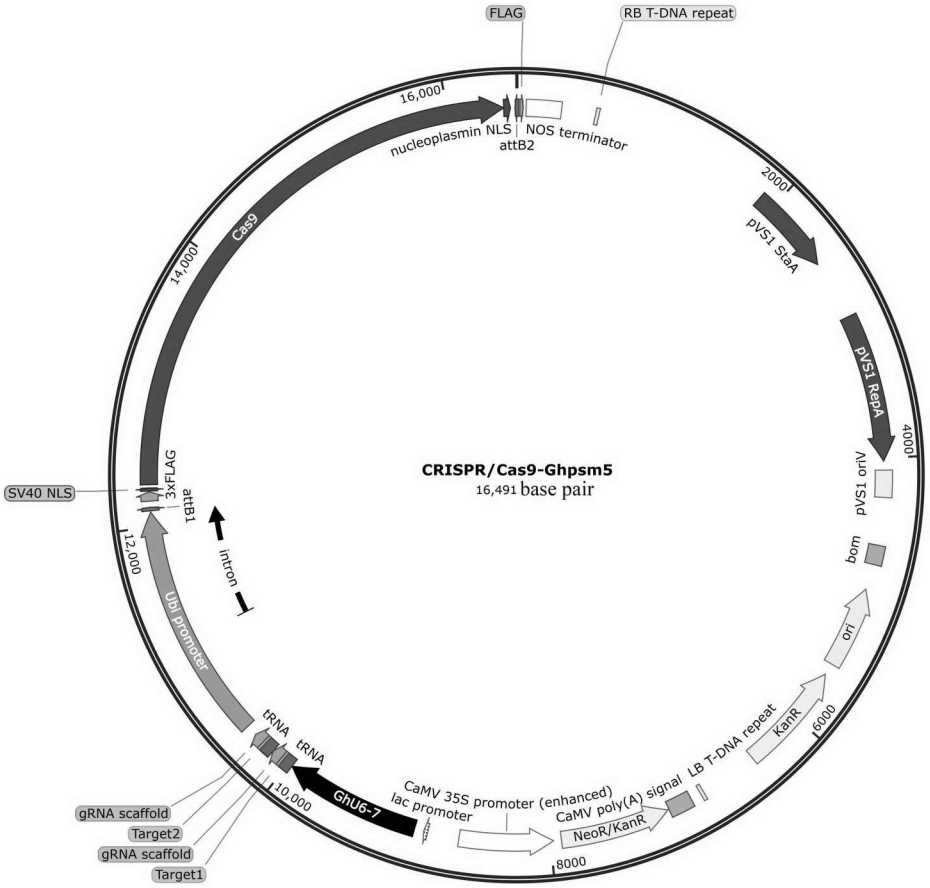

FIG.1

RECESSIVE PHOTOPERIOD-SENSITIVE GENIC MALE STERILE GENE Ghpsm5 AND USE THEREOF IN COTTON

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the priority of Chinese Patent Application No. 202211463130.X, entitled "Recessive photoperiod-sensitive genic male sterile gene *Ghpsm5* and use thereof in cotton" filed with the China National Intellectual Property Administration on November 22, 2022, which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

[0002] The present disclosure belongs to the technical field of genetic engineering and agricultural crop genetic breeding technology, in particular to a recessive photoperiod-sensitive genic male sterile (RPGMS) gene *Ghpsm5* and use thereof in cotton.

BACKGROUND ART

[0003] Cotton is one of the major crops in the world, which is currently cultivated in more than 70 countries. China, India and the United States are the major cotton producers. The main task of cotton scientific research is to improve the yield and quality of cotton. Heterosis is one of the most successful biological phenomena applied in agriculture, and cotton is also a cash crop with obvious heterosis. The hybrid cotton has the advantages as to nutritional growth, yield and resistance to stresses such as high temperature, humidity, drought, leanness and disease, so the application of hybrids has become an important means for cotton breeding. The United States began the research on the utilization of cotton heterosis in the 1940s. It was not until 1970s that the cytoplasmic male sterile line with Hacknessy cotton was developed, and the three-line method was realized. The breeding of hybrid cotton in China has been developing rapidly. The three-line method using cytoplasmic male sterile lines, the two-line method using recessive nuclear male sterile lines and the hand-emasculation assisted pollination method have been applied. The utilization of hybrid cotton in China reached its peak in 2007. Hybrid cotton accounted for more than 90% of the cotton area in the Yangtze River Basin. However, the hybrid seeds are mainly produced by hand-emasculation assisted pollination. After 2007, the cost of hybrid cotton seeds rose sharply with the increase of labor costs, resulting in a rapid reduction of hybrid cotton planting area. The successive success of the "three-line method" and "two-line method" hybrid rice has produced huge social and economic benefits, while the selection of restorer lines of cotton "cytoplasmic male sterile line" is difficult, and there is no excellent combination of advantages. 50% of fertile plants have to be pulled out during seed production of cotton genic male sterile line, and the yield of seed production is limited, so the production of cotton hybrid is still dominated by hand-emasculation. The preparation of a "dual-use line" material that can be used as a sterile line and propagate itself is the key technology for the application of cotton hybrids, and is also a worldwide challenge.

[0004] There are many kinds of genic male sterile lines found in cotton. Up to now, 17

different types of genic male sterile lines have been found, including 9 recessive genic male sterile lines, namely *ms1*, *ms2*, *ms3*, *ms5ms6*, *ms8ms9*, *ms13*, *ms14*, *ms15* and *ms16*, and 8 dominant genic male sterile lines, namely Ms4, Ms7, Ms10, Ms11, Ms12, Ms17, Ms18 and Ms19. Among the 17 male sterile lines, 12 were found in upland cotton, and 5 were found in sea island cotton. The lines *ms2* and Ms4 are completely sterile, *ms1* and *ms3* are only partially sterile, and *ms8ms9* shows no anther dehiscence. Molecular markers located on chromosome are found in *ms5*, *ms8* and *ms9*. The research on genic male sterile gene is in the stage of searching for markers, and there are no patent and report on the above related genes.

[0005] The institute of cotton research of Chinese Academy Agricultural Sciences developed a veriscent cotton photoperiod-sensitive genic male sterile (PGMS) mutant Zhong9106 using the space mutation breeding technology, which is male sterile under long sunshine conditions with an illumination period of more than 13.5 hours, and fertile under short sunshine conditions with an illumination period of less than 13.5 hours and a daily average temperature of more than or equal to 21.5°C. Zhang Chaojun obtained a recessive photoperiod-sensitive genic male sterile mutant *psm4* (ZL201810132189.8) by culturing a variety of tissues from cotton material W10 and identified molecular markers related to the RPGMS trait (ZL202010869171.3). The gene *Ghpsm5*, which controls the RPGMS of cotton, was identified by verifying the gene function of the molecular marker associated intervals. The RPGMS material *ps201* was obtained by editing the *Ghpsm5* gene.

SUMMARY

[0006] An objective of the present disclosure is to provide a RPGMS gene *Ghpsm5* and use thereof in cotton. Based on the gene and/or the promoter thereof, RPGMS materials of cotton or other plants are prepared by making use of plant fertilitychanges incuded by photoperiod.

[0007] The present disclosure provides a cotton RPGMS gene *Ghpsm5*. The amino acid sequence of the protein encoded by the RPGMS gene *Ghpsm5* includes the sequence set forth in SEQ ID NO:2, or the amino acid sequence that has an identity of more than 75% with the amino acid sequence set forth in SEQ ID NO:2.

[0008] The present disclosure also provides a cotton RPGMS gene *Ghpsm5*. The nucleotide sequence of the RPGMS gene *Ghpsm5* includes the nucleotide sequence set forth in SEQ ID NO:1, or the nucleotide sequence that encodes the amino acid sequence of a derivate protein with the function of regulating anther dehiscence obtained by replacing and/or deleting and/or adding one or more amino acid residues of SEQ ID NO:2.

[0009] The present disclosure also provides a promoter regulating the expression of the cotton RPGMS gene *Ghpsm5*. The nucleotide sequence of the promoter is set forth in SEQ ID NO:3.

[0010] The present disclosure also provides a use of the cotton RPGMS gene *Ghpsm5* or the promoter in preparing a PGMS plant material.

[0011] In one embodiment, under a condition that the sunshine duration is greater than or equal to 12.5 hours, the RPGMS plant material has abnormal pollen development and

no anther dehiscence, resulting in male sterility. Under a condition that the sunshine duration is less than or equal to 12.0 hours, the pollen development and the anther dehiscence are normal, and the male fertility is restored. Under a condition that the sunshine duration is greater than 12.0 hours but less than 12.5 hours, some anthers close to the base of flowers occasionally crack and disperse pollens with normal vitality but small numbers (generally less than 5 anthers), which make it difficult for the cotton to self-cross and set bolls, but may still be used for hybrid seed production. The RPGMS cotton material that blooms in long sunshine may be transformed into male fertile after 15-18 days of short sunshine treatment. The specific transformation time is affected by the development speed of buds. The transformation time of *Ghpsm5* gene editing material *ps201* is 17 days when it is treated in August in Anyang city; The RPGMS cotton material that blooms in short sunshine and is normally fertile, may be transformed into male sterile after 15-18 days of long sunshine treatment. The specific transformation time is affected by the development speed of buds. The transformation time of *ps201* is 17 days when it is treated in August in Anyang city.

[0012] The present disclosure also provides a method for preparing a RPGMS plant material, including regulating and/or changing an activity and/or expression of the cotton RPGMS gene *Ghpsm5*.

[0013] In one embodiment, a method for the regulating and/or changing includes one or more of gene editing, RNAi, antisense RNA and DNA methylation.

[0014] In one embodiment, a method for the regulating and/or changing includes constructing an expression vector by using the promoter through genetic engineering to express the protein, RNA and/or DNA sequence that can affect the development of male organs.

[0015] In one embodiment, the male organ includes anthers.

[0016] The present disclosure also provides a use of the RPGMS material in plant breeding.

[0017] Beneficial effects: The present disclosure provides a cotton RPGMS gene *Ghpsm5*. The sequence of *Ghpsm5* gene in normal cotton plants is changed, so that *Ghpsm5* gene cannot be expressed normally. Under a condition that the sunshine duration is greater than or equal to 12.5 hours, the pollen of the cotton is inactive and has no anther dehiscence, resulting in male sterility. Under a condition that the sunshine duration is less than or equal to 12.0 hours, the pollen development and the anther dehiscence are normal, and the male fertility is restored. Under a condition that the sunshine duration is greater than 12.0 hours but less than 12.5 hours, some anthers close to the base of flowers may crack and disperse pollens with normal vitality but small numbers (generally less than 5 anthers), which make it difficult for the cotton to self-cross and set bolls, but may still be used for hybrid seed production.

[0018] Changing the *Ghpsm5* gene has no effect on female fertility, and *Ghpsm5* has no relationship with the low-temperature yellowing of *psm1*. Therefore, the gene may be used for cotton breeding, and cotton RPGMS lines may be prepared by inhibiting the expression of this gene through biotechnologies.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] In order to explain the embodiments of the present disclosure or the technical solutions in the prior art more clearly, the following will briefly introduce the drawings needed in the embodiments. It is obvious that the drawings in the following description are only some embodiments of the present disclosure. For those skilled in the art, other drawings can also be obtained from these drawings without an inventive step.

[0025] FIG.1 is a schematic diagram of plant CRISPR/Cas9-*Ghpsm5* vector;

[0026] FIG.2 show a process of cotton genetic transformation CRISPR/Cas9-*Ghpsm5* (A) and photos of each growth and development stage (B), in which a is hypocotyl segment, b and c are early callus, d is late callus, e is embryogenic callus, f: globular embryo, g is heart-shaped embryo, h is torpedo embryo, i is cotyledon embryo, and j is regenerated cotton seedling; the scale of a, b, c, d and e is 5mm, the scale of f, g, h, i and j is 1mm;

[0027] FIG.3 show the expression and editing site analysis results of the cotton RPGMS line *ps201* obtained by gene editing; a, b, c and d are male fertile plants, anthers and stigma on the day of flowering, normal pollen dispersal anther and activity detection of pollen grains (active) of *ps201* under the Southern winter breeding conditions at the south breeding base of the institute of cotton research of Chinese Academy Agricultural Sciences in Yacheng, Hainan Province, respectively; e, f, g and h are plants (leaves removed manually), anthers and stigma on the day of flowering, uncracked anther, activity detection of pollen grains (inacive) extracted manually from *ps201* under normal male sterile conditions in the experimental field of the institute of cotton research of Chinese Academy Agricultural Sciences in Anyang, Henan Province, respectively; i and j are the editing site.

DETAILED DESCRIPTION OF THE EMBODIMENTS

[0019] The present disclosure provides a cotton RPGMS gene *Ghpsm5*. The amino acid sequence of the protein encoded by the RPGMS gene *Ghpsm5* includes the sequence set forth in SEQ ID NO:2, or the amino acid sequence that has an identity of more than 75% with the amino acid sequence set in SEQ ID NO:2.

[0020] In the present disclosure, the RPGMS material *psm5* is mainly used. Closely linked molecular markers of this trait are obtained through genome sequencing, association analysis and molecular marker research on the hybrid offspring of *psm5* and W10. The cotton RPGMS gene is obtained through the functional study of candidate genes in the marker region, and named as *Ghpsm5*. Changing the sequence of *Ghpsm5* gene in normal cotton plants may make *Ghpsm5* unable to be expressed normally. Under a condition that the sunshine duration is greater than or equal to 12.5 hours, the pollen of the cotton is inactive and has no anther dehiscence, resulting in male sterility. Under a condition that the sunshine duration is less than or equal to 12.0 hours, the pollen development and the anther dehiscence are normal, and the male fertility is restored. Under a condition that the sunshine duration is greater than 12.0 hours but less than 12.5 hours, some anthers close to the base of the flower may crack and disperse pollens with normal vitality but small numbers (generally less than 5 anthers), which make it difficult for the cotton to self-cross and set boll, but may still be used for hybrid seed production. Changing the *Ghpsm5* gene has no effect on female fertility, and

Ghpsm5 has no relationship with the low-temperature yellowing of *psm1*. The W10 somatic regeneration plant and various mutants, such as *psm5*, involved in the present disclosure have been publicized in the article (Breeding of the photosensitive male sterile line *psm5* in cotton and the pattern of fertility transformation; Research on Creation and Characteristics of photoperiod Sensitive Genetic Male Sterility Mutant *psm4*), and the author promises to distribute them to the public within 20 years from the application date.

[0021] The term "identity" in the present disclosure refers to the sequence similarity with natural nucleic acid sequence. The term"identity" involves DNA molecules, cDNA molecules or RNA molecules of the nucleotide sequences set forth in SEQ ID NO:1 and/or SEQ ID NO:3, and/or protein composed of amino acid residues set forth in SEQ ID NO:2, and/or nucleotide sequences with 75% or higher, or 85% or higher, or 90% or higher, or 95% or higher identity thereto. Identity may be evaluated with naked eyes or computer software. When a computer software is used to evaluate the identity between related sequences, the identity between two or more sequences may be expressed as a percentage (%).

[0022] The present disclosure also provides a cotton RPGMS gene *Ghpsm5*, where a nucleotide sequence of the RPGMS gene *Ghpsm5* includes the nucleotide sequence as set forth in SEQ ID NO:1, or the nucleotide sequence of a derivate protein with the function of regulating anther dehiscence obtained by replacing and/or deleting and/or adding one or more amino acid residues set forth in SEQ ID NO:2. The cotton RPGMS gene *Ghpsm5* is isolated from *Gossypium hirsutum*.

[0023] The present disclosure also provides a promoter that regulates the expression of the cotton RPGMS gene *Ghpsm5*. The nucleotide sequence of the promoter is set forth in SEQ ID NO:3.

[0024] In particular, the sequence having 75% or higher identity with the nucleotide sequence of the *Ghpsm5* gene or/and the promoter and encoding the protein set forth in SEQ ID NO:2 is derived from the nucleotide sequence of the present disclosure and is equivalent to the sequence of the present disclosure.

[0025] The present disclosure also provides a use of the cotton RPGMS gene *Ghpsm5* or the promoter in the preparation of RPGMS plant material.

[0026] In the present disclosure, manipulations may be at the DNA level or RNA level to abolish the ability of the *Ghpsm5* gene to be completely expressed and/or to be translated into proteins. Alternatively, the promoter can be manipulated at the DNA level to deprive its ability to initiate the expression of downstream genes , thus changing the expression of *Ghpsm5* gene. Therefore, a RPGMS material of cotton or other plants is created. In normal field cultivations, under a condition that the sunshine duration is greater than or equal to 12.5 hours, the RPGMS material of the plant has abnormal pollen development and no anther dehiscence, resulting in male sterility. Under a condition that the sunshine duration is less than or equal to 12.0 hours, the pollen development and the anther dehiscence are normal, and the male fertility is restored. Under a condition that the sunshine duration is greater than 12.0 hours but less than 12.5 hours, some anthers close to the base of flowers occasionally crack and disperse pollens with normal vitality but small numbers (generally less than 5 anthers), which make it

difficult for the cotton to self-cross and set bolls, but may still be used for hybrid seed production.

[0027] The present disclosure also provides a method for preparing a RPGMS plant material, including regulating and/or changing an activity and/or expression of the cotton RPGMS gene *Ghpsm5*.

[0028] A method for the regulating and/or changing includes one or more of gene editing, RNAi, antisense RNA and DNA methylation, and preferably further includes construction of an expression vector using the promoter through genetic engineering method to express the protein, RNA and/or DNA sequences that may affect the development of a male organ. The male organ of the present disclosure preferably comprises anthers.

1. In the present disclosure, the *Ghpsm5* gene and/or the promoter sequence are changed by using at least one of the methods including gene editing, RNAi, antisense RNA, DNA methylation, etc., thus changing the activity and/or expression of the *Ghpsm5* gene. Therefore, an RPGMS material of cotton or other plants is created. The nucleotide sequence of the RPGMS gene *Ghpsm5* comprises any one of a) - d): a) a DNA molecule or cDNA molecule encoded by SEQ ID NO:1; b) a cDNA molecule or genomic DNA molecule encoding the protein set forth in SEQ ID NO:2 and having an identity of 75% or more with the nucleotide sequence set forth in SEQ ID NO:1; c) a cDNA molecule or genomic DNA molecule encoded by SEQ ID NO:1 and Hybridizing with the nucleotide sequence of SEQ ID NO:1 under strict conditions; d) a DNA molecule that is inversely complementary to the DNA molecule in a) or b) or c). The nucleotide sequence of the promoter comprises any one of 1) - 3): 1) a DNA molecule with the nucleic acid sequence set forth in SEQ ID NO:3; 2) a genomic DNA molecule with 75% or more identity with the nucleotide sequence in 1); 3) a DNA molecule that is inversely complementary to the DNA molecule in 1) or 2).

[0029] In the present disclosure, there is no special restriction on the operation steps of the method that may cause the change in *Ghpsm5* gene and/or promoter sequence, and those in conventional methods in the art may be used.

[0030] The present disclosure also provides a use of the RPGMS material prepared by the method in plant breeding.

[0031] In order to further explain the present disclosure, the RPGMS gene *Ghpsm5* and the use thereof in cotton provided by the present disclosure will be described in detail in combination with the drawings and examples, but it should not be understood as limiting the protection scope of the present disclosure.

[0032] The experimental methods in the following examples are conventional methods unless otherwise specified.

[0033] The materials, reagents, etc. used in the following examples can be obtained commercially unless otherwise specified.

[0034] The public can obtain the *Gossypium hirsutum* CCIR 24 (Wang Xinyong, Liu Yining. The performance and cultivation techniques of CCIR 24 in northern Xinjiang [J]. China Cotton, 1998, 25 (1): 31-31.) in the following examples from the National Cotton Germplasm Medium Term Bank of the institute of cotton research of Chinese Academy Agricultural Sciences. The biological material is used only to repeat the

relevant experiments of the present disclosure and cannot be used for other purposes.

[0035] The *Agrobacterium tumefaciens* LBA4404 in the following examples is a product of Beijing Dingguo Changsheng Biotechnology Co., Ltd., whose catalog number is MCC026.

[0036] Example 1

[0037] Cotton RPGMS material obtained by editing *Ghpsm5* gene via gene editing technology

[0038] 1. Construction of editing vector

[0039] DNA fragment containing 2 gRNAs was ligated with a linear CRISPR/Cas9 plasmid obtained by BSAI enzyme digestion to obtain a ligated product. The ligated product was transformed into competent cells of *Escherichia coli*. Monoclones were picked up for positive detection to obtain a positive monoclone. Plasmids were extracted from the positive monoclone to obtain a CRISPR/Cas9-Ghpsm5 recombinant vector (FIG.1).

[0040] gRNA:

[0041] SEQ ID NO:4: CCAAGCTCCCACATTAGAAA CGG

[0042] SEQ ID NO:5: CATTCCAGAAAGCAAACAAC AGG

[0043] Primers used for positive detection:

[0044] CAS9-F (SEQ ID NO:6): 5'- ACACAGGAGCGTTTATATAAGCGA -3',

[0045] CAS9-R (SEQ ID NO:7): 5'- TGGTTTGTTGGTCGCCGTTAG -3'.

[0046] The reaction conditions for PCR amplification were: pre-denaturation at 94°C for 5 min; 94°C for 30s, 58°C for 30s, 72°C for 1min, 30 cycles; and extension at 72°C for 5min.

[0047] 2. Creation of the RPGMS material by gene editing technology

[0048] CRISPR/Cas9-*Ghpsm5* recombinant vector was transformed into *Agrobacterium tumefaciens* LBA4404 to obtain a recombinant *Agrobacterium tumefaciens* containing CRISPR/Cas9-*Ghpsm5*, namely LBA4404/*Ghpsm5*.

[0049] Agrobacterium-mediated genetic transformation was carried out according to the methods in the literature (Firoozabady E, DeBoer D L, Merlo D J, et al. Transformation of cotton (*Gossypium hirsutum* L.) by Agrobacterium tumefaciens and regeneration of transgenic plants [J]. Plant Molecular Biology, 1987, 10(2): 105-116.). The hypocotyl of *Gossypium hirsutum* CCIR 24 was transformed by LBA4404/*Ghpsm5* to transfer the gene to be edited into the cotton genome. Kanamycin was used for screen. Transgenic cotton was obtained after regeneration of the hypocotyl. The transgenic cotton was transplanted (FIG.2). The T1 generation inbred seeds were harvested from T0 generation kanamycin-resistant plants. The molecular detection of PGMS individuals in T1 generation was carried out.

[0050] Leaves were taken to extract DNA. With the DNA as the template, and the following primers as primers, PCR amplification was carried out. The amplification product was recovered and connected to the T vector, which was then transferred into *Escherichia coli*. Monoclones were selected for sequencing to detect the gene editing. It was found after the sequencing that there were a 5-base deletion in target 1 segment and a 2-base deletion in target 2 segement of the Ghpsm5 gene in the RPGMS plant (The site sequence and detection results are shown in i and j of FIG.3).

[0051] Ghpsm5-F1 (SEQ ID NO:8): 5'- GGGCAGGATCGGAGATTGTT -3'

[0052] Ghpsm5-R1 (SEQ ID NO:9): 5'- GCATCGGAACCCAACAGGA -3'

[0053] Ghpsm5-F2 (SEQ ID NO:10): 5'- GGTTCTCGGTTGTTGCCAAT -3'

[0054] Ghpsm5-R2 (SEQ ID NO:11): 5'- AGATAGGACAGCTACACAGGC -3'

[0055] The gene-edited material was planted continuously. It was found that the PGMS characteristic was maintained from T1 generation to T3 generation. The gene-edited material was named as *ps201* (FIG.3).

[0056] 3. Genetic analysis of fertility of gene-edited male sterile material

[0057] Tested materials: The cross was carried out with the non-transgenic CCIR 24 as the male parent and the RPGMS material *ps201* obtained by gene editing as the female parent. The F_1 generation was normal and fertile. The F_2 generation included RPGMS individuals. The proportion of fertile and RPGMS individuals was 3:1, which conformed to the genetic law of single recessive gene. It was found that the RPGMS trait and the gene editing site were co-segregated through molecular identification.

[0058] The method of molecular identification: F_2 generation was planted in the experimental field of the institute of cotton research of Chinese Academy Agricultural Sciences in Anyang. Male fertile plants and male sterile plants were identified after their flowering. Among 136 F_2 plants, 32 male sterile plants were identified. DNA of 32 male sterile plants was extracted separately, and then 23 of them were mixed equally to obtain a mixed sample. The mixed sample and the remaining 9 plants, in total 10 plants, were detected together. Three fertile plants in the field were taken to detect one editing site. The results showed that there were no unedited sequences detected in male sterile single plants and mixed samples. 30 normal fertile single plants were selected for detection, among which 17 heterozygous single plants with edited sequence and non-edited sequence and 13 single plants without gene editing were detected.

[0059] 3.1 Specific detection methods were as follows

[0060] a: The leaves of F2 generation plants planted in the field were taken back to the laboratory with ice box and stored in the refrigerator at - 80°C. DNA was extracted from leaves by Cetyltrimethylammonium Bromide (CTAB) method.

[0061] b: The sequence of the two editing sites was amplified from DNA by using KOD high-fidelity enzyme. Bands with the same size as the target segment were recycled. The target fragment was linked to a T vector and transformed into *Escherichia coli*. The *Escherichia coli* was coated on Luria-Bertani (LB) solid medium plate containing kanamycin, and put upside down in a 37°C incubator overnight (12-24 hours).

[0062] c: (1) Growing monoclones were observed and picked. Kana antibiotic was added to LB in the ratio of 1 ml: 1 μL in the ultra-clean bench and gently shake up. 300-500 μL of the above LB solution to the centrifuge tube. Then the monoclones were taken to the centrifuge tube with a pipette tip. The LB solution with the monoclones was shaken in a shaker at 37°C for more than 3 hours for detection. The *Escherichia coli* with the same size as the target fragment was sequenced.

[0063] d: The sequencing result was compared with the target sequence to determine whether editing was done.

[0064] 4. Corresponding time of photoperiod of gene-edited RPGMS line

[0065] *ps201* was planted in flowerpots during the normal growth period of cotton in Anyang, Henan Province. After the cotton entered the flowering period, it was observed that *ps201* was male sterility, with the anthers not cracking and the pollens having no vitality. The flowerpots were moved into the room for shading treatment in the afternoon, and moved outdoor for normal lighting after 20pm. 17 days after the flowerpots were removed, when, the plant with a light duration at less than 12.0 hours started to disperse pollens normally, and the plant with more than 12.5 hours did not disperse pollens. It was thus inferred that *ps201* was male sterile when the sunshine duration was more than 12.5 hours, and fertile when the sunshine duration was less than 12.0 hours.

[0066] *ps201* was planted in the south breeding base of the institute of cotton research of Chinese Academy Agricultural Sciences in Yacheng, Hainan Province in late October. *ps201* was normally fertile at flowering in December. By the middle of April of the next year, after the southern winter breeding, the sunshine duration in Hainan began to be more than 12 hours. *ps201* turned into male sterility after 17 days of sunshine. The basal anthers of a small number of flowers occasionally dispersed pollens. When the cotton was planted in Anyang, Henan Province, and the sunshine duration changed from 12.5 hours to 12 hours during the period from the middle to the end of September, dispersed pollens from a small amount of anther was observed. 17 days after the sunshine duration became less than 12 hours, the cotton began to disperse pollens and set bolls like normal cotton, showing male fertility characteristics.

[0067] Example 2

[0068] RPGMS lines prepared from gene editing material

[0069] 1. The RPGMS plant obtained in Example 1 was planted in an area where the sunshine was less than 12.0 hours and self-pollinated, such that an RPGMS line was generated.

[0070] 2. The RPGMS line material obtained from step 1 was hybridized with normal fertile material. The RPGMS material was isolated from the offspring. The offspring material was planted in an area with more than 12.5 hours of sunshine duration. New cotton material with RPGMS characteristics was selected from the offspring material.

[0071] Example 3

[0072] Use of the RPGMS line in Breeding

[0073] Normal expression of *Ghpsm5* gene will be destroyed by gene editing technology, resulting in recessive photoperiod-sensitive genic male sterility. In view of the fact that the cotton regions in China and the world's major cotton producing countries have more than 12.5 hours of sunshine in the normal growth stage of cotton and the RPGMS materials are all male sterile, such materials could be used as female parent for hybrid seed production. In the winter of tropical regions of the world where cotton grows normally, the fertility of the RPGMS material could be restored by placing it in an area where the sunshine hours are less than 12 hours (such as Sanya City, Hainan Province, China) and through self-crossing. The normally planted cotton without gene editing (except male sterile materials caused by other reasons) show normal fertility, and could be used as a male parent to cross with an RPGMS material to prepare hybrid with normal fertility. Therefore, normally planted cotton could be used

as restorer lines for hybrid seed production, and the hybrids could be used for cotton hybrid production and breeding.

[0074] 1. The RPGMS line obtained in Example 1 or Example 2 was used to cross with normal fertile cotton to cultivate hybrids.

[0075] 2. The RPGMS line obtained in Example 1 or Example 2 was used to cross with normal fertile cotton to select excellent fertile material in the hybrid offspring to cultivate new varieties. The excellent RPGMS material in hybrid progeny was bred to cultivate RPGMS lines.

[0076] Example 4

[0077] Use of the RPGMS line in Intelligent breeding

[0078] 1. The RPGMS line was cultivated by the method of Example 1-3 and DNA sequencing was performed.

[0079] 2. The RPGMS line sequenced in step 1 was planted to investigate the agronomic traits.

[0080] 3. The cross was carried out with the excellent line as the male parent and the RPGMS plant as the female parent. The agronomic traits of the male parent and F_1 were investigated, and DNA of the male parent was sequenced.

[0081] 4. A breeding model was established based on the parent traits, DNA sequences and the traits of F1 generation to form a parent selection algorithm for RPGMS lines.

[0082] 5. The candidate male parent material was sequenced. The traits of hybrid F1 of the material and the RPGMS line were predicted by model calculation. The combinations with poor prediction results were eliminated, and the combinations with heterosis were prepared. Sequencing of hybrid breeding was carried out, and the agronomic traits of F1 generation were investigated. Steps 3 and 4 were repeated and the results were fed back to the model to improve the model. An intelligent breeding approach based on the RPGMS lines was formed.

[0083] Although the above embodiments give a detailed description of the present disclosure, they are only part not all of embodiments of the present disclosure. Those skilled in the art can also obtain other embodiments according to these embodiments without an inventive step, which are within the protection scope of the present disclosure.

WHAT IS CLAIMED IS:

1. A cotton recessive photoperiod-sensitive genic male sterile (RPGMS) gene *Ghpsm5*, wherein an amino acid sequence of a protein encoded by the RPGMS gene *Ghpsm5* comprises the sequence set forth in SEQ ID NO:2, or a sequence that has more than 75% identity with the amino acid sequence set forth in SEQ ID NO:2.

2. A cotton RPGMS gene *Ghpsm5*, wherein a nucleotide sequence of the RPGMS gene *Ghpsm5* comprises the nucleotide sequence set forth in SEQ ID NO:1, or a nucleotide sequence of a derivate protein with the function of regulating anther dehiscence obtained by replacing and/or deleting and/or adding one or more amino acid residues to the protein set forth in SEQ ID NO:2.

3. The cotton RPGMS gene *Ghpsm5* according to claim 1 or 2, wherein a nucleotide sequence of the RPGMS gene *Ghpsm5* comprises any one of a) - d):

a) a DNA molecule or cDNA molecule encoded by SEQ ID NO:1;

b) a cDNA molecule or genomic DNA molecule encoding the protein set forth in SEQ ID NO:2 and having 75% or more identity with the nucleotide sequence set forth in SEQ ID NO:1;

c) a cDNA molecule or genomic DNA molecule encoded by SEQ ID NO:1 that Hybridizes with the nucleotide sequence of SEQ ID NO:1 under strict conditions;

d) a DNA molecule that is inversely complementary to the DNA molecule in a) or b) or c).

4. A promoter that regulates the expression of the cotton RPGMS gene *Ghpsm5* according to any one of claims 1 to 3, wherein the nucleotide sequence of the promoter comprises any one of 1) - 3):

1) a DNA molecule with the nucleic acid sequence set forth in SEQ ID NO:3;

2) a genomic DNA molecule with 75% or more identity with the nucleotide sequence in 1);

3) a DNA molecule that is inversely complementary to the DNA molecule in 1) or 2).

5. The promoter according to claim 4, wherein the nucleotide sequence of the promoter is set forth in SEQ ID NO:3.

6. Use of the cotton RPGMS gene *Ghpsm5* according to any one of claims 1 to 3 or the promoter according to claim 4 or 5 in the preparation of RPGMS plant material.

7. The use according to claim 6, wherein under a condition that the sunshine duration is greater than or equal to 12.5 hours, pollens of the RPGMS plant material are inactive and have no anther dehiscence, resulting in male sterility; under a condition that the sunshine duration is greater than 12.0 hours but less than 12.5 hours, anthers close to the base of flowers may crack and disperse pollenswith normal vitality but small numbers, which make it difficult for the cotton to self-cross and set bolls, but may still be used for hybrid seed production; under a condition that the sunshine duration is less than or equal to 12.0 hours, pollen development and anther dehiscence are normal, and male fertility is restored.

7. A method for preparing an RPGMS plant material, comprising regulating and/or changing an activity and/or expression pattern of the cotton RPGMS gene *Ghpsm5*

according to any one of claims 1 to 3.

8. The method according to claim 7, wherein a method for the regulating and/or changing comprises one or more of gene editing, RNAi, antisense RNA and DNA methylation.

9. The method according to claim 7, wherein a method for the regulating and/or changing comprises constructing an expression vector by using the promoter according to claim 4 or 5 through genetic engineering to express a protein, RNA and/or DNA sequence that can affect the development of a male organ.

10. The method according to claim 9, wherein the male organ comprises anthers.

11. A method for preparing a cotton RPGMS material comprising editing the cotton RPGMS gene *Ghpsm5* according to any one of claims 1 to 3 with gene editing technology, wherein the editing comprises the following steps:

A) ligating a DNA fragment containing gRNAs set forth in SEQ ID NO:4 and SEQ ID NO:5 with a CRISPR/Cas9 linear plasmid obtained by BSAI enzyme digestion to obtain a ligated product, transforming the ligated product into a competent cell of *Escherichia coli*, selecting a monoclone for positive detection to obtain a positive monoclone, and extracting a plasmid from the positive monoclone to obtain a CRISPR/Cas9-Ghpsm5 recombinant vector;

B) transforming the CRISPR/Cas9-*Ghpsm5* recombinant vector into *Agrobacterium tumefaciens* LBA4404 to obtain a recombinant *Agrobacterium tumefaciens* containing CRISPR/Cas9-*Ghpsm5*, namely LBA4404/*Ghpsm5*; and

C) transforming hypocotyl of *Gossypium hirsutum* CCIR 24 by using LBA4404/*Ghpsm5* through agrobacterium-mediated genetic transformation to transfer *Ghpsm5* into cotton genome, screening by kanamycin, then obtaining transgenic cotton after regeneration of the hypocotyl, and transplanting the transgenic cotton.

12. The method according to claim 11, further comprising harvesting T1 generation inbred seeds from kanamycin resistant plants of T0 generation after transplanting the transgenic cotton, and carrying out molecular detection of RPGMS individuals in T1 generation.

13. The method according to claim 12, wherein the molecular detection comprises: extracting DNA from leaves, taking the DNA as a template, using the sequences set forth in SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10 and SEQ ID NO:11 as primers, performing PCR amplification, recovering an amplification product, ligating the amplification product to a T vector, transferring a T vector ligated to the amplification product into *Escherichia coli*, selecting a monoclone for sequencing, and detecting gene editing;

an editing target 1 segment of the Ghpsm5 gene in RPGMS plant has 5 bases deletion and a target 2 segment has 2 bases deletion.

14. The method according to claim 11, wherein the sequence of primers used for the positive detection is set forth in SEQ ID NO:6 and SEQ ID NO:7.

15. The method according to claim 11, wherein procedures of the PCR amplification for positive detection comprise: pre-denaturation at 94°C for 5 min; 94°C for 30s, 58°C for 30s, 72°C for 1min, 30 cycles; and extension at 72°C for 5min.

16. Use of the RPGMS plant material prepared by the method according to any one

of claims 7-10 in plant breeding.

17. A method for cultivating an RPGMS line from the cotton RPGMS material prepared by the method according to any one of claims 11-15, comprising the following steps:

planting the cotton RPGMS material in an area where the sunshine duration is less than 12.0 hours to obtain the RPGMS line after self-pollination and propagation;

hybridizing the RPGMS line with a normal fertile material, and isolating an RPGMS material from a progeny, planting the RPGMS material from the progeny in an area where the sunshine duration is more than 12.5 hours, selecting a new cotton material with RPGMS characteristics from the RPGMS material from the progeny.

DRAWINGS

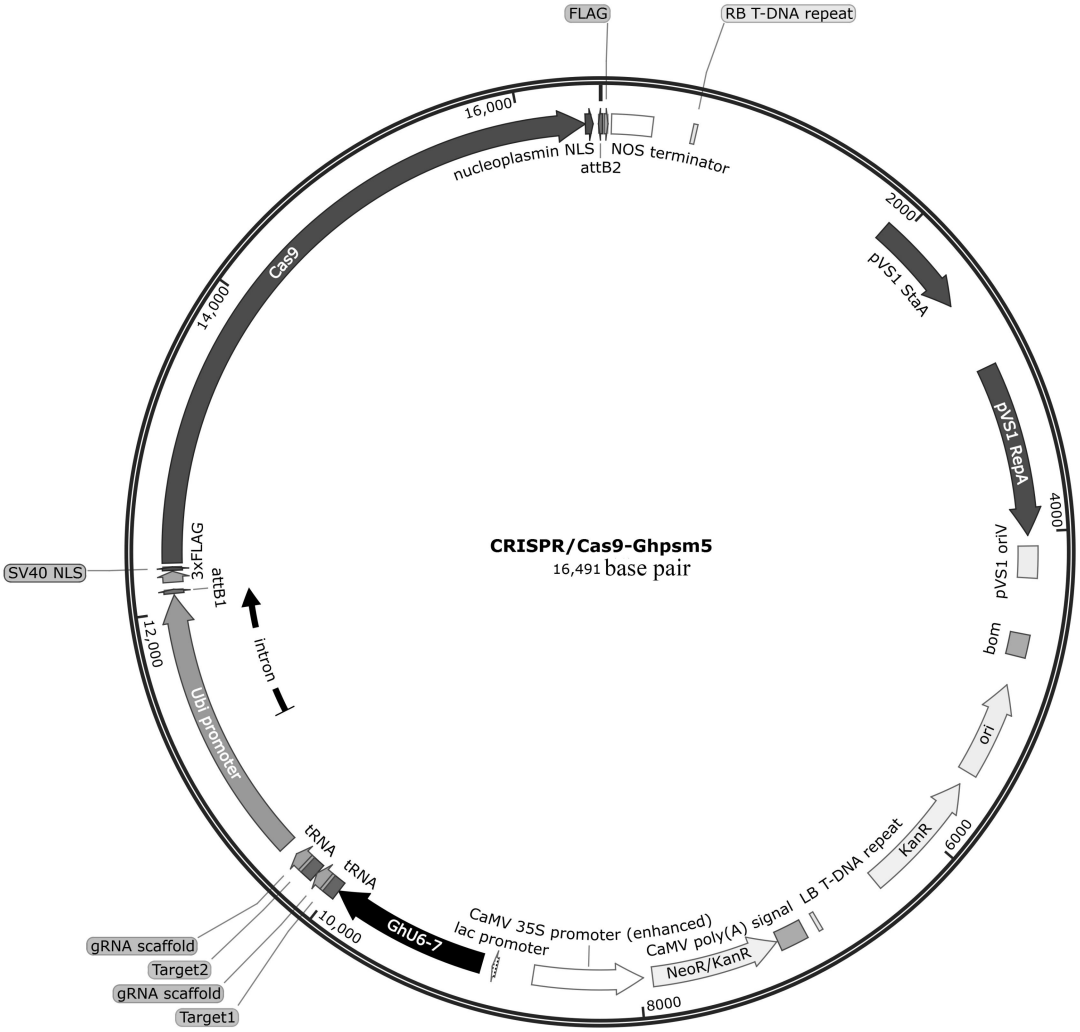

FIG.1

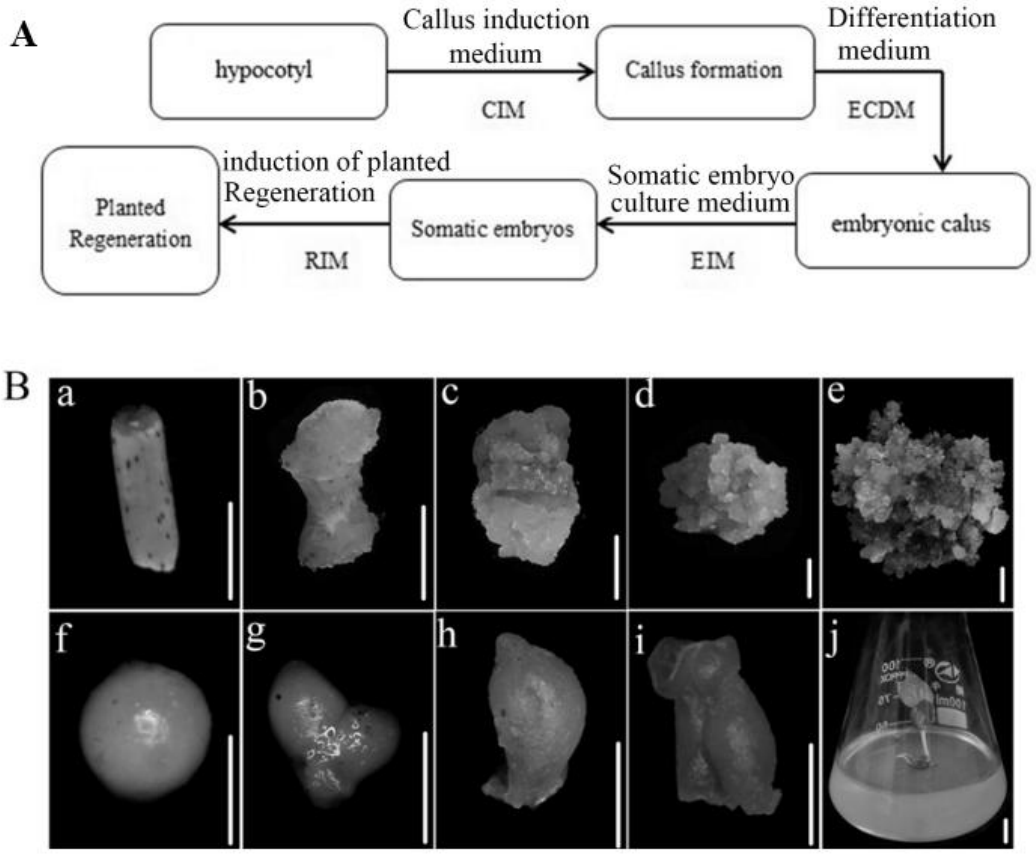
A
hypocotyl
Callus induction medium
CIM
Callus formation
Differentiation medium
ECDM
embryonic calus
Somatic embryo culture medium
EIM
Somatic embryos
induction of planted Regeneration
RIM
Planted Regeneration
B
a
b
c
d
e
f
g
h
i
j

FIG.2

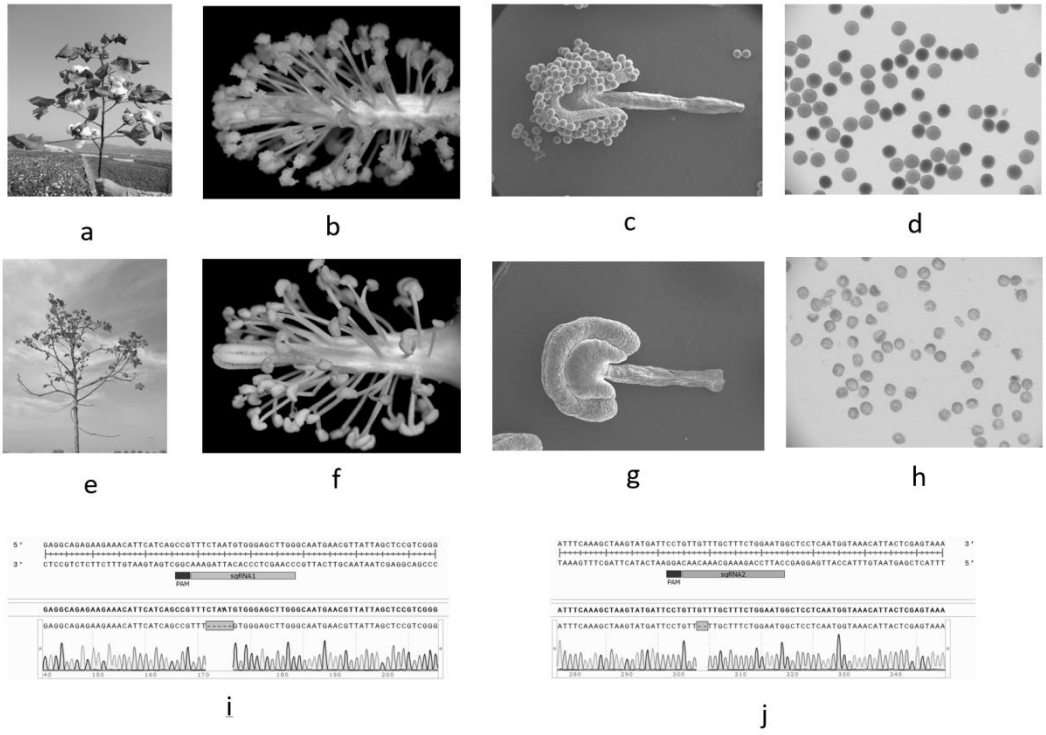
a
b
c
d
e
f
g
h
i
j

FIG.3

Sequence Listing

1	**Sequence Listing Information**	
1-1	File Name	SEQUENCE LISTING.xml
1-2	DTD Version	V1_3
1-3	Software Name	WIPO Sequence
1-4	Software Version	2.0.0
1-5	Production Date	2023-08-15
1-6	Original free text language code	
1-7	Non English free text language code	
2	**General Information**	
2-1	Current application: IP Office	
2-2	Current application: Application number	
2-3	Current application: Filing date	
2-4	Current application: Applicant file reference	GWPCTP20230604805
2-5	Earliest priority application: IP Office	CN
2-6	Earliest priority application: Application number	202211463130.X
2-7	Earliest priority application: Filing date	2022-11-22
2-8en	Applicant name	INSTITUTE OF COTTON RESEARCH OF CAAS
2-8	Applicant name: Name Latin	
2-9en	Inventor name	
2-9	Inventor name: Name Latin	
2-10en	Invention title	RECESSIVE PHOTOPERIOD-SENSITIVE GENIC MALE STERILE GENE Ghpsm5 AND USE THEREOF IN COTTON
2-11	Sequence Total Quantity	17

3-1	**Sequences**	
3-1-1	Sequence Number [ID]	1
3-1-2	Molecule Type	DNA
3-1-3	Length	1026
3-1-4	Features	**source 1..1026**
	Location/Qualifiers	mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-1-5	Residues	atggaggcag agaagaaaca ttcatcagcc gtttctaatg tgggagcttg ggcaatgaac 60 gttattagct ccgtcgggtt aatcatggtc aacaagcaac tcatgtcccc taccggtcat 120 gccttcgctt ttgcgacaac attaactggg ttccactttt gtacaactgc cttaatcggt 180 ttggtgtcaa atgccactgg ttacacaaca aaaaagaatg ttcctttgtg ggagcttctt 240 tggttctcgg ttgttgccaa tacttcaatt accgcgatga acttgagcct catgttgaat 300 tcagttggat tttatcagat ttcaaagcta agtatgattc ctgttgtttg ctttctggaa 360 tggctcctca atggtaaaca ttactcgagt aaagtcaaga tggcagtggt ggtggtggtg 420 gtgattggtg taggtgtttg tacagttact gatgtaaaaa ttaattcgca tggatttctt 480 tgtgcctgtg tagctgtcct atctacctcc ttacaacaaa tttcaattgg gtccttacag 540 aagaagtact caataggatc ttttgaactg cttagccaaa cagctccaat ccaagcttta 600 tctcttttgt tgcttggtcc attcgttgat tacttcctca ctggcaagtt actagcaagt 660 tataagatct cctcagctgc attttttttc atagtactgt catgttcctt agctgttttc 720 tgcaacataa gccagtacct gtgcattgga cgattctcgg cggtgtcttt ccaagtgcta 780 ggacacatga aaacagtatg cgtgttgata ttaggatgga tgctgtttga ctcagagctg 840 acattgaaaa acatactagg gatgagcata gctgtgaatg ggatgataat atatagttgg 900 gcagttgagg gagataagcc aatgcactac cgaaaagatg ctacatcaga tgtaaagcta 960 ctgataaagc aagtggacgg ctcatcacta ctcaaggacg ttgagcttgg caaatctcaa 1020 ggatag 1026
3-2	**Sequences**	
3-2-1	Sequence Number [ID]	2
3-2-2	Molecule Type	AA
3-2-3	Length	341
3-2-4	Features	**source 1..341**
	Location/Qualifiers	mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-2-5	Residues	MEAEKKHSSA VSNVGAWAMN VISSVGLIMV NKQLMSPTGH AFAFATTLTG FHFCTTALIG 60 LVSNATGYTT KKNVPLWELL WFSVVANTSI TAMNLSLMLN SVGFYQISKL SMIPVVCFLE 120 WLLNGKHYSS KVKMAVVVVV VIGVGVCTVT DVKINSHGFL CACVAVLSTS LQQISIGSLQ 180 KKYSIGSFEL LSQTAPIQAL SLLLLGPFVD YFLTGKLLAS YKISSAAFFF IVLSCSLAVF 240 CNISQYLCIG RFSAVSFQVL GHMKTVCVLI LGWMLFDSEL TLKNILGMSI AVNGMIIYSW 300 AVEGDKPMHY RKDATSDVKL LIKQVDGSSL LKDVELGKSQ G 341
3-3	**Sequences**	
3-3-1	Sequence Number [ID]	3
3-3-2	Molecule Type	DNA
3-3-3	Length	2500
3-3-4	Features	**source 1..2500**
	Location/Qualifiers	mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-3-5	Residues	tatcacatta aatgaactcg cttctggcag atgaagtttt agttttgtct tcaggatcct 60 gctacgtcta gcatgcttga aagtttgtcc aatcctcagc aaaaagacca gattgaggaa 120 agaatggcac gtatcaaaga ggatccatca ttgaaacata ttctagaaga aatagagaca 180 ggtggtcccg cagccatgat gaggtgggtg atcaaattac tagtagcatc atagtattat 240 agtgttgtgg gcacaacagt ttcttcttta gaacgtatgt agatgtccgc atttagctat 300 ttttcactaa attaataaca gaaaggcttc tggattaggt actggaatca taaagaagtt 360 ctccagaagt tgggtgaagc aatgggtctt gcagattcag gagatgcagc catttctgtt 420 ggtaaggctg cagccgatga agatgatgaa gtagggaatg aggatgagtc aattgtacat 480 cattgtgcta gtgttggtga tgttgaggta ggacagagtg aatttttgta catgtatttt 540 tcgcctttga tagataatct aaaaagtctc gactgtattt gtggtttagg gtctgaaaac 600 tgcactagcc tctggtgctg acaaggatga agaagatgca gaggggagga cggcattaca 660 ttttgcatgt ggttatggcg aggtatgata attttataat tgaaatctgg gtagtcccta 720 ctgctgcatt gcctgactaa tgaagtagtt ttgtctatcg aggacaaact tgtgtggaaa 780 atctgatggg tttaataact gattgttaca agtattcaat actagtctca aatgccggtg 840 gacacctcta gtctagtagt ttaggagagt ttgtaggaga ttgggttatt gggctagttt 900 taggttatgt ttcttagaaa agatttcagg caaagggata gattccatgt catgtttttt 960 acccgggggt aaagctaata cactagtgat tttattattg tagatgaaat gtgcacaaat 1020 cctagttgaa gctggagcaa gagtggatgc tttggacaag aataagaata ctgcacttca 1080 ttatgcagct ggttatggaa ggaaagactg tgtggccctt ctactggaga acggggctgc 1140 tgtgtaagta aacaaactcg ttgggtaacc attgatatgt atattgactt gtttatgtaa 1200 tttatttatt ttatttttatt ttttatgtca gcacactcca aaacatggat ggcaagaccc 1260 ccattgaagt agccaagctg aacaatcagc atgaggtagt aaagttgttg gagaaagatg 1320 cgtttttgtg agttgggagg gggcattgcg cccatgaatt aaattgtacc tcgaggaaaa 1380 gggaatggga tgaaggtaaa gtggtgaact caaaacaata ataaataaa atatactgaa 1440 tacagttata tgaacgaaag atctgtagat gaagactggt agacaacaac aatgtttatt 1500

		gtgttttgtt aatcacagtt tcatcactta aattctagaa ttattgtata atgcttaatt	1560
		gataaattaa tctaattgta atttgttggg cttaatgcca aaaaagttcg gcttaaaagc	1620
		aatgttttgg aaattagatt ggtggtctaa tcagtcaaaa tgtatcggtc gtttgtaaat	1680
		aatttatgta aatataagat gaaaatgtga ccaaaaaaga gataagatat caacaataaa	1740
		tgtgacaaac aaattataat agtcattttg accatacata aatggtctac atttatcttg	1800
		tcacggttga ttatttaagg agtatattta tcttctagat agatgatgat aagattaata	1860
		tagattaaac aaagtttact tgtgcttcat gcaaagaaaa gacatttcat gtgcgacgta	1920
		tacatatacc tacagaattt atagttgaca aaatgttgtc gttgaaggaa gctatataag	1980
		tgatgagata aatttaaata aaaagaggaa attcaaagga aagaacaaaa aaggtgtgag	2040
		agaaaaaaga aaagggaggt ggggggggagg ccaagaagct accatctgcc aaactggagt	2100
		aaaaatagga tccacactct tcacaccggt ttcttacttt ctatttttat ttttcacaaa	2160
		tttaaaaaga gattagggca attgacattt tccaaatact ttaacaatcg atgatctact	2220
		actcaccatg tcattcgttc ttgttcaaag agaattaaat cgtaaatata taaatttatg	2280
		taactattaa taaaaaaaga aaattggaac ataattcgga tccaactact gtatataaag	2340
		tcgtgatcgt tcaaaattta catgtttcag attttctcgt atatcttgct ctgagctgcc	2400
		tctgcgtgcg ggggcaggat cggagattgt ttggtcggcg aagtttcaga tctgtctttg	2460
		tagtccaatc gactggtaat attcgatttg gattgcgaca	2500
3-4	**Sequences**		
3-4-1	Sequence Number [ID]	4	
3-4-2	Molecule Type	DNA	
3-4-3	Length	23	
3-4-4	Features	**source 1..23**	
	Location/Qualifiers	mol_type=other DNA	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-4-5	Residues	ccaagctccc acattagaaa cgg	23
3-5	**Sequences**		
3-5-1	Sequence Number [ID]	5	
3-5-2	Molecule Type	DNA	
3-5-3	Length	23	
3-5-4	Features	**source 1..23**	
	Location/Qualifiers	mol_type=other DNA	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-5-5	Residues	cattccagaa agcaaacaac agg	23
3-6	**Sequences**		
3-6-1	Sequence Number [ID]	6	
3-6-2	Molecule Type	DNA	
3-6-3	Length	24	
3-6-4	Features	**source 1..24**	
	Location/Qualifiers	mol_type=other DNA	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-6-5	Residues	acacaggagc gtttatataa gcga	24
3-7	**Sequences**		
3-7-1	Sequence Number [ID]	7	
3-7-2	Molecule Type	DNA	
3-7-3	Length	21	
3-7-4	Features	**source 1..21**	
	Location/Qualifiers	mol_type=other DNA	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-7-5	Residues	tggtttgttg gtcgccgtta g	21
3-8	**Sequences**		
3-8-1	Sequence Number [ID]	8	
3-8-2	Molecule Type	DNA	
3-8-3	Length	20	
3-8-4	Features	**source 1..20**	
	Location/Qualifiers	mol_type=other DNA	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-8-5	Residues	gggcaggatc ggagattgtt	20
3-9	**Sequences**		
3-9-1	Sequence Number [ID]	9	
3-9-2	Molecule Type	DNA	
3-9-3	Length	19	
3-9-4	Features	**source 1..19**	
	Location/Qualifiers	mol_type=other DNA	
		organism=synthetic construct	
	NonEnglishQualifier Value		

3-9-5	Residues	gcatcggaac ccaacagga	19
3-10	**Sequences**		
3-10-1	Sequence Number [ID]	10	
3-10-2	Molecule Type	DNA	
3-10-3	Length	20	
3-10-4	Features	**source 1..20**	
	Location/Qualifiers	mol_type=other DNA	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-10-5	Residues	ggttctcggt tgttgccaat	20
3-11	**Sequences**		
3-11-1	Sequence Number [ID]	11	
3-11-2	Molecule Type	DNA	
3-11-3	Length	21	
3-11-4	Features	**source 1..21**	
	Location/Qualifiers	mol_type=other DNA	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-11-5	Residues	agataggaca gctacacagg c	21
3-12	**Sequences**		
3-12-1	Sequence Number [ID]	12	
3-12-2	Molecule Type	DNA	
3-12-3	Length	55	
3-12-4	Features	**source 1..55**	
	Location/Qualifiers	mol_type=other DNA	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-12-5	Residues	gaaacattca tcagccgttt ctaatgtggg agcttgggca atgaacgtta ttagc	55
3-13	**Sequences**		
3-13-1	Sequence Number [ID]	13	
3-13-2	Molecule Type	DNA	
3-13-3	Length	55	
3-13-4	Features	**source 1..55**	
	Location/Qualifiers	mol_type=other DNA	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-13-5	Residues	ctttgtaagt agtcggcaaa gattacaccc tcgaacccgt tacttgcaat aatcg	55
3-14	**Sequences**		
3-14-1	Sequence Number [ID]	14	
3-14-2	Molecule Type	DNA	
3-14-3	Length	50	
3-14-4	Features	**source 1..50**	
	Location/Qualifiers	mol_type=other DNA	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-14-5	Residues	gaaacattca tcagccgttt gtgggagctt gggcaatgaa cgttattagc	50
3-15	**Sequences**		
3-15-1	Sequence Number [ID]	15	
3-15-2	Molecule Type	DNA	
3-15-3	Length	55	
3-15-4	Features	**source 1..55**	
	Location/Qualifiers	mol_type=other DNA	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-15-5	Residues	aagctaagta tgattcctgt tgtttgcttt ctggaatggc tcctcaatgg taaac	55
3-16	**Sequences**		
3-16-1	Sequence Number [ID]	16	
3-16-2	Molecule Type	DNA	
3-16-3	Length	55	
3-16-4	Features	**source 1..55**	
	Location/Qualifiers	mol_type=other DNA	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-16-5	Residues	ttcgattcat actaaggaca acaaacgaaa gaccttaccg aggagttacc atttg	55
3-17	**Sequences**		
3-17-1	Sequence Number [ID]	17	
3-17-2	Molecule Type	DNA	
3-17-3	Length	53	

3-17-4	Features	**source 1..53**
	Location/Qualifiers	mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-17-5	Residues	aagctaagta tgattcctgt tttgctttct ggaatggctc ctcaatggta aac 53