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# Analysis of short fruiting branch gene and Marker-assisted selection with SNP linked to its trait in upland cotton

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## Abstract

**Background:** With the rapid development of genomics, many functional genes have been targeted. Molecular marker assisted selection can accelerate the breeding process by linking selection to functional genes.

**Methods:** In a study of upland cotton (*Gossypium hirsutum* L.), the F<sub>2</sub> segregated population was constructed by crossing X1570 (short branches) with Ekangmian-13 (long branches) to identify the short fruiting branch gene and marker-assisted selection with SNP (Single Nucleotide Polymorphisms, SNP) linked to its trait.

**Result:** The result demonstrated that linked SSR marker BNL3232 was screened by BSA (Bulked segregant analysis, BSA) method; one SNP locus was found, which was totally separated from the fruiting branches trait in upland cotton.

**Conclusion:** It was verified that this SNP marker could be used for molecular assisted selection of cotton architecture.

**Keywords:** Short fruit-branch, Cotton, Gene, Marker-assisted selection

## Background

Plant architecture is an important breeding target trait, and fruiting branch type is a primary determinant of plant architecture in upland cotton (*Gossypium hirsutum* L.). Fruiting branches, as the name implies, are fruit-bearing branches of cotton plants that can bear bolls and are usually located in the upper middle of the main stem. The short fruiting branch cotton type is identified by plants with only one to two fruiting positions per branch and short fruiting branch internodes (Du 1996). An advantage of this growth habit is that after cutting off the top of the plant, the plant does not continue to produce additional vegetative growth, concentrating nutrient and carbohydrate supply for developing bolls and accelerating boll maturation (Du et al. 1996). In addition, plants with short fruiting branches allow additional light transmission into the plant canopy

and are suitable for high-density planting (Wang and Yang 2003). Plants with short fruiting branches also do not need pruning, which decreases inputs and improves production costs. Growth of leaves and branches can be controlled completely by high-density planting of short fruiting branch upland cotton, so that fertilizer demand is decreased compared with normal fruiting branch varieties (Yang et al. 2001).

Additionally, plant architecture is an important characteristic that affects cotton plant adaptability, yield, and fiber quality. An enhanced understanding of the molecular-genetic regulation of plant morphological developmental can aid in modifying relevant agronomic traits to obtain high yielding cotton varieties (Song and Zhang 2009). Moreover, with the change of the quantity and structure of the rural labor force in China, traditional labor-intensive management measures are losing their economic viability for cotton cultivation and production in the Yangtze River Basin of China. Selection of cultivars with short fruiting branches also allows less cultivation and chemical application, improving the environmental impact and sustainability of cotton fields in

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Yangtze River Basin of China. Cotton breeding should focus on those varieties suitable for shortening the cotton growing period, sowing after wheat or edible rape, and being picked by machine (Liu et al. 2013). Therefore, the short fruiting branch becomes an important selected target in cotton breeding (Zhang et al. 2006).

Classical genetic studies have shown that the traits of fruiting branch belong to quality traits. Cluster gene *cl1* and *cl3* from the upland cotton are located on Chromosome 16 (D07) (Hau et al. 1980; Silow 1946; Ni et al. 2007). The *cl2* gene from *G. barbadense* is located on Chromosome 7 (A07) (Thadani 1923). Hu (2016) established an F<sub>2</sub> population from a cross between *G. hirsutum* mutant T586 and *G. barbadense* Hai7124 for rough mapping *cl* gene, and *cl* was located on the Chr16 chromosome and linked to SSR marker BNL1694 (Hu and Zhou 2006). In recent years, QTL mapping and sequencing techniques were used to finely map the short fruiting branch traits of *Gossypium barbadense*. F<sub>2</sub> population was constructed by the nulliplex-branch Pima cotton variety, Xinhai-18 crossed with the normal branch upland cotton line, TM-1, the nulliplex-branch trait was found to be controlled by the recessive gene *gb\_nb 1*, localized at the 600 kb interval on Chr 16 (Chen et al. 2014); In 2015, *cl* gene was mapped between maker SWU07707 and SWU08487 within an interval of 69 kb physical distance of *G. raimondii* genome, and there is only one annotated gene, *Gorai.001G121800*, which is associated with flowering time candidate gene (Zhai 2015). Although there are several SSR markers linked to short fruiting branch trait in *G. barbadense*, there are still no reports on the location and linkage markers of the short-branch trait gene of *G. hirsutum*. In this study, the sequence analysis and uncovering of markers linked to short fruiting branch traits derived

from two *G. hirsutum* L. mutants were used to improve markers of the functional genes and make molecular marker-assisted selection in upland cotton.

## Methods

### Plant materials

In this study, parent cotton material X1570 and Ekangmian-13 were used for constructing population. Both belong to *G. hirsutum*. Ekangmian-13 was a national certified cotton variety by Huimin Agricultural in Hubei Province, and had normal fruiting branches with more than two nodes; X1570 is a type of early maturity material obtained from the Duck cotton which originated from Deltapine 15 (Yu and Liu 1957), and characterized by short fruiting branches trait with only 1–2 fruiting positions (Fig. 1). The growth period duration of X1570 is about 95–100 days, with 50 days of flowering time. The difference flowering time of the first position between two adjacent fruiting branches is 4–5 days, and the interval of flowering between two adjacent nodes of the same fruiting branch is only 1–2 days, less than with ordinary varieties of 5–7 days (Bednarz and Nichols 2005). This feature which concentrates flowering and boll opening is suitable for cotton harvest by machine.

During flowering, the short fruiting branch trait was observed visually. It was found that X1570 flowered about 8–10 days earlier than Ekangmian-13 when planted in Hainan and Hubei province. An F<sub>2</sub> population comprised of 155 plants through crossing X1570 with Ekangmian-13 was constructed for genetic analysis and locating the *cl* gene with 367 SSR makers coming from Chr\_16 to the reference of CMD (Cotton marker database) by using bulked segregant analysis based on molecular markers.



**Fig. 1** a Ekangmian13 phenotype of opening boll; b X1570 phenotype of opening boll (Oct, 2015)

### DNA and RNA extraction

Cotton genomic DNA was extracted using CTAB method, a cotton genomic rapid extraction method, according to Paterson's CTAB (Hexadecyltrimethyl ammonium bromide) method of 1993 (Paterson et al. 1993). RNA was isolated from young leaves using RNAPrep Pure Plant Kit. DNA was quantified by a Nanodrop 2000 UV-Vis spectrophotometer machine; RNA was quantified by 18 s rRNA and 28 s rRNA in agarose gel electrophoresis.

### Distinguishing SNP locus with dCAPS makers

Derived cleaved amplified polymorphic (dCAPS) is one of the methods to verify SNP loci. It is rare that the SNP is justly located at the restriction enzyme site. By bringing the mismatch base in the amplification primer on the basis of the Cleaved Amplification Polymorphism Sequence (CAPS) marker, a new SNP of the restriction endonuclease site can be formed to result in similar polymorphisms as CAPS markers, which is dCAPS method. SNP sites can be converted to PCR-based molecular markers by CAPS or dCAPS. Application methods and steps are as follows:

First, the dCAPS primer sequence (F: 5'-TTCAACACCAACAAgCAGGT-3'; R: 5'-ggTCACTAggAC-CAggAACAA-3') was designed based on the *Gorai.001-G121800\_ATC* gene contain SNP locus. Polyacrylamide gel electrophoresis was clear without polymorphism.

Second, the PCR production was digested with endonuclease enzymes Mun I in a water bath at 37 °C for 2~4 h. The enzyme digestion system: Mun I 1 µL, 10 × M Buffer 2 µL, 0.1% BSA 2 µL, DNA 3 µL, Sterile water 2 µL.

Third, digested products were subjected to polyacrylamide gel electrophoresis, adding 2 µL of loading buffer, electrophoresed in 8% polyacrylamide gel and 1 × TBE electrophoresis buffer. After electrophoresis was completed, the polyacrylamide gel was stained in 0.1% AgNO<sub>3</sub> solution for 10 min. Then, it was quickly washed twice with purified water and placed in the color reagent for 10 min until the electrophoresis strip was clear. Finally, it was cleaned by running water.

### Quantitative analysis of gene ATC by real-time PCR

According to the results of blasting homologous gene sequence, the primers of *Gorai.001G121800\_ATC* gene located on D07 were designed based on the conserved and specific (no homology with other genes) gene segments. PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real Time) was used for fluorescence quantitative PCR analysis, the primers sequence are as follows:

ATCQ1F: CACCCTGGTGATGACAGACC.  
 ATCQ1R: TGTTTGGCCTTGGCATTTCG.  
 ATCQ2F: AGTGACAGATATCCCCGGCA.  
 ATCQ2R: GCTTCTCACTGTTTGCCTGC.

Internal reference sequence:

actin-F:TCACGGAAGCACCTCTCAAC.  
 actin-R:ACAAAGAGAGAACGGCCTGG.

## Results

### Genetic analysis of the short fruiting branch trait

According to the chi-square test of the two types of branch traits in F<sub>2</sub> population, the segregation corresponded to the Mendelian segregation ratio of 3: 1 ( $\chi^2 = 0.286 < \chi^2(0.05, 1) = 3.84$ ) (Table 1). The result indicated that the trait of short fruiting branch is a pair of recessive genes.

### Validation of SSR markers

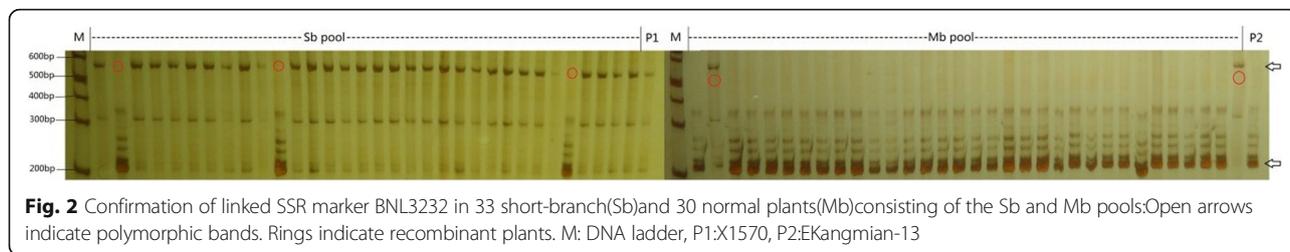
Classical genetic studies have shown that short fruiting branch trait is controlled by the gene originated from Chr\_16 (Hau et al. 1980; Silow 1946) in *G. hirsutum*. BSA was used to rapidly identify 367 SSR markers, which were selected from Chr\_16 of tetraploid cotton linked to the short fruiting branch trait. Thirty-three short fruiting branch plants (referred to as Sb pool) and 33 multiple node-branch plants (referred to as Mb pool) were randomly selected from the F<sub>2</sub> population, and an equal amount of DNA from each plant in each group was mixed to form two pools. The result (Fig. 2) showed that the SSR marker BNL3232 is linked to the short-fruiting branches, and the exchange ratio is only 7.6%. Therefore, it is supposed that gene *Gorai.001-G121800\_ATC* originated from gene *cl1* in upland cotton.

### Analysis of ATC gene expression by RT-PCR

Quantitative PCR results showed that the expression level of *Gorai.001G121800\_ATC* gene was very low, the Cq value of Actin was only 20, and the Cq value of *Gorai.001G121800\_ATC* gene was about 35, which indicated that the abundance of ATC gene was very low, agreeing with previous results reported by Anagnostis Argiriou in 2008 (Argiriou et al. 2008). The expression level of this gene is also different in X1570 and Ekangmian-13 (as shown in Fig. 3), especially during the budding stage. The relative expression of the gene in leaves of Ekangmian-13 was significantly higher than in X1570, which had such little expression of this gene that it could not be detected by equipment. This indicates that the gene plays an important role in determining the type of fruit branch.

**Table 1** Segregation ration of fruiting branch in F<sub>2</sub> population

Traits	Number	$\chi^2$
Normal branch	122	0.286
Short fruiting branch	33	
Total	155	



**Sequence analysis of ATC gene**

The DNA template isolated from parent plants of X1570 and Ekangmian-13 was selected to amplify the genome region between the ATG codon to TGA codon according to the open reading frame(ORF) of the ATC gene. The region contained the exon and intron segments and were respectively connected to the PMD18-T plasmid vector. Then, 6 positive clones selected from both amplified production above were sequenced, for a total of 12. The primers are as follows.

ATCF1 ATGGCAAACACTGTCAGATCCTCT.  
 ATCR1 TTAGCGTCTTCTAGCAGCTGTTTC.

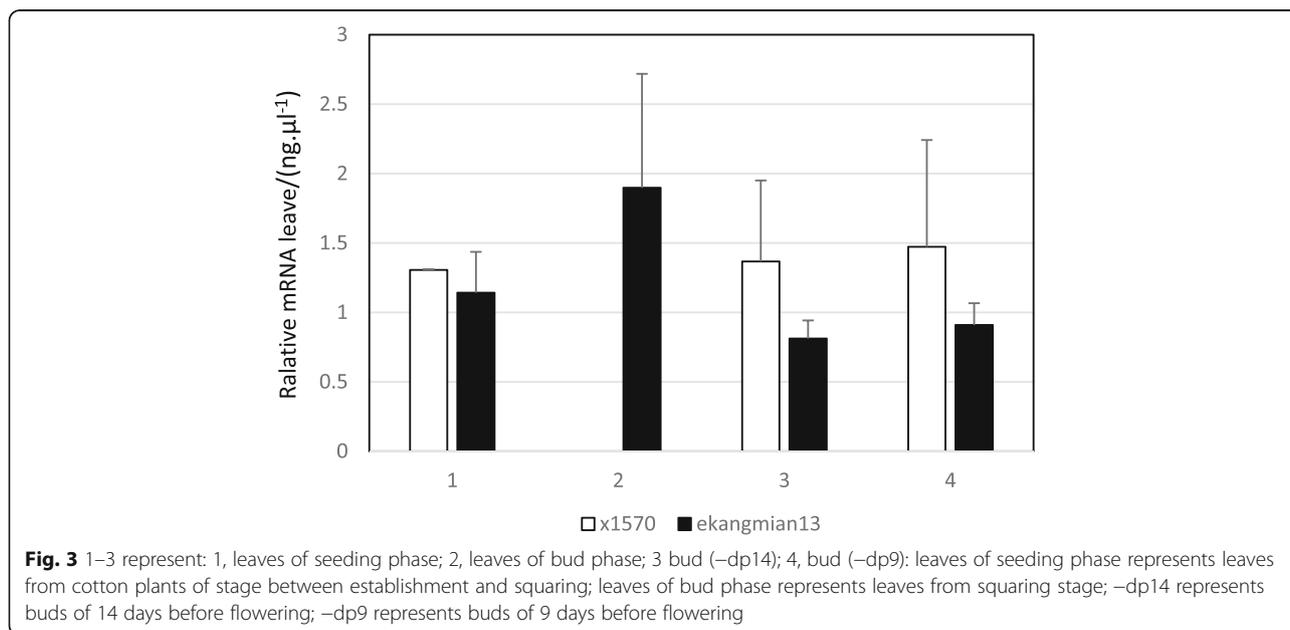
The *Gorai.001G121800\_ATC* gene has a total length of 1052 bp and contains four exons with a total length of 525 bp and three introns with a total length of 527 bp. It encodes 175 amino acids and is homologous to *Arabidopsis thaliana TFL1/ATC*. The 12 pairs of sequences mentioned above was blasted by software DNAMAN. It could be seen that there were five positions where there was a base variation. They were on the 83rd base of the first one exon, 371st base of the second exons, and 935th, 962rd, 992rd base of the fourth exon. However, only the base at position 371 of the second exon had

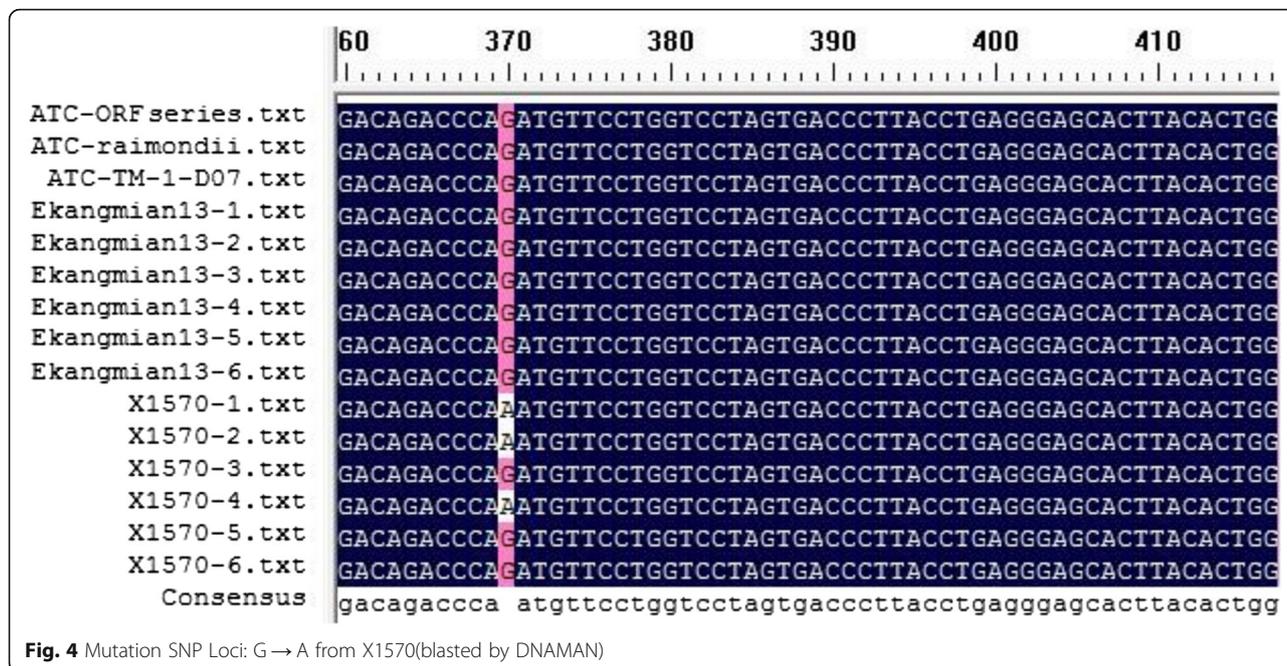
mutations on X1570 and no mutation on Ekangmian-13, and the other four loci had mutations among the three upland cotton varieties. The *Gorai.001G121800\_ATC* gene of 371 base of X1570 was characterized with G transferring A, which resulted in amino acid arginine (*Arg*) transferring lysine (*Lys*). It was guessed that the GA mutation loci from X1570 (shown in Fig. 4) created the short fruiting branch trait.

**Validation SNP marker by dCAPS**

The dCAPS primer sequence (F: 5'-TTCAACACC AACAAgCAGGT-3 ' ; R: 5'-ggTCACTAggACCAggAA CAA-3') was designed based on the *Gorai.001-G121800\_ATC* gene containing mutation *SNP\_GH1570*. The production of PCR can be digested by Mun I enzyme from the *SNP\_GH1570* loci. Polyacrylamide gel electrophoresis was clear without polymorphism.

The results of dCAPS (shown in Fig. 5) indicated that a band restricted by enzyme Mun I at the position of 226 bp appeared in the 30 plants with short fruiting branch randomly selected from F<sub>2</sub> of × 1570 × Ekangmian-13. In contrast,there were no electrophoretic bands at the same position of 226 bp



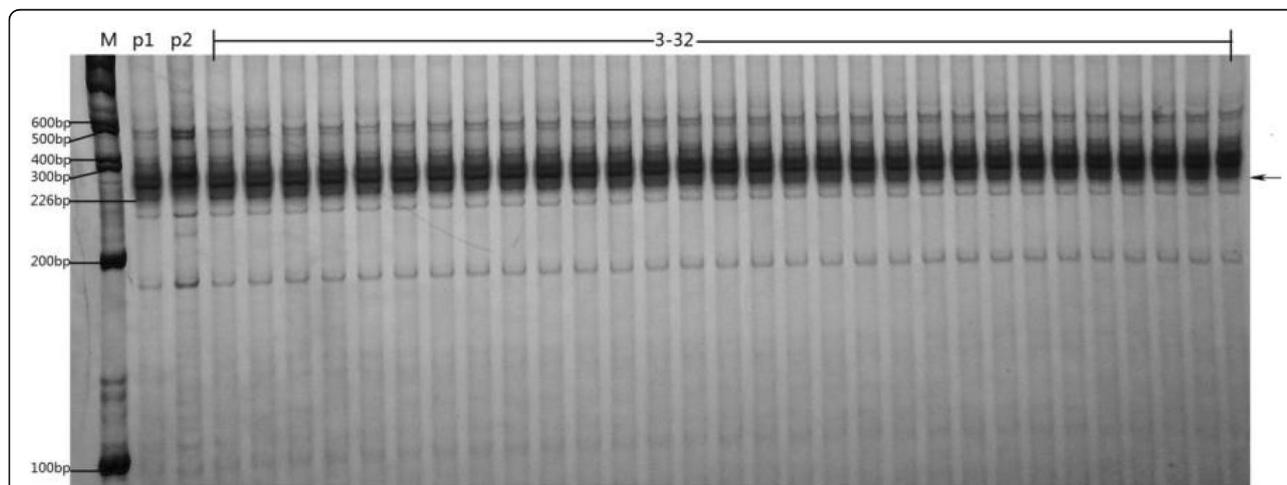


observed in the normal fruiting branch plants. Thus, it was proved that the mutation SNP\_GH1570 was 100% co-segregated with short fruiting branch trait.

**Discussion**

The result of a chi-square test on short fruiting branch trait of F<sub>2</sub> populations in upland cotton indicated that the trait is a quality trait controlled by a recessive gene, the same as classical genetics and the result of Chen (Zhang et al. 2006; Hau et al. 1980; Chen et al. 2014). Because of the relatively narrow kinship and low polymorphism of *G. hirsutum*, only the SSR marker BNL3232 linked to the short fruiting branch trait was

found on chromosome D07 by BSA method with SSR marker selection. There was a significant difference of the expression level of *ATC* gene between two cotton varieties derived from Upland cotton at the budding stage. Further sequence analysis revealed the GA mutation SNP loci of the *ATC* gene from X1570 was 100% co-segregated with short fruiting branch trait by dCAPS marker technique. The SSR markers BNL3232 associated with short fruiting branch traits existed exchanging, which could not be isolated 100% totally. However, that could be achieved by using the SNP markers obtained from the trait-related genes, which was more accurate in breeding selection.



**Fig. 5** Confirmation of co-segregated SNP marker in 32 short fruiting branches M: marker, p1: “X1570” with short fruiting branch, p2: “Ekangmian-13” with normal branch, 3–32: 30 short fruiting branch plants randomly selected from F<sub>2</sub> of × 1570 × Ekangmian-13

This SNP loci can be used for plant architecture selection. Because the short fruiting branch trait was controlled by a single recessive gene, the backcrossing process requires many times of selfing and identification tests to purify the short fruiting branch plants, time-consuming, costing a lot of labor and material. And the normal fruiting branch of plants are susceptible to environmental factors to be mistaken as short fruiting branches, it is difficult to distinguished from the phenotype of difference traits in the breeding process (Song et al. 2005; Dong et al. 2013; Cui 2011; Ai 2012). Therefore, the *SNP\_GH1570* loci was 100% co-segregated with the short fruiting branch traits, which was simple and convenient for molecular marker-assisted selection. The *SNP\_GH1570* loci could be identified accurately from the heterozygous plants in breeding process without interference.

The *ATC* gene may be associated with flowering time traits. The study of Corrinne E in 2015 indicated that inhibition of *ATC* gene expression can promote early flowering of cotton (Grover et al. 2015). In the parents or  $F_2$  population of X1570 and Ekangmian-13, the flowering time of X1570 characterized with short fruiting branch were earlier than that of Ekangmian-13 for 8–10 days. In addition, it was reported that the *ATC* gene is one of the key genes controlling the flowering time, and the gene encodes the TF1 protein. The main function of this protein is to maintain the vegetative growth and the unlimited growth of the inflorescence (Fu et al. 2011). The *Arabidopsis thaliana TFL1* gene inhibits forming flower primarily by inhibiting the expression of the integrating genes *LEAFY (LFY)* of flowering pathway and the flower mesenchymal tissues gene *AP1* and *CAULIFLOWER (CAL)* gene (Conti and Bradley 2007). *TFL1* is another important member of *TF / TFL1* gene family, which inhibits the formation of floral primordia on stem apical meristems and delays the transformation of plant from vegetative growth to reproductive growth (Hanzawa et al. 2007). Therefore, *Gorai.001G121800* both controls short fruiting branch trait and flowering time. That similar phenomenon had been reported in previous research. Overexpression of *PtFT* gene in *Prunus salicina* not only resulted in early flowering, but also changed the plant architecture and increased the proliferation of nulliplex-branches (Srinivasan et al. 2012). Overexpression of *AtFT* gene in cotton mediated by virus could change the shape of leaves which made the palmate leaves transfer lanceolate leaves, meanwhile shorten the internodes of cotton, reduced the amount of fruit branches and axons, make the fruit more concentrated. Those changes altered plant architecture but didn't affected development of flower organ (McGarry et al. 2013).

Furthermore, it was found that the plants characterized with short fruiting branch in the  $F_2$  and  $F_{2:3}$  population exhibited early flowering, had a concentrated boll opening

period, and a shortened growth period. To be brief, the short fruiting branch trait was associated with early flowering and maturity traits, consistent with the characteristics of the X1570 parent line. Although those agronomic traits caused thin and weak plants at seedling stage, they didn't reduce yield, which means that the advanced maturity of short fruiting branch varieties can play an important role in the breeding of conventional and early mature varieties. Early maturity is one of the important traits for cotton cultivation following wheat or rape in a double cropping system, and fruit branch type is a determinant of plant architecture and maturity in cotton. The short fruiting branch trait improves early maturity, encourages increased planting density, and improves light energy utilization in high density cropping (Dong et al. 2011). Moreover, it is necessary to transfer the short fruiting branch trait to high-yield varieties because of its advantage, such as high planting density, without demand of pruning, suitable for picking up cotton by machine, and so on. This integration can improve the current limitations associated with plants with the short fruiting branch trait, including low lint percentage, low yield and short fiber length (Li 2012). And plants that are homozygous for the short branch trait can be identified rapidly, a benefit from these traits being controlled by a single recessive gene, which can shorten the breeding period greatly. Consequently, it is expected that appropriate varieties suitable for Yangtze River can be achieved through backcrossing the short fruiting branch mutant X1570 with high-yield, high-quality, disease-resistant materials. To achieve the purpose of simple and high-efficient planting cotton by reducing investment without reducing yield, this short fruiting branch trait mutant characterized with short growth period can be used to shorten the cotton growing season to reduce labor cost and fertilizer inputs.

## Conclusion

The  $F_2$  segregate population was constructed by using the X1570 crossed with Ekangmian-13 to analyze the short fruiting branch gene and marker-assisted selection with SNP linked to its trait. The result demonstrated that linked SSR marker BNL3232 was screened by BSA method; one *SNP\_GH1570* locus was found, which was totally separated from the fruiting branches trait in upland cotton. It was verified that this *SNP\_GH1570* was 100% co-segregated with short fruiting branch trait, which could be used for molecular assisted selection of cotton architecture in upland cotton.

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### Availability of data and materials

All authors must include an “Availability of Data and Materials” section in their manuscript detailing where the data supporting their findings can be found.

### Authors’ contributions

Zhang YC carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. Feng CH participated in the sequence alignment. Qin HD, Bie S participated in the design of the study and performed the statistical analysis. Wang XG, Zhang JH, Xia SB conceived of the study and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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