Journal of Cotton Research

RESEARCH

Open Access

Identification of SSR markers linked to the abscission of cotton boll traits and mining germplasm in Cotton

SHUI Guangling[1†], LIN Hairong[1†], MA Xiaomei[2], ZHU Bo[3], HAN Peng[1], AINI Nurimanguli[4], GUO Chunping[1], WU Yuanlong[1], PAN Zhenyuan[1], YOU Chunyuan[5], SONG Guoli[6*] and NIE Xinhui[1*]

Abstract

Background Cotton is an economically important crop. It is crucial to find an effective method to improve cotton yield, and one approach is to decrease the abscission of cotton bolls and buds. However, the lack of knowledge of the genetic and molecular mechanisms underlying cotton boll abscission traits has hindered genetic improvements.

Results Pearson's correlation analysis revealed a significant positive correlation between boll abscission rates 1 (AR1) and boll abscission rates 2 (AR2). A genome-wide association study was conducted on 145 loci that exhibited high polymorphism and were uniformly distributed across 26 chromosomes (pair). The study revealed 18, 46, and 62 markers that were significantly associated with boll abscission, fiber quality, and yield traits ($P < 0.05$), explaining 1.75%–7.13%, 1.16%–9.58%, and 1.40%–5.44% of the phenotypic variation, respectively. Notably, the marker MON_SHIN-1584b was associated with the cotton boll abscission trait, whereas MON_CGR5732a was associated with cotton boll abscission and fiber quality traits. Thirteen of the marker loci identified in this study had been previously reported. Based on phenotypic effects, six typical cultivars with elite alleles related to cotton boll abscission, fiber quality, and yield traits were identified. These cultivars hold great promise for widespread utilization in breeding programs.

Conclusions These results lay the foundation for understanding the molecular regulatory mechanism of cotton boll abscission and provide data for the future improvement of cotton breeding.

Keywords SSR, Genome wide association studies, Abscission, Favorable alleles, Cotton, Genetic improvement

[†]Shui Guangling and Lin Hairong contributed equally to this work.

*Correspondence:
Song Guoli
sglzms@163.com
Nie Xinhui
xjnxh2004130@126.com
[1] Key Laboratory of Oasis Ecology Agricultural of Xinjiang Bingtuan, Agricultural College, Shihezi University, Shihezi, Xinjiang 832003, China
[2] Cotton Institute, Xinjiang Academy of Agriculture and Reclamation Science, Shihezi, Xinjiang 832000, China
[3] Agricultural Science Research Institute of the 5th Division of Xinjiang Production and Construction Corps, Shuanghe, Xinjiang 833408, China
[4] Modern Agricultural College, Kashi University, Kashi, Xinjiang 844000, China
[5] National Key Laboratory of Crop Genetic Improvement, College of Plant Sciences & Technology, Huazhong Agricultural University, Wuhan, Hubei 430070, China
[6] National Key Laboratory of Cotton Bio-Breeding and Integrated Utilization, Institute of Cotton Research, Chinese Academy of Agricultural Sciences, Anyang, Henan 455000, China

Background

Cotton is one of the leading economic crops, providing the most important natural textile fiber, oil, and biofuel worldwide (Cheng et al., 2020). China is one of the world's largest producers, consumers, and importers of cotton. The National Bureau of Statistics of China reported that China produced 5.98 million tons of cotton in 2022. Currently, Xinjiang is the main cotton-producing area in China and produces an annual average of 5.39 million tons of cotton, representing 90% of the total value of cotton crops (http://data.stats.gov.cn). The critical period for cotton yield is the flowering and bolling stage, which accounts for 58.7%–60.4% of the total cotton growth period (Wang et al., 2017). Furthermore, these stages are the peak risk periods for the abscission of flower buds and cotton bolls (Guinn et al., 1990). Abscission is a premature aging symptom during cotton growth and includes flower bud abscission before flowering and cotton boll abscission after flowering. It is a physiological phenomenon and complex trait that is influenced by multiple factors, including genetic and non-genetic factors such as weather events. In production, the abscission rate is generally between 60%–70%; however, when it exceeds 80%, the cotton yield declines (Yu et al., 1999; Zhang et al., 2021a, Zhang et al., 2021b). Therefore, reducing the abscission rate of flower buds and cotton bolls is a primary goal to improve cotton yield. Many studies have shown that the abscission rate varies among different cultivars. In upland cotton, the abscission rate of flower buds is significantly higher than that of cotton bolls, and abscission occurs more frequently on the central fruit branch (Wang et al., 2021). A comparison of four different sea island cotton varieties showed that the abscission rate of high-quality varieties was higher than that of high-yield varieties (Hu et al., 2020). The abscission rate in sea island cotton was 50%, with the abscission rate of flower buds higher than that of bolls. Varieties with high abscission rates were found to have low yields (Zhang et al., 2007).

Abscission refers to the detachment of leaves, branches, flowers, fruits, and other organs from the parent plant, and the separation layer is called the abscission zone (AZ). During abscission, cell enlargement begins and activates gene expression, increasing the activity of cell wall-degrading enzymes, including cellulase, polygalacturonases, and pectin methyl esterase; ultimately leading to the detachment of the organ (Li et al., 2015). Several studies have suggested that organ shedding depends on the activation of the AZ (Patharkar et al., 2018). In the abscission layer, the INFLORESCENCE DEFICIENT IN ABSCISSION (IDA) peptide functions as a ligand of the receptor-like kinases HAESA and HAESA-LIKE2 (HAE/HSL2), which are presumed to activate the mitogen-activated protein kinase (MAPK) cascade that includes MKK4/5 (MAPKK) and MPK3/6 (MAPK). The MADS-domain transcription factor AGAMOUS like-15, KNAT1, and KNAT2/6 participate in *Arabidopsis* floral shedding. EVERSHED, CAST AWAY, NEVERSHED, and somatic embryogenesis receptor kinase1 are thought to negatively regulate IDA and play a role in floral organ abscission in *Arabidopsis* (Glazinska et al., 2017).

Many studies have shown that ethylene and auxins play an important role in the abscission of leaves, flowers, young fruits, and mature fruits and that the endogenous ethylene content increases before organ abscission (Goldental-Cohen et al., 2017). LcEIL2/3 is involved in the regulation of ethylene biosynthesis and cell wall remodeling during litchi fruit ripening by inducing ethylene production which results in the activation of fruit abscission (Ma et al., 2020). The expression of genes related to expansins, pectinases, and cellulases is induced by the interaction between ethylene and auxin, which leads to the separation of an organ from the plant. The abscission of plant organs is associated with changes in the auxin gradient across the AZ and is affected by ethylene (Glazinska et al., 2017). Abscission is accompanied by a series of physiological responses, including the activity of cell wall-degrading enzymes and polygalacturonases (Du et al., 2014). In *Arabidopsis*, flower organ abscission is dependent on the IDA precursor protein, and the IDA-HAE/HSL2 pathway activates downstream mitogen-activated protein (MAP) kinase cascades (Meng et al., 2016). The transcription factor AGAMOUS-like 15 binds to the HAE promoter, resulting in the delayed abscission of floral organs in *Arabidopsis* (Rahul et al., 2015). Previous studies have shown that exogenous application of gibberellic acid induces the translationally controlled tumor protein, which is widely expressed in the roots and stems of cotton, leading to the abscission of cotton bolls (Zhang, 2018). Other studies showed that the *BOP1* promotes the abscission layer to stimulate leaf shedding (Chang et al., 2015). Transcriptome analysis of separation layer formation between leaves and petioles in cotton identified eight miRNAs exclusively involved in flower abscission (Guo et al., 2018). In addition, cytokinins and ethylene can induce cotton leaf abscission (Xu et al., 2019).

In this study, 145 polymorphic simple sequence repeat (SSR) markers were selected, and genetic diversity analysis was performed among a population containing 238 varieties. The phenotypes of flower bud and cotton boll abscission traits in four different environments were determined. Furthermore, allelic variants associated with abscission and typical carrier cultivars with elite alleles were identified using genome-wide association studies (GWAS). Our results provide a basis for understanding

the regulatory mechanism of cotton boll abscission and for advancing molecular breeding in cotton, potentially leading to improved cotton yield through molecular breeding.

Materials and methods

Plant materials and field experiments

To evaluate the abscission traits, a total of 178 *Gossypium hirsutum* and 60 *G. barbadense* cultivars were collected from the Institute of Cotton Research Center of Shihezi Academy of Agricultural Sciences, Shihezi, Xinjiang, China (Table S1) and planted in two locations in Shihezi (SHZ) and Kuerle (KEL), Xinjiang, China during 2018 and 2019. A randomized block design including two replicates was used, with consistent chemical control, drip irrigation with plastic mulch, and regular field management. Experimental plots consisted of two rows, 2.5 m long each, with a plant-to-plant spacing of 0.1 m within a row and row spacing of (0.66 + 0.10) m.

Phenotype investigation and statistical analysis

To determine the phenotype of the abscission traits, ten consecutive cotton plants were labeled in the middle of each line. Ten days after flowering, the number of boll abscissions and non-abscissions was investigated, and the abscission rate was calculated as the boll abscission rate 1 (AR1). At the full boll stage, a second survey of the boll abscission rate was conducted, denoted as the boll abscission rate 2 (AR2). AR1 and AR2 are calculated in the same manner as the rate of boll abscission (AR). AR was calculated according to the formula AR (%) = The number of boll abscissions/total number of bolls × 100%. The study also included additional measurements during the flowering period (FP), from seedling to flowering, and the entire growth period (WGP), from seedling to boll opening. During the cotton boll opening period, 20 spontaneously opened bolls were collected from the middle parts of the plants from each replicate for each accession. To measure cotton fiber yield, the seed cotton weight (seed plus fuzz, SW) and lint weight (LW) of each cotton sample were measured, and the lint percentage (LP) was calculated as follows: LP (%) = LW (g) /SW (g) × 100%. During the cotton boll opening period, lint samples derived from 20 normally opened bolls of each variety were collected, and the fiber quality was determined using the HVI 1000 Automatic Fiber Determination System (Uster Technologies, Switzerland), including the fiber upper half mean length (FUHML), fiber strength (FS), and micronaire value (MV).

The variance, normality statistical analyses, and basic statistics, including extreme values, mean value, standard deviation, coefficient of variation, and statistics for the correlation between traits were performed using SPSS v22.0 software (IBM Corp., Armonk, NY, USA). Correlation analyses and boxplots between environments were drawn using R software (Guo et al., 2021).

Population structure

The structural analysis was conducted using Structure v2.3.4 with an admixture model. To obtain the optimal K value, the K value that maximized the statistic ΔK was calculated as described by Pritchard et al. (2000). The number of clusters was set from 1 to 10, and 10 independent runs were conducted for each value of K. The parameters used were as follows: length of burn-in period of 10 000, the number of MCMC steps after burn-in of 100 000, and the number of populations assumed to be 2–10.

Genetic diversity analysis and GWAS

The SSR primers used in this study were obtained from the interspecific maps of *G. barbadense* and *G. hirsutum* cultivars constructed by State Key Laboratory of Crop Genetic Improvement at Huazhong Agricultural University, Wuhan, China (Li et al., 2012). From these, we chose 75 SSR primer pairs that exhibited polymorphism among the different varieties for the association analysis. According to the method of Muktar et al. (2019), polymorphic information content (PIC) was calculated using PowerMarker v3.25 (http://statgen.ncsu.edu/powermarker/downloads.htm). The association between phenotypic and genotypic traits was detected using TASSEL v2.1 software with MLM (G + P + Q + K) models (Bradbury et al., 2007).

Results

Phenotype analysis of cotton boll abscission among 238 cotton accessions

To evaluate the cotton boll abscission traits in the natural population, the AR1, AR2, FP, and WGP were determined in four environments. Phenotype analysis of the four traits showed that the coefficient of variation of FP was minimal, ranging from 2.56%–6.07%, and the largest coefficient of variation was found in AR1, ranging from 19.27%–63.79% (Table 1). This indicated that the AR1 trait was more influenced by environmental factors. A wide range of phenotypic variation was observed across the 238 accessions in four environments. AR1, AR2, and WGP varied from 0.43%–63.30%, 11.67%–74.94%, and 111.50–159.50 d, respectively (Table 1). The absolute values of skewness and kurtosis for most of the cotton boll abscission traits were less than 1.00, except for AR1 of 2018 KEL and WGP of the 2019 KEL. The boxplot

Table 1 Statistics of cotton the abscission of cotton boll traits in cotton

Trait	Env	Min	Max	Mean	SD	CV%	Skewness	Kurtosis	H^2
AR1 /%	2018KEL	2.11	31.32	10.45	4.58	43.83	0.90	1.73	0.6
	2018SHZ	0.43	41.45	14.72	9.39	63.79	0.58	-0.57	
	2019KEL	21.22	63.30	41.03	7.91	19.27	0.12	-0.43	
	2019SHZ	1.65	34.68	13.51	6.73	49.81	0.71	0.27	
AR2 /%	2018KEL	23.01	73.89	43.31	9.25	21.36	0.50	0.38	0.6
	2018SHZ	11.67	58.65	33.74	10.87	32.22	0.53	-0.49	
	2019KEL	35.15	74.94	55.54	6.81	12.26	-0.12	-0.04	
	2019SHZ	15.64	60.17	38.91	7.94	20.41	0.01	-0.07	
FP /d	2018KEL	77.85	88.65	83.85	2.15	2.56	-0.27	-0.40	0.75
	2018SHZ	66.25	85.90	77.77	4.72	6.07	-0.58	-0.65	
	2019KEL	69.00	80.00	73.22	2.24	3.06	-0.58	-0.09	
	2019SHZ	72.80	91.00	82.17	4.09	4.98	-0.53	-0.67	
WGP /d	2018SHZ	132.55	148.00	140.37	3.64	2.59	0.41	-0.39	0.32
	2019KEL	111.50	145.00	132.04	4.65	3.52	-0.39	1.28	
	2019SHZ	135.55	159.50	147.40	4.54	3.08	0.30	-0.05	

AR1 rates of boll abscission 1, *AR2* rates of boll abscission 2, *FP* flowering period, *WGP* whole growth period, *CV* coefficient of variation, H^2 heritability (broad sense)

analysis showed that the changes in AR2 were relatively stable between years, and AR2 in KEL was higher than that in SHZ. A large phenotypic variation in the abscission traits was found between different growing sites. In addition, FP and WGP were more stable than AR1 and AR2 in all four environments (Fig. 1). Correlation analysis showed that yield-related traits were positively correlated with each other, whereas there was no significant correlation with AR1. A positive relationship was found between SW and FP ($P<0.01$) and between LW and FP ($P<0.01$) (Fig. 2). There was a positive correlation between FUHML and FS, whereas there was a significant negative correlation between MV and FUHML, and MV and FS. These results provide a solid foundation for the identification of pleiotropic loci through GWAS.

Diversity analysis of molecular markers

To identify the efficiency and polymorphism of the markers, an analysis was performed using 75 SSR primer pairs with 145 loci (Li et al., 2012). The results showed that the 145 loci amplified clear fragments in each of the 238 accessions, which suggested that these SSR markers had high reproducibility, abundant polymorphisms, and uniform distribution on the 26 cotton chromosomes (pairs). There was an average of 2.8 SSR primer pairs per chromosome, ranging from 1–5, and the average distance

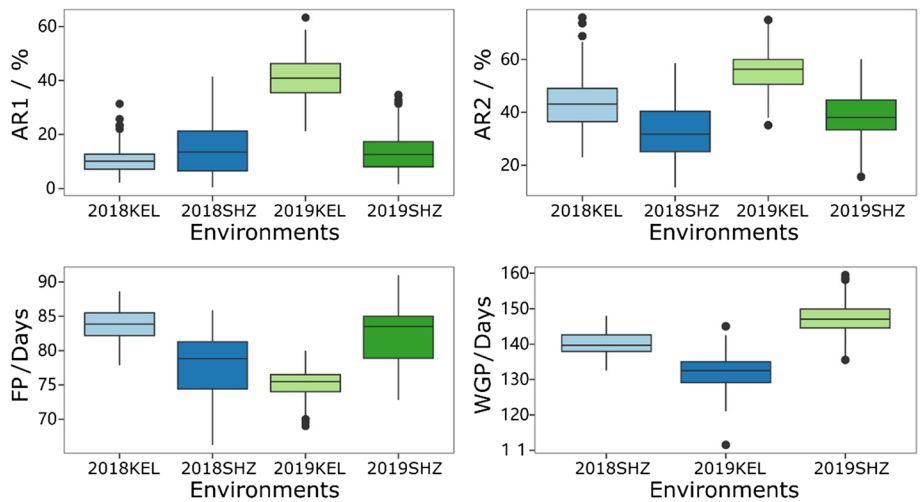

Fig. 1 Boxplots for cotton boll abscission traits in the four environments

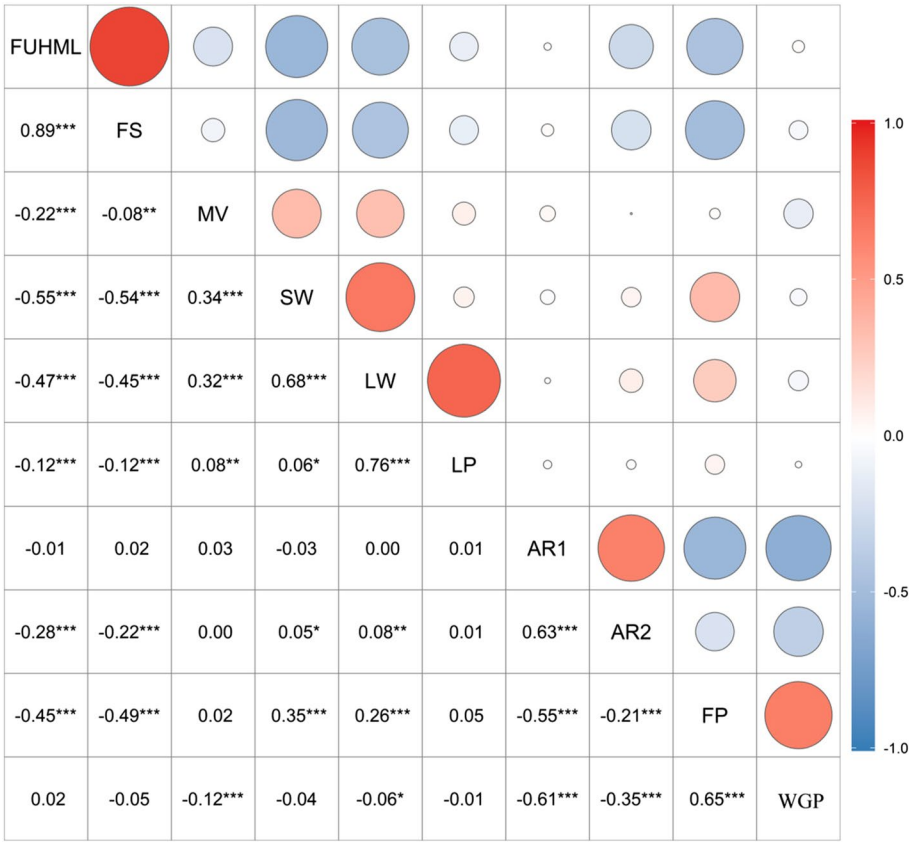

Fig. 2 The correlation of cotton boll abscission and other traits in the four environments. FUHML, fiber upper half mean length; FS, fiber strength; MV, micronaire value; SW, seed cotton weight; LW, lint weight; LP, lint percentage; AR1, rate of boll abscission 1; AR2, rate of boll abscission 2; FP, flowering period; and WGP, whole growth period. *** represents significant correlation at $P < 0.01$ level, ** significant correlation at $P < 0.05$ level, * significant correlation at $P < 0.1$ level

between the markers was 31.68 cM (centimorgan). The PIC at each locus ranged from 0.022–0.521, with an average of 0.345 (Table S2). NAU3774b was highly polymorphic and located on chromosome D13. The results indicate that these SSR markers had abundant variants in the population.

Population genetic structure analysis

Population structure analyses based on the 145 polymorphic markers were performed (Fig. 3). The LnP (D) values gradually increased as the K-value increased from 1 to 10, and ΔK based on rate of change of LnP (K) between successive K values, and a sharp peak of ΔK was observed at K = 2 (Fig. 3), indicating that the 238 cotton cultivars could be divided into two subpopulations. The maximum likelihood method was used to assign each cotton cultivar to a cluster. Subcluster-1 contained 174 varieties, including 39.08% of the Xinluzao series (normally with middle to long growth period), 43.10% of the Xinluzhong series (normally with early maturation), 2.30% of the Xinhai series (sea-island cultivars), and 15.52% of the Xincai series (naturally color cotton); and subcluster-2 contained 62 varieties, including 95.16% of the Xinhai series and 4.84% of the Xinluzhong series.

GWAS for the cotton boll abscission traits

Association analysis was performed on the phenotypes of 238 cultivars from the four environments with 145 SSR loci (Fig. S1). Eighteen loci associated with cotton boll abscission were identified across two or more environments, and the phenotypic variance explained by these loci ranged from 1.75%–7.13%, with an average of 3.61% (Table S3). Two SSR loci, MON_SHIN-1584b and NBRI_HQ526730b, associated with AR1 were identified, and the phenotypic variance explained by these loci was 3.02% and 3.13%, respectively. Four SSR loci associated with AR2 were identified, with phenotypic variance explained rates varying from 2.79%–6.24% and an average of 4.22%. Of these, NAU3419c showed the highest and

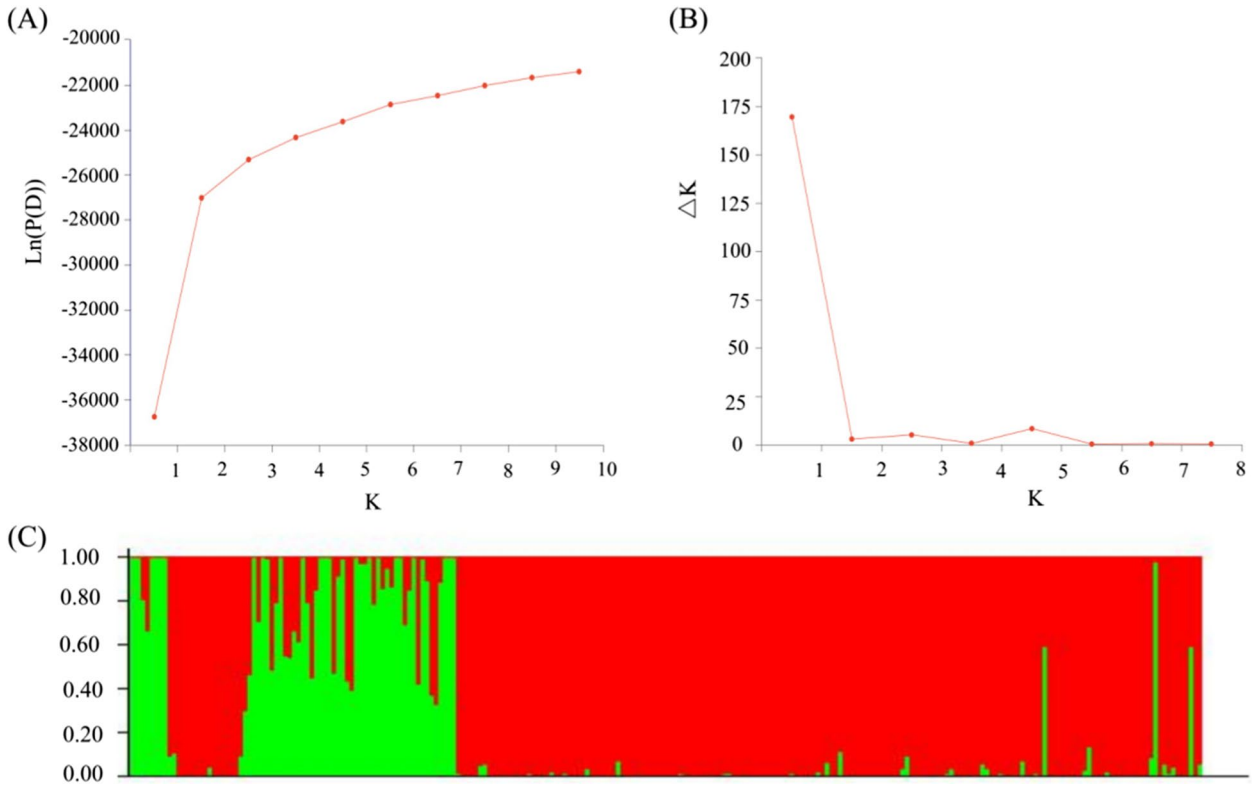

Fig. 3 Population structure analysis. **A** Ln(P(D)) for K ranging 1 to 10; **B** Lines chart showing Evanno's DK. The number of identified sub-populations was two (K = 2); **C** Population genetic structure of cotton cultivars at K = 2, based on inferred ancestry (Q matrix)

MON_SHIN-1584b exhibited the lowest rate of phenotypic variation explained. Eight SSR loci associated with FP were detected, and the range of phenotypic variance explained rates was 1.75%–6.59%, and the mean value was 3.18%. MON_DPL0504aa exhibited the highest and HAU1968a exhibited the lowest rate of phenotypic variation explained. Four SSR loci associated with WGP were detected, and the range of phenotypic variance explained rates was 2.56%–7.13%, with a mean value was 4.13%. HAU2588b showed the highest and HAU1952bb exhibited the lowest rate of phenotypic variation explained.

GWAS for fiber quality and yield traits

To investigate the effect of the cotton boll abscission traits on cotton fiber quality and yield, an association analysis was conducted (Fig. S1). Forty-six loci were found to be associated with fiber quality traits, with phenotypic variance explained rates ranging from 1.16%–9.58% and a mean value of 3.66%. Among them, 18 SSR loci (phenotypic variance explained rates was 1.35%–4.10%), 15 SSR loci (phenotypic variance explained rates was 1.16%–4.10%), and 13 SSR loci (phenotypic variance explained rates was 2.71%–9.58%) were associated with FUHML, FS, and MV, respectively (Table S3). NAU3827b (FUHML), HAU1952bc (FS), and HAU2588a (MV) were the loci with the highest rates of phenotypic variation explained. Gh330c (FUHML), BNL2535ba (FS), and MON_CGR6012b (MV) were the loci with the lowest phenotypic variation explained rates. Sixty-two loci associated with yield traits were identified (Fig. S1), with phenotypic variance explained rates ranging from 1.40%–5.44% and a mean value of 2.87%. Among these, 18 SSR loci (phenotypic variance explained rates was 1.48%–4.06%), 30 SSR loci (phenotypic variance explained rates was 1.40%–4.25%), and 14 SSR loci (phenotypic variance explained rates was 1.78%–5.44%) were associated with SW, LW, and LP, respectively (Table S3). MON_DPL0504ab (SW and LW) and HAU2588a (LP) were the loci with the highest phenotypic variation explained rates. MON_CGR5007b, HAU2846c, and HAU1968a exhibited the lowest rates of phenotypic variation. The results of GWAS were compared with those reported in previous studies (Table 2). Thirteen marker loci from our study had been identified in previous studies, seven of which were associated with the previously reported phenotypes. The remaining six loci showed differences in their associated phenotypes, suggesting that they may be newly discovered loci that require further

Table 2 Markers associated with multiple effect traits

Locus-Allele	Chr	BP	Trait	Other research traits
MON_CGR5732a	Chr05	155	AR2, WGP, MV	LP, FS (Xia et al., 2014; Shao et al., 2014)
MON_CGR6012b	Chr05	155	AR2, MV	FUHML (Shao et al., 2014)
BNL3043a	Chr19	296	LW	MV (Liu et al., 2013)
NAU2126a	Chr19	186	FUHML, FS	LW, FUHML (Shao et al., 2014, Lin et al., 2009)
NAU2126b	Chr19	178	FUHML, FS, LW, SW	LW, FUHML (Shao et al., 2014, Lin et al., 2009)
NAU3346ba	Chr15	305	FUHML, FS	FE (Han 2013)
NAU3346bb	Chr15	289	FUHML, FS	FE (Han 2013)
NAU3827a	Chr18	149	FS, LW, SW	LW (Yu et al., 2013)
NAU3827b	Chr18	136	FUHML, FS, LW, SW	LW (Yu et al., 2013)
MUSS422aa	Chr01	207	MV, LP, LW	MV (Han 2013)
MUSS422ab	Chr01	191	MV, LP, LW	MV (Han 2013)
DPL0062	Chr21	137	LW, SW	MV (Wang et al., 2015)
NAU3298a	Chr25	385	LW, SW	FS, SW (Sun et al., 2012; Zhao 2016)

FUHML fiber upper half mean length, *FS* fiber strength, *MV* micronaire value, *SW* seed cotton weight, *LW* lint weight, *LP* lint percentage, *AR1* rate of boll abscission 1, *AR2* rate of boll abscission 2, *FP* flowering period, *WGP* whole growth period, *BP* basic pair

investigation. These SSR markers could be used to comprehensively improve traits related to cotton boll abscission, fiber quality, and yield.

Identification of favorable alleles and typical materials for cotton boll abscission traits

Based on the effect values of allelic variant loci, six typical materials (Xinluzhong 36, Xincai 12, Xinhai 63, Xinhai 37, Xinluzao 30, and Xinluzhong 26) associated with the abscission of cotton boll traits, were identified (Table 3). MON_SHIN-1584b and NBRI_HQ526730b were associated with AR1. In Xinluzhong 36, MON_SHIN-1584b had a negative phenotypic effect (–0.16%), whereas NBRI_HQ526730b had a positive phenotypic effect (0.52%).

Four allelic loci, MON_SHIN-1584b, MON_CGR5732a, MON_CGR6012b, and NAU3419c, were associated with AR2. Among them, MON_SHIN-1584b had a negative phenotypic effect (–0.60%) in Xinhai 63. MON_CGR5732a, MON_CGR6012b, and NAU3419c were found in Xincai12; however, the phenotypic effects of each locus were different. MON_CGR5732a (0.16%) and MON_CGR6012b (0.68%) had a positive phenotypic effect, whereas NAU3419c (–0.50%) had a negative phenotypic effect.

Eight allelic loci were associated with FP, including HAU1952bc, HAU1968a, MON_DPL0504aa, and MON_DPL0893a, had a positive phenotypic effects. The typical carrier cultivar for the polymerization of the four alleles was Xinhai 37. HAU1952bc (0.73 d) was the allelic variation locus with the greatest positive phenotypic effect. The typical carrier cultivar for the HAU3071aa and NAU5172b alleles was Xinluzao 30. HAU3071aa (0.07 d) was the locus with a positive phenotypic effect. The typical carrier cultivar for the NAU3774a and NAU3774b alleles was Xinluzhong 26. NAU3774a (0.14 d) was the locus with a positive phenotypic effect. Of the four loci associated with WGP, HAU2588b (–1.17 d) and HAU1952bb (–0.26 d) showed the maximum negative phenotypic effect, whereas MON_CGR5732b (0.66 d) and MON_CGR5732a (1.91 d) had a positive phenotypic effect. The typical carrier cultivar for the polymerization of the four alleles was Xinluzao 30.

This study identified seven excellent allelic loci, MON_CGR5732b, MON_Shin-1584b, MON_CGR5732a, DPL0062, MGHES31a, NAU2126b, and HAU2588b (Table S4). A total of 12 typical carrier cultivars (four materials had a positive phenotypic effect and seven materials had a negative phenotypic effect) were identified by those loci. Two allelic loci (MON_CGR5732b and MON_SHIN-1584b) were associated with AR1 and AR2, and were identiifed in a total of five typical carrier cultivars (Xinluzao 28, Xinluzhong 1, Xinluzhong 28, Xinluzhong 35, and Xinluzhong 86). Xinluzao 28, Xinluzhong 1, Xinluzhong 28, and Xinluzhong 86 showed a negative phenotypic effect. The two allelic loci associated with FP and WGP were MON_CGR5732a and DPL0062, respectively. These loci were identified in three typical carrier cultivars (Xinluzao 30, Xinhai 37, and Xinluzhong 26), all of which exhibited positive phenotypic effects. The loci associated with the yield and quality traits were DPL0062, MGHES31a, HAU2588b, MON_CGR5732a, and NAU2126b. These loci were identified in four typical cultivars, three of which (Xinhai 9, Xinhai 12, and Xincai 3) exhibited negative phenotypic effects, and only Xinluzao 81 showed a positive phenotypic effect.

Table 3 Allelic variation of loci significantly associated with the cotton boll abscission traits and their corresponding phenotypic effects

Traits	Locus-Allele	Phenotypic effect	Typical materials
AR1 /%	MON_SHIN-1584b	–0.16	Xinluzhong 36
	NBRI_HQ526730b	0.52	Xinluzhong 36
AR2 /%	MON_CGR5732a	0.16	Xincai 12
	MON_CGR6012b	0.68	Xincai 12
	NAU3419c	–0.50	Xincai 12
	MON_SHIN-1584b	–0.60	Xinhai 63
FP /d	HAU1952bc	0.73	Xinhai 37
	HAU1968a	0.28	Xinhai 37
	MON_DPL0504aa	0.60	Xinhai 37
	MON_DPL0893a	0.05	Xinhai 37
	HAU3071aa	0.07	Xinluzao 30
	NAU5172b	–0.34	Xinluzao 30
	NAU3774a	0.14	Xinluzhong 26
	NAU3774b	–0.35	Xinluzhong 26
WGP /d	HAU2588b	–1.17	Xinluzao 30
	HAU1952bb	–0.26	Xinluzao 30
	MON_CGR5732b	0.66	Xinluzao 30
	MON_CGR5732a	1.91	Xinluzao 30

These twelve typical carrier cultivars contained multiple favorable allele loci and possess high breeding value, with the potential to decrease the abscission rate of cotton buds and bolls and also enhance cotton fiber yield and quality.

Discussion

The abscission of cotton flower buds and bolls is a quantitative trait controlled by micro-effect polygenes and is the result of the interaction between genotype and environmental factors. Previous studies have indicated that the abscission of flower buds and cotton bolls is caused by disasters, extreme weather events, and the cotton species, ultimately leading to serious losses in yield and quality (Goldental-Cohen et al., 2017; Cheng et al., 2020). In this study, the phenotypes of flower bud and cotton boll abscission traits in 238 Xinjiang cotton varieties across various environments were compared and analyzed. The average coefficient of variation of the traits ranged 3.06%–44.18%. This indicates that the cultivars used in this study had abundant genetic variation among the four boll abscission traits. Correlation analysis results showed that FP was positively correlated with SW and LW, while negatively correlated with AR, which is consistent with previous studies (Li et al., 2021, 2018; Su et al., 2016). The early maturation of cotton can lead to an increase in both weight of seed cotton and lint per boll, but it may also reduce the boll-setting rate. Therefore, it is important to find a balance between cotton yield per boll and the overall yield. When cultivating early maturing cotton, this balance could be achieved by increasing the planting density and the number of cotton bolls to further enhance cotton yield. FP, WGP, AR1, and AR2 can be used as important references to measure the cotton yield, and a comprehensive evaluation of cotton yield traits could be performed based on these indicators. This approach potentially improves cotton flower buds and boll shedding, ensures cotton yield and quality, and improves cotton economic benefits. Based on above considerations, this study used these four cotton boll abscission traits, in addition to three yield and three quality traits, to carry out a GWAS and mined elite allelic loci which have a significant influence on cotton breeding.

In recent years, GWAS have been extensively used to dissect the natural allelic variation responsible for complex quantitative traits in crops (Wang et al., 2019; Liu et al., 2020; Zhang et al., 2020). A large number of genomic loci and candidate genes have been identified for fiber development (Thyssen et al., 2019; Zhao et al., 2022), yield formation (Shen et al., 2019; Lei et al., 2020), and early maturity-related traits (Zhang et al., 2021a). However, fewer loci have been identified for flower bud or cotton boll abscission traits. In this study, we identified seven loci that were associated with not only cotton boll abscission traits but also fiber quality and yield traits (Table S3). These loci were divided into three categories. The first category included loci that were significantly associated with the boll abscission rate. MON_SHIN-1584b was significantly correlated with AR1 (explaining 3.02% of the phenotypic variation) and AR2 (explaining 3.38% of the phenotypic variation). The second category consisted of loci that were significantly associated with both AR2 and MV. MON_CGR5732a and MON_CGR6012b were significantly correlated with AR2 (explaining 4.46% and 2.79% of the phenotypic variation, respectively) and MV (explaining 4.30% and 2.71% of the phenotypic variation, respectively). The third category included HAU1952bc, HAU1968a, MON_DPL0504aa, and NAU5172b which were significantly correlated with FP, FUHML, SW, and LW. The explained phenotypic variation ranged from 1.84%–6.59%. We hypothesized that these seven loci exhibit pleiotropy and may control multiple highly correlated traits. Additionally, we identified six carrier cultivars related to boll abscission based on multi-effect marker loci.

The marker loci ($P < 0.05$) associated with the four cotton boll abscission traits in this study were

compared with other reported QTLs in cotton. Thirteen marker loci identified in our study were consistent with those identified in previous studies, seven of which were associated with the same traits. Six loci were related to the boll abscission rate, but only two (MON_CGR5732a and MON_CGR6012b) were co-located with other traits. Previous studies have shown that MON_CGR5732a, located on chromosome 5, is associated with LP and FS with a high explanation rate for phenotypic variation. MON_CGR6012b, located on chromosome 12, is associated with FUHML and has a high explanation rate for phenotypic variation. These results demonstrate that these markers can be detected in multiple populations, however, this locus was only associated with yield and quality traits in previous studies.

Traditional crop breeding has the disadvantages of a long cycle time and low efficiency, for cotton boll abscission traits are controlled by multiple genes. In this study, we applied GWAS to identify SSR loci that are closely associated with cotton boll abscission traits which will be beneficial for molecular marker-assisted selection (MAS) and will accelerate future breeding progress. Most of the associated loci in this study have not been previously reported. This may be because of the low level of marker coincidence and the greater influence of the environment and cultivar on some QTLs. Therefore, different results have been detected under different analysis conditions. In the future, associated regions can be delineated according to the results, and high-density SNP markers can be developed in associated regions. Combining this information with functional gene annotation would allow the identification of marker loci closely linked to target traits, which can be applied to the assisted selection of related traits to accelerate the process of variety breeding. In addition, it provides a reference for fine mapping and candidate gene mining of QTLs for yield and important agronomic traits in the future.

Conclusion

To identify related markers and allelic variation in the cotton boll abscission traits, an association analysis was performed using 238 cotton cultivars in China based on 145 SSR markers. Seven SSR markers were consistently identified in this study and previous reports, suggesting their genetic stability and significant potential for further research to quantify their impact on traits. Our study provides valuable information for MAS in cotton breeding, provides new resources for yield improvement in future cotton breeding efforts, and offers a foundation for fine gene mapping.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42397-024-00180-3.

Additional file 1: Supplementary Table S1. Information for the 238 cotton accessions. **Supplementary Table S2.** The polymorphism information content of the SSRs. **Supplementary Table S3.** SSR loci associated with the abscission of cotton boll traits, yield-related traits, and fiber quality-related traits. **Supplementary Table S4.** The phenotypic effect of typical cultivars. **Supplementary Fig. S1.** Manhattan plot for the abscission of cotton bolls traits, yield and fiber quality related traits

Acknowledgements
We would like to thank the anonymous reviewers for their valuable comments and helpful suggestions; these have greatly helped us improve the quality of the manuscript.

Authors' contributions
Nie XH, Song GL, and You CY designed the experiments. Nurimanguli A, Guo CP, Ma XM, and Zhu B performed the experiments. Shui GL wrote the main manuscript and prepared all the figures. Lin HR and Hang P performed the data analysis. Wu YL and Pan ZY revised and polished the manuscript. All authors contributed to the interpretation of results and have read and approved the final manuscript.

Funding
This work was sponsored by the State Key Laboratory of Cotton Biology Open Fund (CB2022A11), the National Natural Science Foundation of China (32260510), the Innovation talent Program in Sciences and Technologies of Xinjiang Production and Construction Corps, China (2021CB028), the Key Programs for Science and Technology Development of Shihezi city, Xinjiang Production and Construction Crops, China (2022NY01), the Science and Technology Planning of Shuanghe city, Xinjiang Production and Construction Crops, China (2021NY02), and the key programs for science and technology development in agricultural field of Xinjiang Production and Construction Corps, China.

Availability of data and materials
The datasets used and analyzed in the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 16 November 2023 Accepted: 28 April 2024
Published: 5 June 2024

References

Bradbury PJ, Zhang Z, Kroon DE, et al. TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics. 2007;23(19):2633–5. https://doi.org/10.1093/bioinformatics/btm308.

Chang J, Jin X, Pang JH, et al. Cloning of abscission zone development gene of *Gossypium hirsutum* L. *GhBOP1* and preliminary analysis of its function. Acta Agriculturae Boreali-Sinica. 2015;30(3):14–9. https://doi.org/10.7668/hbnxb.2015.03.003. (in Chinese with English abstract).

Cheng G, Zhang L, Wei H, et al. Transcriptome analysis reveals a gene expression pattern associated with fuzz fiber initiation induced by high

temperature in *gossypium barbadense*. Genes. 2020;11(9):1066. https://doi.org/10.3390/genes11091066.
Du M, Li Y, Tian X, et al. The phytotoxin coronatine induces abscission-related gene expression and boll ripening during defoliation of cotton. PLoS One. 2014;9(5): e97652. https://doi.org/10.1371/journal.pone.0097652.
Glazinska P, Wojciechowski W, Kulasek M, et al. *De novo* transcriptome profiling of flowers, flower pedicels and pods of *lupinus luteus* (yellow lupine) reveals complex expression changes during organ abscission. Front Plant Sci. 2017;8:641. https://doi.org/10.3389/fpls.2017.00641.
Goldental-Cohen S, Burstein C, Biton I, et al. Ethephon induced oxidative stress in the olive leaf abscission zone enables development of a selective abscission compound. BMC Plant Biol. 2017;17(1):87. https://doi.org/10.1186/s12870-017-1035-1.
Guinn G, Dunlap JR, Brummett DL. Influence of water deficits on the abscisic acid and indole-3-acetic acid contents of cotton flower buds and flowers. Plant Physiol. 1990;93(3):1117–20. https://doi.org/10.1104/pp.93.3.1117.
Guo N, Zhang Y, Sun X, et al. Genome-wide identification of differentially expressed miRNAs induced by ethephon treatment in abscission layer cells of cotton (*Gossypium hirsutum*). Gene. 2018;676:263–8. https://doi.org/10.1016/j.gene.2018.08.057.
Guo CP, Pan ZY, You CY, et al. Association mapping and domestication analysis to dissect genetic improvement process of upland cotton yield-related traits in China. J Cotton Res. 2021;4:10. https://doi.org/10.1186/s42397-021-00087-3.
Han J. QTL Mapping of Fiber Quality and Yield Components in Ultra High Strength Upland Cotton. Nanjing Agricultural University; 2013. https://doi.org/CNKI:CDMD:2.1014.216852. (in Chinese with English abstract).
Hu KM, Li XT, Bai YL, et al. Study on the dynamics of the abscission of square and boll in cotton (*Gossypium barbadense* L.) varieties. China Cotton. 2020;47(7):20–6. https://doi.org/10.11963/1000-632X.hkmlx.20200714. (in Chinese).
Lei JJ, Shao PX, Guo CP, et al. Genetic dissection of allelic loci associated with economic traits of upland cottons in Xinjiang. Cotton Sci. 2020;32(3):185–98. https://doi.org/10.11963/1002-7807.ljjnxh.20200509. (in Chinese with English abstract).
Li X, Yuan D, Wang H, et al. Increasing cotton genome coverage with polymorphic SSRs as revealed by SSCP. Genome. 2012;55(6):459–70. https://doi.org/10.1139/g2012-032.
Li C, Wang Y, Ying P, et al. Genome-wide digital transcript analysis of putative fruitlet abscission related genes regulated by ethephon in litchi. Front Plant Sci. 2015;6:502. https://doi.org/10.3389/fpls.2015.00502.
Li C, Wang Y, Ai N, et al. A genome-wide association study of early-maturation traits in upland cotton based on the CottonSNP80K array. J Integr Plant Biol. 2018;60(10):970–85. https://doi.org/10.1111/jipb.12673.
Li L, Zhang C, Huang J, et al. Genomic analyses reveal the genetic basis of early maturity and identification of loci and candidate genes in upland cotton (*Gossypium hirsutum* L.). Plant Biotechnol J. 2021;19(1):109–23. https://doi.org/10.1111/pbi.13446.
Lin ZX, Feng CH, Guo XP, et al. Genetic analysis of major QTLs and epistasis interaction for yield and fiber quality in upland cotton. Sci Agric Sin. 2009;42(9):3036–47. https://doi.org/10.3864/j.issn.0578-1752.2009.09.004. (in Chinese with English abstract).
Liu XF, Li JW, Yu XK, et al. Identification of QTL for cottonseed oil and protein content in upland cotton (*Gossypium hirsutum* L.) based on a RIL population. Mol Plant Breed. 2013;11(5):520–8. https://doi.org/10.3969/mpb.011.000520. (in Chinese with English abstract).
Liu S, Li C, Wang H, et al. Mapping regulatory variants controlling gene expression in drought response and tolerance in maize. Genome Biol. 2020;21(1):163. https://doi.org/10.1186/s13059-020-02069-1.
Ma X, Yuan Y, Wu Q, et al. LcEIL2/3 are involved in fruitlet abscission via activating genes related to ethylene biosynthesis and cell wall remodeling in litchi. Plant J. 2020;103(4):1338–50. https://doi.org/10.1111/tpj.14804.
Meng XZ, Zhou JG, Tang J, et al. Ligand-induced receptor-like kinase complex regulates floral organ abscission in *Arabidopsis*. Cell Rep. 2016;14(6):1330–8. https://doi.org/10.1016/j.celrep.2016.01.023.
Muktar MS, Teshome A, Hanson J, et al. Genotyping by sequencing provides new insights into the diversity of Napier grass (*Cenchrus purpureus*) and reveals variation in genome-wide LD patterns between collections. Sci Rep. 2019;9(1):6936. https://doi.org/10.1038/s41598-019-43406-0.
Patharkar OR, Walker JC. Advances in abscission signaling. J Exp Bot. 2018;69(4):733–40. https://doi.org/10.1093/jxb/erx256.
Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000;155:945–59. https://doi.org/10.1093/genetics/155.2.945.
Rahul O, Patharkar CJ, Walker. Floral organ abscission is regulated by a positive feedback loop. Proc Natl Acad Sci USA. 2015;112(9):2906–11. https://doi.org/10.1073/pnas.1423595112.
Shao QS, Zhang FJ, Tang SY, et al. Identifying QTL for fiber quality traits with three upland cotton (*Gossypium hirsutum* L.) populations. Euphytica. 2014;198(1):43–58. https://doi.org/10.1007/s10681-014-1082-8.
Shen C, Wang N, Huang C, et al. Population genomics reveals a fine-scale recombination landscape for genetic improvement of cotton. Plant J. 2019;99(3):494–505. https://doi.org/10.1111/tpj.14339.
Su J, Pang C, Wei H, et al. Identification of favorable SNP alleles and candidate genes for traits related to early maturity via GWAS in upland cotton. BMC Genomics. 2016;17:687. https://doi.org/10.1186/s12864-016-2875-z.
Sun FD, Zhang JH, Wang SF, et al. QTL mapping for fiber quality traits across multiple generations and environments in upland cotton. Mol Breed. 2012;30(1):569–82. https://doi.org/10.3389/fpls.2018.01364.
Thyssen GN, Jenkins JN, McCarty JC, et al. Whole genome sequencing of a MAGIC population identified genomic loci and candidate genes for major fiber quality traits in upland cotton (*Gossypium hirsutum* L.). Theor Appl Genet. 2019;132(4):989–99. https://doi.org/10.1007/s00122-018-3254-8.
Wang H, Huang C, Guo H, et al. QTL mapping for fiber and yield traits in upland cotton under multiple environments. PLoS ONE. 2015;10(6): e0130742. https://doi.org/10.1371/journal.pone.0130742.
Wang X, Deng Z, Zhang W, et al. Effect of waterlogging duration at different growth stages on the growth, yield and quality of cotton. PLoS ONE. 2017;12(1): e0169029. https://doi.org/10.1371/journal.pone.0169029.
Wang C, Tang S, Zhan Q, et al. Dissecting a heterotic gene through Graded-Pool-Seq mapping informs a rice-improvement strategy. Nat Commun. 2019;10(1):2982. https://doi.org/10.1038/s41467-019-11017-y.
Wang Q, Chen H, Han Y, et al. Effect of spatial-temporal light competition on cotton yield and yield distribution. Agronomy. 2021;11:2346. https://doi.org/10.3390/agronomy11112346.
Xia Z, Zhang X, Liu YY, et al. Major gene identification and quantitative trait locus mapping for yield related traits in upland cotton (*Gossypium hirsutum* L.). J Integr Agric. 2014;13(2):299–309. https://doi.org/10.1016/S2095-3119(13)60508-0.
Xu J, Chen L, Sun H, et al. Crosstalk between cytokinin and ethylene signaling pathways regulates leaf abscission in cotton in response to chemical defoliants. J Exp Bot. 2019;70(5):1525–38. https://doi.org/10.1093/jxb/erz036.
Yu J, Zhang K, Li S, et al. Mapping quantitative trait loci for lint yield and fiber quality across environments in a *Gossypium hirsutum* × *Gossypium barbadense* backcross inbred line population. Theor Appl Genet. 2013;126(1):275–87. https://doi.org/10.1007/s00122-012-1980-x.
Yu Y, Cheng GW, Lin H, et al. Research on regulation of the square and boll abscission in Xinjiang cotton area. J Xinjiang Agric Univ. 1999;22(1):29–33. (in Chinese).
Zhang SQ. Cloning and expression analysis of genes related to abscission of cotton boll abscission layer. J South Agric. 2018;49(5):832–8. https://doi.org/10.3969/j.issn.2095-1191.2018.05.02. (in Chinese with English abstract).
Zhang P, Guo G, Wu Q, et al. Identification and fine mapping of spot blotch (*Bipolaris sorokiniana*) resistance gene *Sb4* in wheat. Theor Appl Genet. 2020;133(8):2451–9. https://doi.org/10.1007/s00122-020-03610-3.
Zhang N, Tian L, Feng L, et al. Boll characteristics and yield of cotton in relation to the canopy microclimate under varying plant densities in an arid area. Peer J. 2021b;9:e12111. https://doi.org/10.7717/peerj.12111.
Zhang J, Jia X, Guo X, et al. QTL and candidate gene identification of the node of the first fruiting branch (NFFB) by QTL-seq in upland cotton (*Gossypium hirsutum* L.). BMC Genomics. 2021a;22(1):882. https://doi.org/10.1186/s12864-021-08164-2.
Zhang SR, Baidengsha MMTAL, Ning XM, et al. Study on the dynamics of the abscission of square and boll in cotton (*Gossypium barbadense* L .) in South Xinjiang. Xinjiang Agric Sci. 2007;44(2):115–21. https://doi.org/10.3969/j.issn.1001-4330.2007.02.002. (in Chinese with English abstract).

Zhao SQ. Analysis on the major gene and multigene mixed inheritance and QTL mapping for early maturity traits in upland cotton. Chinese Academy of Agricultural Sciences. 2016. https://doi.org/10.3969/j.issn.201600501. (in Chinese with English abstract)
Zhao H, Chen Y, Liu J, et al. Recent advances and future perspectives in early-maturing cotton research. New Phytol. 2022;237(4):1100–14. https://doi.org/10.1111/nph.18611.