RESEARCH NOTE

Mapping of genes for flower-related traits and QTLs for flowering time in an interspecific population of *Gossypium hirsutum* × *G. darwinii*

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Introduction

Gossypium darwinii possesses favourable genes but are unavailable in cultivated cotton, that are difficult to directly transfer into cultivated cotton by conventional breeding due to interspecific incompatibility. Molecular map will provide the foundation for the genetic dissection of important traits and facilitate utilization in breeding by marker-assisted selection (MAS). However, only one molecular map from a cross between *G. hirsutum* and *G. darwinii* has been reported by Chen (2013), while no morphological traits or quantitative trait loci (QTLs) were mapped on it. Here, we produced and reported the first single sequence repeat (SSR) (PCR-based) genetic backbone-map and mapped genes for flower-related traits (Y_1^a on chr A13; *R2* on A7; P_1 on A5; L^o on D1) and three QTLs for flowering time (FT) on chrs D1 and D11, which would greatly enhance the use of *G. darwinii*-specific desirable genes in future cotton breeding programmes.

Materials and methods

Plant materials and DNA extraction

Here, 181 F_2 population that produced from a cross between *G. hirsutum* (TM-1) and *G. darwinii* were used. The former has been self-pollinated more than 60 times, recognized as genetic standard line (figure 1a in electronic supplementary material at http://www.ias.ac.in/jgenet/) and nonphotoperiod sensitivity (day-neutral), whereas the latter, a wild tetraploid species, possesses many distinctive traits (figure 1b in electronic supplementary material) and photoperiod sensitivity (undesirable trait). Total genomic DNA was extracted from young leaves as per Paterson *et al.* (1993) with some modifications.

SSR analysis, PCR amplification and electrophoresis

A total of 630 SSR markers with an average intermarker distance of 5–10 cM, evenly spaced across 26 chromosomes of upland cotton were selected based on an earlier linkage map (Zhao *et al.* 2012). All SSR primer information can be obtained from http://www.cottonmarker.org. SSR-PCR amplifications were performed and electrophoresis of the products was run as described by Zhang *et al.* (2000).

Phenotypic analysis

F_2 individuals were investigated for morphological traits. Yellow petal, yellow pollen, petal with red spot and okra leaf that were very similar to the paternal parent, *G. darwinii* and F_1 were scored as present (1). Cream petal, cream pollen, no petal with red spot and normal leaf that were similar to the maternal parent, TM-1 was scored as absent (0). The FT (no. of days from planting to the first flower blooming of each F_2 individuals) was recorded to evaluate photoperiod sensitivity.

Genotyping analysis and testing for segregation distortion

All 630 SSR primer pairs were first used to screen polymorphisms between the two parents. Polymorphic markers were then used to survey 181 individuals of the F_2 population. All distinctive and unambiguous polymorphic bands were scored as 1 or 0. Missing data were noted as '–'. At each locus, *G. hirsutum* is denoted as A and *G. darwinii* is denoted as B. The expected allelic ratio of F_2 was 1 : 1 (A : B). Expected genotypic ratio was 1 : 2 : 1 (AA : AB : BB) for codominant markers or 3 : 1 (dominant : recessive) for dominant markers in the F_2 population. The observed ratios for each marker were tested for deviation from the expected values with a χ^2

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goodness-of-fit test ($P < 0.05$). A region with at least three adjacent loci showing significant segregation distortion was defined as the segregation distorted region (Paillard *et al.* 2003).

Construction of genetic linkage map and location of traits

JoinMap 3.0 (Van Ooijen and Voorrips 2001) was used to construct a complete linkage map. A logarithm of odds (LOD) threshold of 4.0 and a 50 cM maximal distance were used to determine all linkage groups, the order of groups on the same chromosomes was arranged in a line. The resulting linkage map was drawn using MapChart ver. 2.2 software (Voorrips 2002). The major QTLs and their effects were determined with WinQTLCart2.5 software (Wang *et al.* 2005) using the composite interval mapping (CIM) method. QTLs with LOD values between 2.0 and 3.0 were defined as suggestive QTLs (Lander and Kruglyak 1995), and QTLs

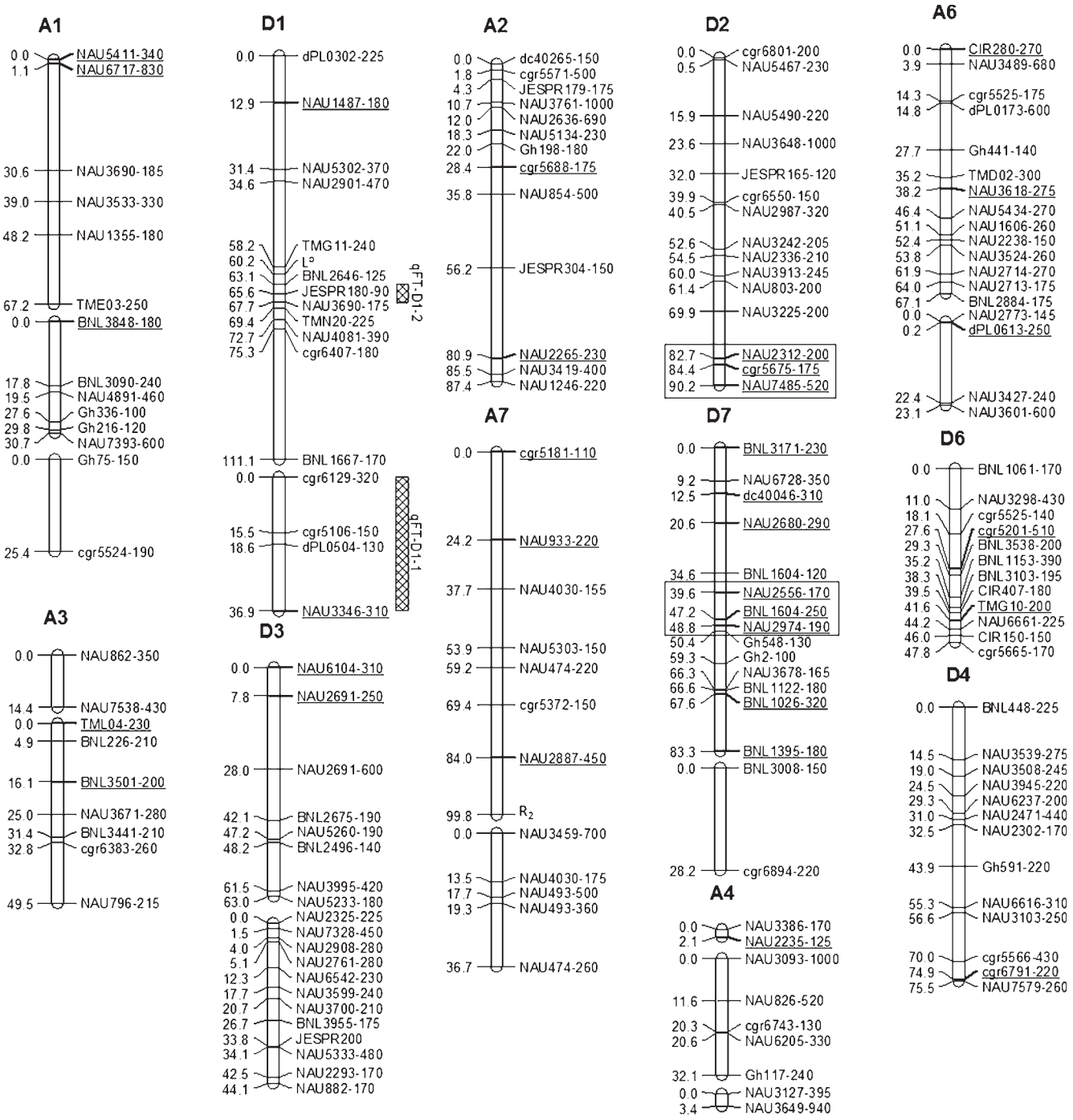

Figure 1. (*continues*)

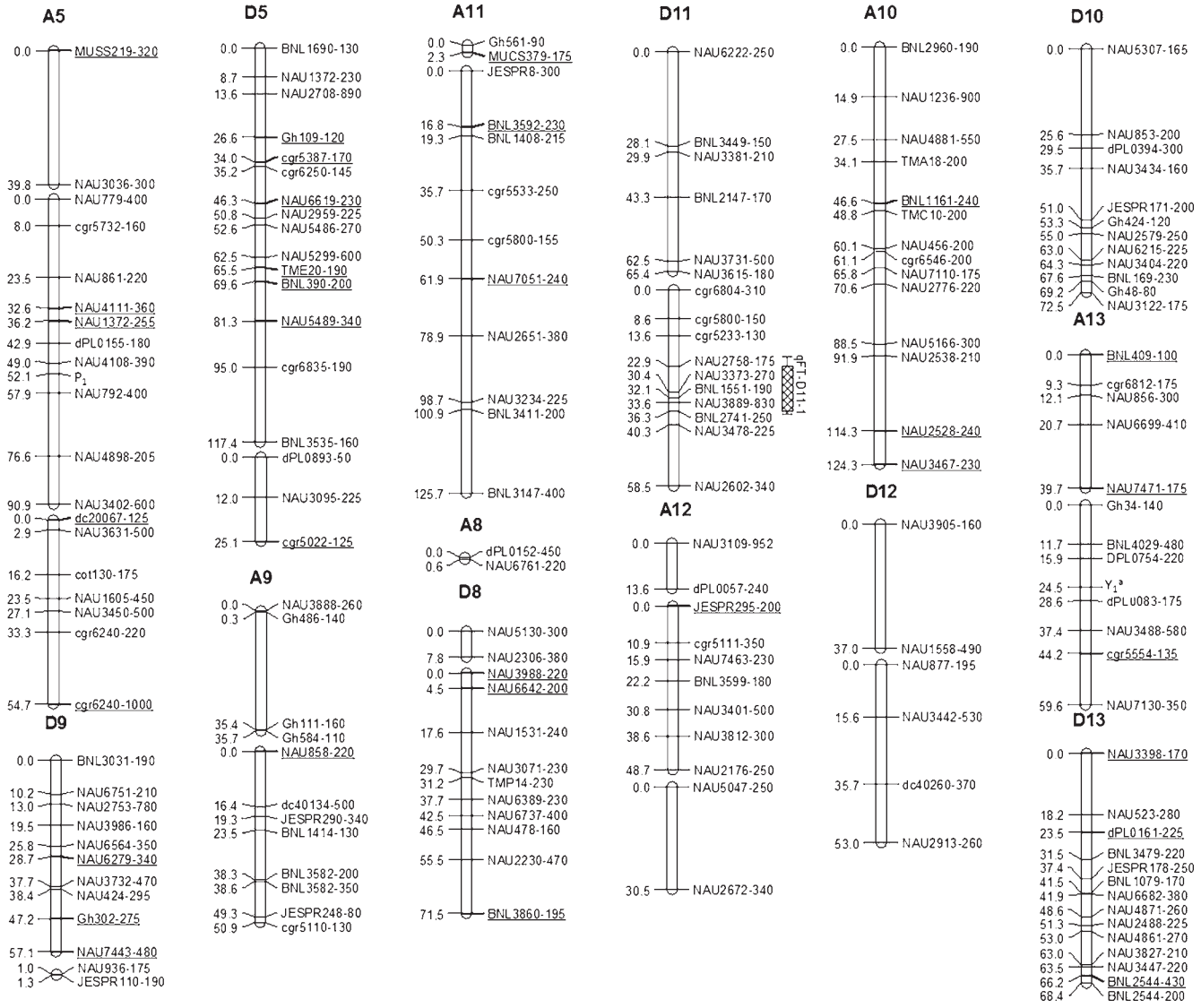

Figure 1. Mapping of discrete characters in interspecific F_2 population. Distorted loci are underlined and SDRs are framed.

with LOD values not less than the threshold value (calculated by a permutation test with 1000 repeats) were defined as significant QTLs (Churchill and Doerge 1994). The name of each QTL includes a 'q' followed by an abbreviation of the trait name, the chromosome or linkage group and a serial number to distinguish different QTLs of the same trait on the same chromosome. Linkage groups were assigned to chromosomes based on anchored markers in the dense linkage map (Zhao *et al.* 2012).

Results and discussion

Construction of genetic linkage map with SSR markers

The results indicated that 388 markers (61.59%) revealed polymorphism between the two parents, yielding 400 polymorphic loci in F_2 individuals. Among the above 400 loci, there were 91 loci dominant, of which 31 loci (7.75%) carried TM-1 (*G. hirsutum*) allele and 60 loci (15.00%) carried *G. darwinii* allele, whereas 309 loci were codominant. Genetic linkage map was constructed using JoinMap 3.0, 342 loci including four morphological traits were mapped on 26 chromosomes of cotton at a LOD $\geq$ 4, including 48 linkage groups. The resultant recombination length reached to 2470.49 cM covering ~55.52% of the cotton genome, based on estimates of the map distance of 4450 cM cotton genome (Rong *et al.* 2004), and the average distance of 7.22 cM between the adjacent markers. There are a total of 160 loci on A genome covering 1255.72 cM with the average distance 7.85 cM and 182 loci on D genome covering 1214.77 cM with the average distance 6.66 cM (table 1 and figure 1 in electronic supplementary material). The density of marker varies between chromosomes ranging from 2.20 (A8) to 15.00 cM (D12), which largely benefits from the greater

number of loci on the D genome. There are 20 markers on chrs A5 and D3, whose polymorphic loci are the most among the 26 chromosomes of cotton. Chromosome A8 had only few markers with only two polymorphic loci. Chr. A5 has the greatest length, covering 185.40 cM, compared with the shortest distance of 2.20 cM of chr. A8. The largest gap between two adjacent loci was 39.7 cM on chr. A5. The number of intervals remaining in the tetraploid map >10 cM is 89, which are almost evenly distributed on the A and D genomes (41 and 48 respectively).

There were 158 (46.20%) eSSR loci, 71 were mapped on A genome and 87 on D genome, with a ratio of A : D = 1 : 1.2. The remaining 184 (53.80%) gSSR loci were mapped, of which 86 were located on A genome and 98 on D genome with a ratio of A : D = 1 : 1.1. Moreover, a few more SSR loci were distributed on the D genome than on the A genome, which suggests that the D genome has a slightly higher DNA sequence divergence rate between tetraploid cotton species than the A genome. There were 85 loci (21.2%) which showed segregation distortion, of which 64 loci (19.1%) located on the map were detected. Twenty-nine distorted segregation loci (19.5%) were on A genome, 14 of them distorted to TM-1, six to *G. darwinii* and nine distorted to F_1. Thirty-five distorted segregation loci (18.8%) were on D genome, 15 loci were distorted to TM-1, eight loci were distorted to *G. darwinii* and 12 were distorted to F_1. The separation of loci ratio on A and D genomes was 1 : 1.2 (table 2 in electronic supplementary material). Only two SDRs were found on two D genome chromosomes, indicating that the distorted loci identified in this study were distributed slightly uneven on the cotton genome. However, the number of SDRs was far lower in this study than that of linkage map of *G. hirsutum* and *G. tomentosum* reported by Hou *et al.* (2013).

Mapping of FT and flower-related traits

Stephens (1954b) reported that the yellow corolla in *G. darwinii* was determined by gene Y_2 which is located on chr. D13 (chr. 18), that was different from Y_1 located on A genome in *G. barbadense*. Here, the segregation ratio for yellow corolla : cream corolla fits a ratio of 3 : 1 ($\chi_c^2 = 0.0902 < \chi_{0.05,1}{}^2 = 3.841$). The yellow corolla is located in the central region of chr. A13, 4.1 cM from the nearest locus, dPL0083-175 and 8.6 cM from dPL0754-220, implying that the gene conferring yellow corolla might be a multiple allele to Y_1 from *G. barbadense*, designated here as Y_1^a to distinguish from Y_1.

In the F_2, 119 individuals had red spot (previously named as R_2) like the parent *G. darwinii* and 62 exhibited no red spot like the maternal parent *G. hirsutum*. We calculated the χ_c^2 value to test whether the segregation ratio for red spot : nonred spot fits a ratio of 3 : 1 or not. The resultant χ_c^2 value was 7.7808 ($P < 0.01$), implying that the ratio does not fit 3 : 1. However, we found that the ratio for red spot : nonred spot extraordinarily approached to 2 : 1 (119 : 62) and further calculated the χ^2 value based on the ratio of 2 : 1. The χ_c^2 value is 0.034 ($P = 80$–90%), implying that the segregation pattern fits a 2 : 1 ratio. We speculated that the viability of gametes carrying the R_2 gene or resultant R_2R_2 zygotes might be low, leading to distorted segregation in the F_2 and a decrease in the rate of the petal with red spot phenotype. Using the software JoinMap 3.0, R_2 is located in the bottom region of chr. A7, the nearest locus, NAU2887-450, consistent with previous reports (Harland 1929; Stephens 1974).

In the F_2, 137 individuals showed yellow pollen (previously named as P_1) like the parent *G. darwinii* and 44 exhibited cream pollen like the maternal parent *G. hirsutum*. The segregation ratio for yellow pollen : cream pollen fits a ratio of 3 : 1 ($\chi_c^2 = 0.0166 < \chi_{0.05,1}{}^2 = 3.841$). P_1 is located in the central region of chr. A5, 3.1 cM from the nearest locus, NAU4106-390 and 5.8 cM from NAU792-400, supporting the previous reports (Stephens 1954a).

In the F_2, 132 individuals showed okra leaf like the parent *G. darwinii* and 49 exhibited normal like the maternal parent *G. hirsutum*. The segregation ratio for okra leaf : normal leaf fits the ratio of 3 : 1 ($\chi_c^2 = 0.3112 < \chi_{0.05,1}{}^2 = 3.841$). Okra leaf ($L^o$) is located in the central region of chr. D1, 2.0 cM from the nearest marker, TMG11-240 and 2.9 cM from BNL2646-125, similar to classical genetic analyses (Endrizzi and Kohel 1966). Andres *et al.* (2014) also identified markers linked to the leaf shape locus on chr. D1 using an intraspecific *G. hirsutum* population.

Flowering is often affected by photoperiod sensitivity. In F_2 population, the minimum number of days to first flower blooming is 89 and the maximum is 136. The average number of days is 115.86 in F_2 population, 90 days for the maternal parent TM-1, but no flowering in growth period for the paternal parent *G. darwinii*. Three significant QTLs for FT were detected, which explained 0.48–11.68% of phenotype variation. Two QTLs, *qFT-D1-2* and *qFT-D11-1*, contributed positive additive effects from TM-1, while the other (*qFT-D1-1*) contributed positive additive effect from *G. darwinii* (table 1; figure 1). In the model plants *Arabidopsis* and rice,

Table 1. Summary of main-effect QTLs for FT.

QTL	Position (cM)	Flanking markers (99%)	LOD value	LOD threshold	Additive effect	Dominant effect	PV (%)	Origin
qFT-D1-2	63.11	BNL2646-125–NAU3690-175	2.74	2.72	−1.08	−4.84	0.48	TM-1
qFT-D1-1	18.71	cgr6129-320–NAU3346-310	2.76	2.72	−3.04	−2.81	1.20	*G. darwinii*
qFT-D11-1	30.41	NAU2758-175–BNL2741-250	3.73	2.72	3.70	−2.71	11.68	TM-1

PV, phenotype variation.

great advances have been made in understanding the photoperiod pathways which control flowering in response to seasonal changes in day length and temperature (Hayama and Coupland 2004). However, studies on FT in cotton are limited and it would facilitate to select early maturity cotton varieties by MAS.

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References

Andres R. J., Bowman D. T., Kaur B. and Kuraparthy V. 2014 Mapping and genomic targeting of the major leaf shape gene (L) in Upland cotton (*Gossypium hirsutum* L.) *Theor. Appl. Genet.* **127**, 167–177.

Chen H. D. 2013 Transcriptome sequencing under drought stress, EST-SSR development and high density genetic linkage map construction in *Gossypium darwinii*. Ph.D. dissertation, Chinese Academy of Agricultural Sciences, Beijing, China.

Churchill G. and Doerge R. 1994 Empirical threshold values for quantitative trait mapping. *Genetics* **138**, 963–971.

Endrizzi J. E. and Kohel R. J. 1966 Use of telosomes in mapping three chromosomes in cotton. *Genetics* **54**, 535–550.

Harland S. C. 1929 The genetics of cotton. I. The inheritance of petal spot in New World cottons. *J. Genet.* **20**, 365–385.

Hayama R. and Coupland G. 2004 The molecular basis of diversity in the photoperiodic flowering responses of *Arabidopsis* and rice. *Plant Physiol.* **135**, 677–684.

Hou M. Y., Cai C. P., Zhang S. W., Guo W. Z., Zhang T. Z. and Zhou B. L. 2013 Construction of microsatellite-based linkage map and mapping of nectarilessness and hairiness genes in *Gossypium tomentosum*. *J. Genet.* **92**, 445–459.

Lander E. and Kruglyak K. 1995 Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat. Genet.* **11**, 241–247.

Paillard S., Schnurbusch T., Winzeler M., Messmer M., Sourdille P., Abderhalden O. *et al.* 2003 An integrative genetic linkage map of winter wheat (*Triticum aestivum* L). *Theor. Appl. Genet.* **107**, 1235–1242.

Paterson A. H., Brubaker C. and Wendel J. 1993 A rapid method for extraction of cotton (*Gossypium* spp.) genomic DNA suitable for RFLP or PCR analysis. *Plant Mol. Biol. Rep.* **11**, 122–127.

Rong J. K., Abbey C., Bowers J. E., Brubaker C. L., Chang C., Chee P. W. *et al.* 2004 A 3347 locus genetic recombination sequence-tagged sites reveals features of genome organization, transmission and evolution of cotton (*Gossypium*). *Genetics* **166**, 389–417.

Stephens S. G. 1954a Interspecific homologies between gene loci in *Gossypium*. I. Pollen color. *Genetics* **39**, 701–711.

Stephens S. G. 1954b Interspecific homologies between gene loci in *Gossypium*. II. Corolla color. *Genetics* **39**, 712–723.

Stephens S. G. 1974 Geographic and taxonomic distribution of anthocyanin genes in New World cottons. *J. Genet.* **61**, 128–141.

Van Ooijen J. W. and Voorrips R. E. 2001 JoinMap ReVersion 3.0: software for the calculation of genetic linkage maps. CPRO-DLO, Wageningen.

Voorrips R. E. 2002 MapChart, software for the graphical presentation of linkage maps and QTLs. *J. Hered.* **93**, 77–78.

Wang S. C., Basten J. and Zeng Z. B. 2005 Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh.

Zhang J., Wu Y. T., Guo W. Z. and Zhang T. Z. 2000 Fast screening of microsatellite markers in cotton with PAGE/silver staining. *Cotton Sci. Sin.* **12**, 267–269.

Zhao L., Lv Y. D., Cai C. P., Tong X. C., Chen X. D., Zhang W. *et al.* 2012 Toward allotetraploid cotton genome assembly: integration of a high-density molecular genetic linkage map with DNA sequence information. *BMC Genomics* **13**, 539.

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