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Note

A complete set of monosomic alien addition lines developed from *Gossypium anomalum* in a *Gossypium hirsutum* background: genotypic and phenotypic characterization

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Gossypium anomalum (B_1B_1) is a valuable wild resource for the genetic improvement of *G. hirsutum* ($A_1A_1D_1D_1$) in terms of fiber quality and disease and pest resistance, but the inherent difficulties in distant hybridization hinder its utilization in breeding programs. Monosomic alien addition lines (MAALs) are powerful tools for interspecific gene transfer. First, to access useful genes from *G. anomalum*, a fertile hexaploid from *G. hirsutum* × *G. anomalum* was obtained and then additional chromosomes were selected using SSR markers in successive backcrosses and self-crossing from BC_2F_1 to BC_4F_4. Finally, a complete set of 13 MAALs were developed. All the MAALs were confirmed by chromosome-specific anchored SSRs and genome-wide resequencing. The MAALs demonstrated abundant variation in morphological, agronomic, yield-related, and fiber quality traits. MAAL_3B had excellent fiber strength and fineness, indicating that the transmitted chromosome may carry desirable genes for the observed phenotypes. This complete set of MAALs will provide important genetic bridge material for the identification and introgression of favorable genes from *G. anomalum* and lay an important foundation for the genetic improvement of cotton.

Key Words: *Gossypium hirsutum*, *Gossypium anomalum*, monosomic alien addition line, complete set, marker-assisted selection, genome resequencing.

Introduction

The most economically valuable cotton species, the Upland cotton *Gossypium hirsutum* L. ($2n = 4x = 52$, $A_1A_1D_1D_1$), evolved from a polyploidization event involving two diploid cottons (Wendel 1989). However, due to domestication and modern plant breeding aimed at obtaining high fiber yields, the genetic diversity of cultivated *G. hirsutum* has narrowed and therefore become vulnerable to biotic and abiotic stresses. Of the 51 species in *Gossypium* L., 47 are wild (Fryxell 1992) and most (44) of these are diploid ($2n = 2x = 26$, BB–GG, KK); diploid wild cottons are abundant in exotic genes and represent a source of genetic diversity (Sun *et al.* 2006). *Gossypium anomalum* (B_1B_1) is a diploid wild cotton species indigenous to the arid to extremely arid parts of Africa (Fryxell 1992, Silow 1941). Although *G. anomalum* is not of direct commercial importance, it possesses several desirable characteristics that Upland cotton lacks, such as good fiber strength, maturity and fineness, and disease and pest resistance (Mehetre 2010, Newaskar *et al.* 2013). Due to differences in chromosome ploidy level and structure, it is difficult to directly transfer genes from *G. anomalum* into Upland cotton using conventional breeding methods.

Developing alien addition lines is an effective intermediate way to introduce genes of *G. anomalum* into Upland cotton. Alien addition lines are formed by adding one or more heterologous chromosomes from an alien donor species to the original genome of the recipient species. As the genetic source is clear, monosomic alien chromosome addition lines (MAALs) are more widely used in the study of exogenous chromosome pairing, beneficial gene transfer, genetic mapping, molecular marker development, genome structure, evolution, recombination, microdissection and construction of chromosome-specific libraries. MAALs have been developed for genetic studies of many crops

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including wheat (Wang *et al.* 2011), rice (Hechanova *et al.* 2018), oat (Dong *et al.* 2018), soybean (Singh *et al.* 1998), rape (Zhu *et al.* 2019), cotton (Chen *et al.* 2014) and potato (Ali *et al.* 2001).

MAALs in the genetic background of Upland cotton have been obtained from a number of diploid *Gossypium* species such as *G. anomalum* (Wang *et al.* 2016), *G. sturtianum* (CC) (Rooney *et al.* 1991), *G. somalense* (E_2E_2) (Zhou *et al.* 2004) and *G. australe* (G_2G_2) (Sarr *et al.* 2011). It is, however, difficult to construct a whole set of diploid cotton MAALs due to the instability of alien chromosomes and time-consuming identification processes. Chen *et al.* (2014) developed a complete set of alien addition lines from *G. australe* in *G. hirsutum*, but two lines were not monosomic. Wang *et al.* (2016) described 11 MAALs derived from *G. anomalum* into *G. hirsutum*, but MAALs of chromosome 1B and 5B were not obtained. To date, no complete set of MAALs from diploid cotton in Upland cotton has been reported.

In our previous studies, we obtained triploid hybrids by crossing *G. hirsutum* with *G. anomalum*. Hybrid seedling plants were then treated with 0.15% colchicine and a putative fertile hexaploid ($A_1A_1D_1D_1B_1B_1$) was obtained, which was confirmed by morphological, cytological and molecular evidences (Zhang *et al.* 2014). In addition, a set of 230 informative *G. anomalum*-specific SSR markers evenly distributed on the chromosomes was identified (Zhai *et al.* 2015). Among the 384 BC_2F_1 lines obtained, 40 were alien addition lines with complicated genetic backgrounds. The objectives of the current study were to i) develop a complete set of MAALs from *G. anomalum* in *G. hirsutum*; and ii) evaluate the *G. anomalum* MAALs set at the genotypic and phenotypic levels.

Materials and Methods

Plant materials

In previous studies, an hexaploid plant, which was doubled by treating triploid hybrids from *G. hirsutum* × *G. anomalum*, was backcrossed as female to Upland cotton var. Su8289, yielding the BC_1F_1 (pentaploid) generation. The pentaploid BC_1F_1 plants were backcrossed as females to Su8289 and a BC_2F_1 segregation population was constructed (Zhai *et al.* 2015, Zhang *et al.* 2014). The recurrent parent, Su8289, was characterized by good boll setting, high and stable yield, and wide adaptability. In the present study, alien chromosome addition lines carrying multiple additional chromosomes were first obtained in the BC_2F_1 generation. From BC_2F_1 to BC_4F_2, marker-assisted foreground selection was used to detect target alien chromosomes in each generation. In BC_4F_3, the alien addition lines were genotyped using the 230 *G. anomalum* genome specific SSR markers. Finally, a complete set of MAALs carrying all 13 *G. anomalum* chromosomes was obtained. Phenotypic evaluation of the MAALs was carried out in the BC_4F_4 population. All the generations were planted at the Experiment Station for Plant Science, Jiangsu Academy of Agricultural Sciences (JAAS), Nanjing, China (N31°36′ E119°10′).

SSR marker analysis

Genomic DNA was extracted from fresh leaves using a modified cetyltrimethylammonium bromide (CTAB) method for cotton (Paterson *et al.* 1993). The primer information for the 230 *G. anomalum*-specific SSR markers is listed in **Supplemental Table 1**. The PCR reaction was performed using a Thermal Cycler-2720 (Thermo Fisher Scientific, MA, USA) in a 10-μL reaction system containing 7.5 μL of PCR Master Mix (1×, TsingKe Biotech, Beijing, China), 0.5 μL of each primer (100 μM) and 1.5 μL of DNA template (20 ng). The polyacrylamide gel electrophoresis (PAGE) was used to separate amplified bands.

Genome resequencing and genotype determination

Genomic DNA of MAALs were extracted from fresh leaves using a Qiagen Plant Genomic DNA Kit. The sequencing library for each MAAL was constructed using an Illumina TruSeq Nano DNA HT Sample Prep Kit with an insertion size of 350 bp. The library constructed for each MAAL was sequenced on an Illumina HiSeq sequencing platform, resulting in 150 bp reads. The clean reads were mapped to the TM-1 genome obtained from Hu *et al.* (2019) using BWA software. Samtools and GATK software were used to identify SNPs between MAALs and TM-1. The SNPs between two parents, Su8289 and *G. anomalum*, and TM-1 were identified using the same program. The Illumina HiSeq sequencing data for the two parents were already available from other parallel studies in our laboratory. Python was used to determine which parent SNP type was the same as the MAAL SNP type within specific bins.

Phenotypic evaluation of the MAALs

The seeds of the MAALs and the recurrent parent, Su8289, were planted at the Experiment Station for Plant Science, Jiangsu Academy of Agricultural Sciences (JAAS), Nanjing, China. MAAL_13B was not included in the field experiment because of its self-incompatibility. The seeds were first sown in plug trays to ensure good seedling emergence and growth rates. Seedlings were transplanted to the field after 30 d in a random complete block design with three replications, one row per replication and 10 plants per row. There were 50 cm between plants and 100 cm between rows. Su8289 was planted among every 10 rows and used as a boundary row.

Plant height (PH), fruit branches per plant (FB) and boll number per plant (BN) were investigated at maturity. PH was measured as the length from the cotyledonary node to the apical bud. The average values of 10 plants per MAAL were taken as the phenotypic values for the three traits. At maturity, 30 open bolls were harvested from the middle of the plants in each plot and utilized to investigate boll weight (BW), lint percentage (LP) and seed index (SI).

Approximately 15 g of lint from each sample was used to test for fiber length (FL), fiber strength (FS), micronaire (MIC), fiber uniformity (FU) and fiber elongation (FE) at the Supervision, Inspection and Test Center of Cotton Quality, Ministry of Agriculture and Rural Affairs, Anyang, China.

Statistical analysis

The mean and standard deviation of the MAAL phenotype data with three replications were calculated using PROC MEAN of SAS/STAT. The significant difference in the phenotypic value between the MAALs and the recurrent parent was tested by the least significant difference (LSD) method performed using PROC ANOVA of SAS/STAT at the significance levels of 0.01 and 0.05.

Results

Development of a complete set of MAALs from G. anomalum

The scheme for the development of the MAALs is illustrated in **Fig. 1**. Alien chromosome addition lines originated at the first generation of the cross between pentaploid and tetraploid cotton (*i.e.* BC_2F_1 in the present study). A total of 40 individual plants were identified as alien chromosome addition lines in BC_2F_1 and the number of alien chromosomes ranged between one and six. Two MAALs were obtained for chromosomes 1B and 10B of *G. anomalum* and named MAAL_1B and MAAL_10B, respectively. One plant contained six additional chromosomes: 2B, 3B, 4B, 5B, 7B and 8B. In order to create a complete set of MAALs, 20 addition lines with representative and relatively simple genetic backgrounds were selected from the 40 plants and backcrossed with Su8289 to obtain the BC_3F_1 generation. Molecular marker screening was carried out in the BC_3F_1 population and individuals containing a heterozygous genotype for target chromosomes and the homozygous genotype of Upland cotton for other chromosomes (as far as possible) were selected. Therefore, at the BC_3F_1 stage, MAALs were obtained for 12 chromosomes except chromosome 4B, which was still associated with chromosome 11B.

In order to develop a complete set of MAALs and to study the transmission rule and frequency of each type of MAAL in the processes of back- and self-crossing, one generation of backcrossing and three generations of self-crossing were carried out. In BC_4F_1 and BC_4F_2, only foreground marker selection was carried out. MAAL_13B was lost during the self-crossing from BC_4F_1 to BC_4F_2, but fortunately one plant of MAAL_13B obtained in BC_4F_1 was preserved in the greenhouse. In BC_4F_3, genome-wide marker screening was carried out on every line. Finally, 12 MAALs of chromosomes 1B to 12B were confirmed.

From BC_3F_1 to BC_4F_4, MAALs were heritable, but their genotypes were separated in each generation. The MAAL offspring were divided into two types, the recurrent parent

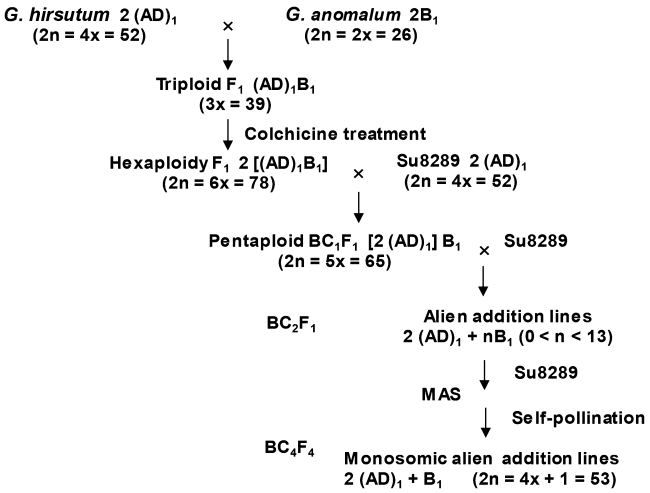

Fig. 1. Development scheme for monosomic alien addition lines (MAALs) derived from *G. anomalum* in *G. hirsutum*. MAS: molecular marker-assisted selection.

type (2n = 52) and the monosomic addition line type (2n + 1 = 53), and no alien homozygous type appeared. The B-genome chromosome transmission rates (*i.e.* the proportion of addition lines in the total plants) of MAALs (except MAAL_13B) were calculated during the process of selfing the BC_4F_3 to form BC_4F_4. The transmission rates among MAALs ranged from 9.09 to 51.33% with an average of 35.31%. MAAL_7B demonstrated the highest transmission rate, while MAAL_5B showed the lowest (**Supplemental Table 2**).

Genotypic characteristics of the MAALs

A major problem in developing Upland cotton-wild diploid relative MAALs is the identification of exogenous chromosomes, which are unstable during transmission. First, to detect MAALs, we used molecular marker-assisted selection involving 230 informative *G. anomalum*-specific SSR markers (Zhai *et al.* 2015). The 230 SSR markers were evenly distributed across the genome, varying from 12 to 27 per chromosome, with a density of 0–35.8 cM and coverage of 86.1–99.49% per chromosome (**Supplemental Table 3**). For each MAAL, the molecular marker bands on additional chromosome showed heterozygous types, while those on the original chromosomes showed the marker types for the background parent Su8289 (**Fig. 2**).

In addition, we used the TM-1 genome as a reference combined with the second-generation sequencing data of the two parents, Su8289 and *G. anomalum*, to carry out high-throughput resequencing and genome comparisons of the 13 MAALs. The total amount of clean sequencing data ranged from 13.45 Gb (MAAL_12B) to 16.88 Gb (MAAL_5B) with an average of 14.60 Gb. Accordingly, the sequencing depth ranged from 5.38× (MAAL_12B) to 6.75× (MAAL_5B) with an average of 5.84× (**Supplemental Table 4**). In addition, the average Q20 reached 97.54% and the average Q30 reached 92.71%, which indicated high

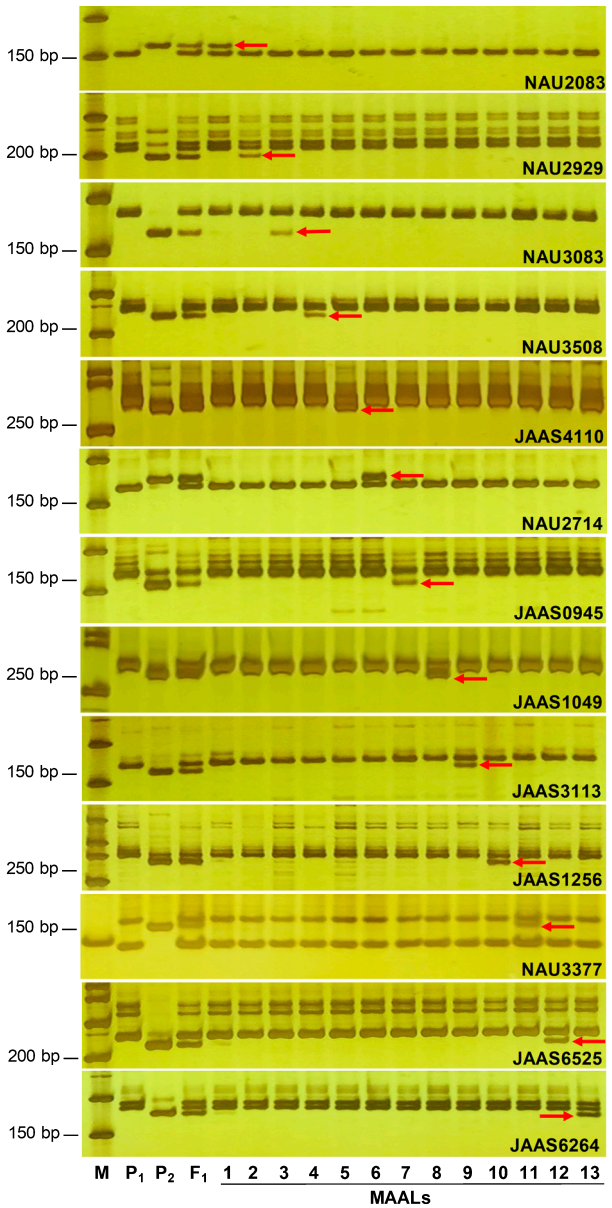

Fig. 2. Amplification patterns of a set of *G. anomalum*-specific SSR markers for identifying alien chromosomes of *G. anomalum* in *G. hirsutum*. A total of 13 SSR markers, one marker per chromosome, were selected for demonstration. The primer sequences and positions in the genome of the markers are listed in **Supplemental Table 1**. M: molecular size marker (50 bp ladder); P_1: *G. hirsutum* var. Su8289; P_2: *G. anomalum*; F_1: (*G. hirsutum* × *G. anomalum*)^2 hexaploid. The 1–13 lanes show that each MAAL possesses a single individual corresponding chromosome from *G. anomalum*. Red arrows indicate specific bands of *G. anomalum* chromosome.

sequencing accuracy. The results showed that heterozygous signals were displayed on the whole corresponding additional chromosome sequences for nine MAALs, except MAAL_2B, 3B, 4B and 5B. MAAL_2B and 3B displayed complementary heterozygous signals on chromosome A02 and A03, whereas MAAL_4B and 5B displayed complementary heterozygous signals on chromosome A04 and A05 (**Fig. 3**). As diploid cotton species, the genetic relationship between *G. anomalum* and *G. arboreum* (A_2A_2) is closer than that between *G. anomalum* and *G. hirsutum*. Compared with A_t group of *G. hirsutum*, large-scale interchromosomal translocation were observed between chromosome 2 and 3, and between chromosome 4 and 5 of *G. arboreum* (Desai *et al.* 2006, Li *et al.* 2014, Rong *et al.* 2004). The breakpoints of translocation were basically the same as those for the heterozygous signal in this study. It was presumed that similar large-scale translocation would exist in the B_1 group compared with the A_t group. Combined with the results from the molecular markers, it was presumed that the separation of heterozygous signals on A02, A03, A04 and A05 were due to the structural characteristics of the genomes and, therefore, MAAL_2B, 3B, 4B and 5B should exist. The results from the resequencing are a powerful demonstration of the existence of MAALs at the level of the nucleotide sequences.

Phenotypic characteristics of the MAALs

Compared with the recurrent parent Su8289, most of the MAALs were late maturing and had poor boll-setting capability. In addition, the MAALs showed abundant variation in morphological, agronomic, yield-related, and fiber quality traits (**Fig. 4**, **Supplemental Fig. 1**).

MAAL_1B plants had dark green leaves with serrated edges, smooth stems and small, shrunken bolls (**Fig. 4**). The PH was significantly ($P < 0.05$) higher than that of the recurrent parent Su8289 and the LP and FL were significantly ($P < 0.05$) lower than those of Su8289 (**Tables 1**, **2**).

MAAL_2B plants produced cone-shape bolls with significantly ($P < 0.01$) larger seeds, resulting in significantly ($P < 0.05$) lower LP than that of Su8289 (**Fig. 4**, **Table 1**).

MAAL_3B had larger leaves and significantly ($P < 0.01$) higher PH than Su8289 (**Fig. 4**, **Table 1**). The plants produced normal FL with significantly ($P < 0.01$) higher FS and significantly ($P < 0.05$) lower MIC than those of Su8289 (**Table 2**).

MAAL_4B plants had small leaves, smooth stems and significantly ($P < 0.05$) smaller BW than that of Su8289 (**Fig. 4**, **Table 1**). The other traits showed no significant difference compared with Su8289.

MAAL_5B plants had smaller leaves, slender bracts and ideal plant heights, which were significantly ($P < 0.01$) shorter than Su8289 individuals (**Fig. 4**, **Table 1**). The plants produced significantly ($P < 0.01$) lower BW and SI (**Table 1**).

MAAL_6B plants had small leaves, hairy stems and very light brown fibers (**Fig. 4**). The plants displayed better boll-setting capability than Su8289, with about 18 bolls per plant, and also produced larger seeds and shorter FL than those of Su8289, both at 0.01 significance levels (**Tables 1**, **2**).

MAAL_7B plants had light purple-red plaques at the base of the petals and significantly ($P < 0.01$) shorter FL

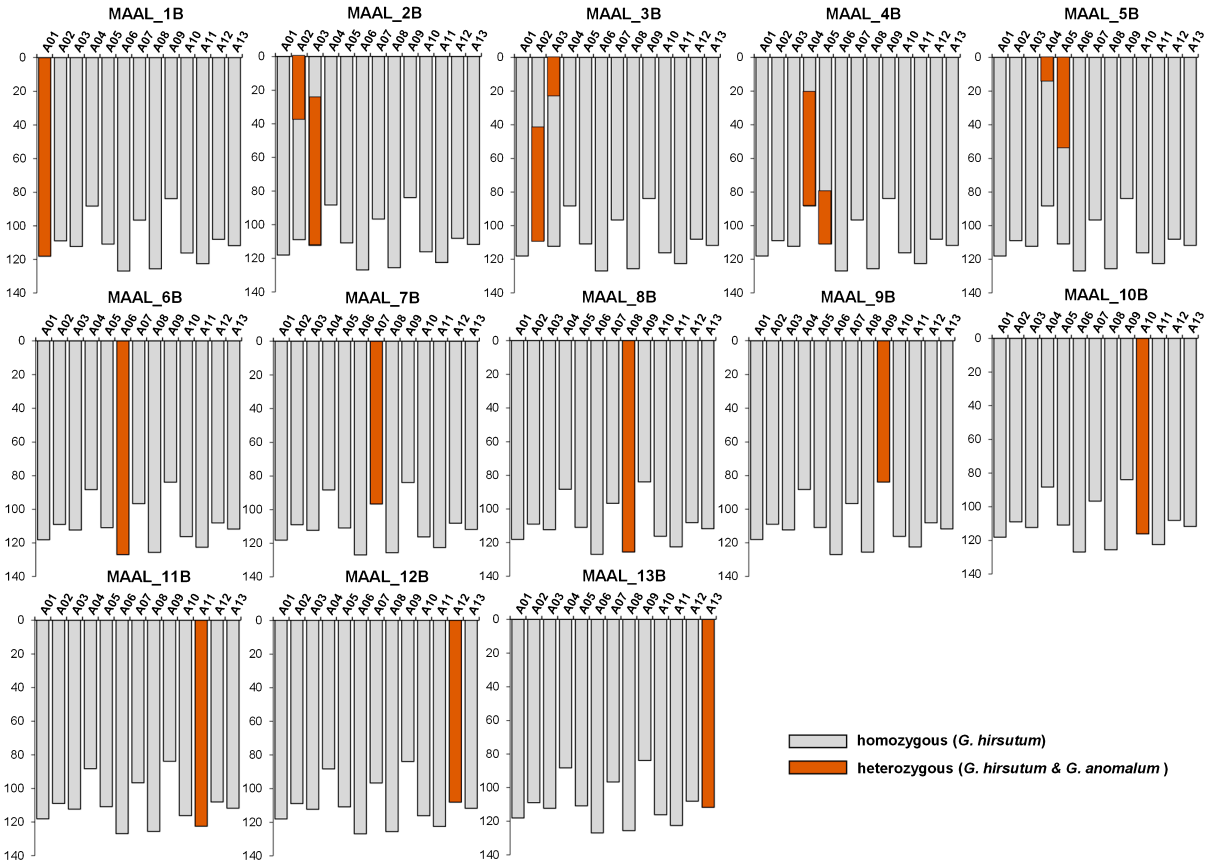

Fig. 3. Graphical genotypes of MAAL_1B to MAAL_13B derived from *G. anomalum* in *G. hirsutum* using genome resequencing data. For each MAAL, the x-axis shows the 13 chromosomes of *G. hirsutum* A-subgenome and the y-axis shows the chromosome length in Mb. The gray column represents the genotype of recurrent parent Su8289 and the orange column represents the heterozygous genotype of *G. anomalum* and *G. hirsutum*. If a whole chromosome shows a heterozygous signal, a new alien chromosome has been added to the genome.

than that of Su8289 (**Fig. 4**, **Table 2**).

MAAL_8B plants had dark green and small leaves, shorter PH, and >40% higher LP, due to the significantly ($P < 0.05$) lower SI (**Fig. 4**, **Table 1**). The FS was higher than that of Su8289 at the 0.05 significance level (**Table 2**).

MAAL_9B plants had a higher PH and smaller bolls with significantly ($P < 0.05$) lower BW than that of Su8289 (**Fig. 4**, **Table 1**). The FS and FE were higher than those of Su8289 at the 0.05 and 0.01 significance levels, respectively (**Table 2**).

MAAL_10B had smaller leaves, smaller bolls and ideal plant heights, which were significantly ($P < 0.01$) lower than that of the Su8289, as well as producing significantly ($P < 0.01$) lower BW and SI (**Fig. 1**, **Table 1**). The FL, FS, FU and FE were all significantly ($P < 0.01$) lower than those of Su8289 (**Table 2**).

MAAL_11B had smaller bolls, smaller seeds and shorter FL than Su8289 (**Fig. 4**, **Tables 1**, **2**).

MAAL_12B had dark green leaves, ideal plant heights which were significantly ($P < 0.01$) shorter than Su8289 individuals, and produced smaller bolls, smaller seeds and shorter FL than those of Su8289 (**Fig. 4**, **Table 1**).

MAAL_13B had poor fertility; the only plant was planted in the greenhouse. MAAL_13B had dark green and smaller leaves and smaller bolls than those of Su8289 (**Fig. 4**).

Discussion

Wild species are important reservoirs of useful genes. Alien chromosome addition lines can be used as bridging materials to introduce good genes from wild species into cultivated species when different ploidy levels are involved. Almost one hundred years ago, the first MAAL was found in distant hybrid progenies of wheat and rye (Leighty and Taylor 1924). MAALs can be regarded as dispersing exogenous genomes into receptor genomes in chromosomal units (Kynast *et al.* 2004). A complete set of alien chromosomes can be obtained by creating a full collection of MAALs. Complete sets of MAAL lines have successfully been constructed in only a few plants, including vegetable and wheat crops in particular (Friebe *et al.* 2000, Mesbah *et al.* 1997).

To date, there has been no report of the construction of a complete set of MAALs for diploid and tetraploid populations of *Gossypium*. In a previous study, MAALs covering

Fig. 4. Plant morphology of the two parents, F_1 and the complete set of MAALs derived from *G. anomalum* in *G. hirsutum*. P_1: *G. hirsutum* var. Su8289; P_2: *G. anomalum*; F_1: (*G. hirsutum* × *G. anomalum*)^2 hexaploid; 1–13: MAAL_1B to MAAL_13B. A: the third leaf from the top; B: petal; C: bract; D: bud; E: stamen; F: boll; G: fiber. Scale bar = 50 mm. The 13 MAALs of *G. anomalum* showed abundant variation in all the A–G aspects.

11 *G. anomalum* chromosomes were developed in the cotton genotype TM-1 background (Wang *et al.* 2016). In the present study, we developed a complete set of MAALs from *G. anomalum* chromosomes in the background of the cotton variety Su8289, which comprehensively shows good performance and makes a suitable recipient. Su8289 is an elite cultivar and superior to TM-1 in lint yield and fiber quality. Therefore, the MAALs created here have better phenotypic traits and offer more value in breeding applications.

During the development of the MAALs from *G. anomalum*, SSR markers were used to screen for lines with alien chromosomes and no cytology was conducted. In addition, at the final stage of the study, the genome composition of each MAAL was confirmed via whole genome sequencing using high-throughput resequencing techniques. These results validated the MAALs at the molecular and genomic levels.

An interesting observation on the transmission of *G. anomalum* chromosomes involved chromosome 13B. While the other 12 chromosomes could be transmitted both by backcrossing and self-crossing, chromosome 13B could only be preserved in the MAAL_13B by backcrossing instead of self-crossing. One plausible explanation for this observation could be due to the co-action of self-incompatibility-related alleles on chromosome 13B of *G. anomalum* and cytoplasmic effects from the receptor parent. One MAAL_13B plant obtained in the BC_4F_1 population was planted in the greenhouse; therefore MAAL_13B lacked field-based phenotypic data. Backcrossing was carried out with MAAL_13B to harvest enough seeds for subsequent studies. The genetic mechanism of the self-incompatibility of MAAL_13B of *G. anomalum* needs to be studied further using cytological and molecular biological methods.

A number of MAALs developed herein displayed potential utility in Upland cotton breeding for the improvement of fiber quality. MAAL_3B had significantly higher FS and lower MIC than Su8289, suggesting that chromosome 3B of *G. anomalum* may contain genes conferring improved fiber strength and fineness. MAAL_8B and 9B also had significantly higher FS than Su8289. Collectively, these results showed that MAALs carrying different chromosomes of *G. anomalum* could harbor alleles that improve

Table 1. Agronomic and yield-related traits of MAALs from *G. anomalum* in *G. hirsutum*

MAAL	Agronomic traits			Yield-related traits		
	PH (cm)	FB	BN	BW (g)	LP (%)	SI (g/100)
MAAL_1B	107.80 ± 5.27*	18 ± 2	7 ± 3**	3.88 ± 0.29	33.14 ± 2.91*	10.27 ± 1.25
MAAL_2B	97.00 ± 7.81	17 ± 0*	7 ± 3**	3.85 ± 0.85	33.19 ± 0.15*	14.20 ± 0.45**
MAAL_3B	109.33 ± 5.25**	18 ± 2	6 ± 1**	4.28 ± 0.59	36.48 ± 0.04	12.63 ± 0.31**
MAAL_4B	92.89 ± 5.55	16 ± 1**	9 ± 3**	3.63 ± 0.08*	39.41 ± 0.38	11.03 ± 0.26
MAAL_5B	78.83 ± 12.95**	17 ± 1*	6 ± 5**	1.54 ± 0.04**	38.82 ± 2.78	8.90 ± 0.08**
MAAL_6B	96.00 ± 1.87	19 ± 1	18 ± 5	5.34 ± 0.33	38.66 ± 1.12	12.33 ± 1.02**
MAAL_7B	100.90 ± 3.70	18 ± 1	10 ± 2**	3.67 ± 0.18*	39.45 ± 0.05	11.23 ± 1.10
MAAL_8B	87.40 ± 3.01*	14 ± 0**	10 ± 2**	3.89 ± 0.74	42.26 ± 2.93	9.47 ± 0.19*
MAAL_9B	105.50 ± 6.18*	18 ± 1	12 ± 2	3.11 ± 0.07**	36.82 ± 1.50	9.63 ± 0.05
MAAL_10B	82.40 ± 4.29**	15 ± 1**	7 ± 2**	2.75 ± 0.50**	39.61 ± 2.37	7.93 ± 0.12**
MAAL_11B	92.80 ± 6.11	18 ± 0	5 ± 2**	3.73 ± 0.03*	38.30 ± 1.34	9.00 ± 0.16**
MAAL_12B	77.17 ± 6.99**	16 ± 2**	17 ± 4	3.69 ± 0.56*	35.33 ± 0.85	9.03 ± 0.21**
Su8289	96.38 ± 10.17	19 ± 1	15 ± 5	5.13 ± 0.27	38.98 ± 1.05	10.77 ± 0.12

PH = Plant height; FB = Fruit branch; BN = Boll number; BW = Boll weight; LP = Lint percentage; SI = Seed index.
*, ** represent 0.05 and 0.01 significance levels, respectively.

Table 2. Fiber quality traits of MAALs from *G. anomalum* in *G. hirsutum*

MAAL	FL (mm)	FS (cN·tex^{-1})	MIC	FU (%)	FE (%)
MAAL_1B	27.93 ± 0.66*	31.60 ± 1.24	5.05 ± 0.21	86.75 ± 0.96	6.78 ± 0.04
MAAL_2B	28.68 ± 1.07	33.02 ± 1.85	4.35 ± 0.49	85.39 ± 0.96	6.65 ± 0.05*
MAAL_3B	30.26 ± 0.41	36.58 ± 0.20**	4.18 ± 0.23*	85.87 ± 0.53	6.73 ± 0.05
MAAL_4B	28.71 ± 0.15	31.15 ± 0.29	5.37 ± 0.02	85.52 ± 0.85	6.73 ± 0.05
MAAL_5B	29.10 ± 0.65	29.90 ± 1.10	5.33 ± 0.09	85.43 ± 1.54	6.77 ± 0.05
MAAL_6B	27.61 ± 0.16**	31.71 ± 1.76	5.37 ± 0.02	85.14 ± 1.24	6.70 ± 0.00
MAAL_7B	27.25 ± 0.75**	31.17 ± 2.18	5.30 ± 0.64	83.87 ± 1.31	6.70 ± 0.00
MAAL_8B	29.58 ± 0.48	35.24 ± 1.14*	4.97 ± 0.43	87.50 ± 0.50	6.75 ± 0.05
MAAL_9B	29.33 ± 0.27	35.17 ± 1.57*	5.15 ± 0.15	86.82 ± 1.48	6.90 ± 0.00**
MAAL_10B	25.51 ± 0.58**	25.98 ± 1.74**	5.36 ± 0.37	82.78 ± 0.80**	6.58 ± 0.08**
MAAL_11B	27.98 ± 0.88*	30.80 ± 0.85	5.07 ± 0.13	85.87 ± 1.90	6.73 ± 0.04
MAAL_12B	27.97 ± 0.58*	29.43 ± 0.53	5.16 ± 0.17	84.84 ± 1.25	6.70 ± 0.00
Su8289	29.50 ± 0.61	32.03 ± 1.98	4.90 ± 0.11	86.00 ± 1.19	6.75 ± 0.05

FL = Fiber length; FS = Fiber strength; MIC = Micronaire; FU = Fiber uniformity; FE = Fiber elongation.
*, ** represent 0.05 and 0.01 significance levels, respectively.

the quality of *G. hirsutum*.

Due to the interaction between genes, the effects of beneficial alleles from *G. anomalum* may be masked by other genes when an entire chromosome is inherited in a MAAL. Therefore, the follow-up application of these MAALs would be to use them as intermediate materials to obtain finer chromosome segments through recombination to develop chromosome segment substitution lines. MAALs have been used to create new materials for plant genetic research. For example, a disomic substitution line from *Orychophragmus violaceus* in *Brassica napus* was selected by crossing the MAALs with a *B. napus* nullisomics expressing novel or specific traits (Ding *et al.* 2013). Additionally, a fertility restorer for cytoplasmic male sterility in *Brassica napus* was developed from doubled haploid lines of one fertile MAAL of rapeseed by microspore culture (Li *et al.* 2019). At present, we are working on creating chromosome segment substitution lines from *G. anomalum* using MAALs. The work reported herein represents one step closer to our goal of extracting beneficial genes from *G. anomalum* for use in Upland cotton improvement.

Author Contribution Statement

XS designed the methods and experiments. SM, ZX, AC, XC, TW and GZ performed the field experiments. SM, ZX and LZ performed the genotyping. SM, ZX, PX, QG, XZ and WN constructed the population. SM, ZX and PX analyzed the data. SM and XS wrote the manuscript.

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Literature Cited

Ali, S.N.H., M.S. Ramanna, E. Jacobsen and R.G.F. Visser (2001) Establishment of a complete series of a monosomic tomato chromosome addition lines in the cultivated potato using RFLP and GISH analyses. Theor. Appl. Genet. 103: 687–695.

Chen, Y., Y.Y. Wang, K. Wang, X.F. Zhu, W.Z. Guo, T.Z. Zhang and B.L. Zhou (2014) Construction of a complete set of alien chromosome addition lines from *Gossypium australe* in *Gossypium hirsutum*: morphological, cytological, and genotypic characterization. Theor. Appl. Genet. 127: 1105–1121.

Desai, A., P.W. Chee, J. Rong, O.L. May and A.H. Paterson (2006) Chromosome structural changes in diploid and tetraploid A genomes of *Gossypium*. Genome 49: 336–345.

Ding, L., Z.G. Zhao, X.H. Ge and Z.Y. Li (2013) Intergeneric addition and substitution of *Brassica napus* with different chromosomes from *Orychophragmus violaceus*: Phenotype and cytology. Sci. Hortic. 164: 303–309.

Dong, Z.B., J. Yu, H. Li, W. Huang, L. Xu, Y. Zhao, T. Zhang, W.Y. Xu, J.M. Jiang, Z. Su *et al.* (2018) Transcriptional and epigenetic adaptation of maize chromosomes in Oat-Maize addition lines. Nucleic Acids Res. 46: 5012–5028.

Friebe, B., L.L. Qi, S. Nasuda, P. Zhang, N.A. Tuleen and B.S. Gill (2000) Development of a complete set of *Triticum aestivum-Aegilops speltoides* chromosome addition lines. Theor. Appl. Genet. 101: 51–58.

Fryxell, P.A. (1992) A revised taxonomic interpretation of *Gossypium* L. (Malvaceae). Rheedea 2: 108–165.

Hechanova, S.L., M.R. Prusty, S.R. Kim, L. Ballesfin, J. Ramos, G.D. Prahalada and K.K. Jena (2018) Monosomic alien addition lines (MAALs) of *Oryza rhizomatis* in *Oryza sativa*: production, cytology, alien trait introgression, molecular analysis and breeding application. Theor. Appl. Genet. 131: 2197–2211.

Hu, Y., J.D. Chen, L. Fang, Z.Y. Zhang, W. Ma, Y.C. Niu, L.Z. Ju, J.Q. Deng, T. Zhao, J.M. Lian *et al.* (2019) *Gossypium barbadense* and *Gossypium hirsutum* genomes provide insights into the origin

and evolution of allotetraploid cotton. Nat. Genet. 51: 739–748.

Kynast, R.G., R.J. Okagaki, M.W. Galatowitsch, S.R. Granath, M.S. Jacobs, A.O. Stec, H.W. Rines and R.L. Phillips (2004) Dissecting the maize genome by using chromosome addition and radiation hybrid lines. Proc. Natl. Acad. Sci. USA 101: 9921–9926.

Leighty, C. and J. Taylor (1924) "Hairy neck" wheat segregates from wheat-rye hybrids. J. Agric. Res. 28: 567–576.

Li, F., G. Fan, K. Wang, F. Sun, Y. Yuan, G. Song, Q. Li, Z. Ma, C. Lu, C. Zou *et al.* (2014) Genome sequence of the cultivated cotton *Gossypium arboreum*. Nat. Genet. 46: 567–572.

Li, P.F., L. Kang, A.F. Wang, C. Cui, L.C. Jiang, S.Z. Guo, X.H. Ge and Z.Y. Li (2019) Development of a fertility restorer for *inap* CMS (*Isatis indigotica*) *Brassica napus* through genetic introgression of one alien addition. Front. Plant Sci. 10: 257.

Mehetre, S.S. (2010) Wild *Gossypium anomalum*: a unique source of fibre fineness and strength. Curr. Sci. 99: 58–71.

Mesbah, M., T.S.M. de Bock, J.M. Sandbrink, R.M. Klein-Lankhorst and W. Lange (1997) Molecular and morphological characterization of monosomic additions in *Beta vulgaris*, carrying extra chromosomes of *B. procumbens* or *B. patellaris*. Mol. Breed. 3: 147–157.

Newaskar, G.S., V.P. Chimote, S.S. Mehetre and A.S. Jadhav (2013) Interspecific hybridization in *Gossypium* L.: characterization of progenies with different ploidy-confirmed multigenomic backgrounds. Plant Breed. 132: 211–216.

Paterson, A.H., C.L. Brubaker and J.F. Wendel (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., C. Abbey, J.E. Bowers, C.L. Brubaker, C. Chang, P.W. Chee, T.A. Delmonte, X.L. Ding, J.J. Garza, B.S. Marler *et al.* (2004) A 3347-locus genetic recombination map of sequence-tagged sites reveals features of genome organization, transmission and evolution of cotton (*Gossypium*). Genetics 166: 389–417.

Rooney, W.L., D.M. Stelly and D.W. Altman (1991) Identification of four *Gossypium sturtianum* monosomic alien addition derivatives from a backcrossing program with *G. hirsutum*. Crop Sci. 31: 337–341.

Sarr, D., J.M. Lacape, M. Rodier-Goud, J.M. Jacquemin, H. Benbouza, A. Toussaint, R. Palm, L. Ahoton, J.P. Baudoin and G. Mergeai (2011) Isolation of five new monosomic alien addition lines of *Gossypium australe* F. Muell in *G. hirsutum* L. by SSR and GISH analyses. Plant Breed. 130: 60–66.

Silow, R.A. (1941) The comparative genetics of *Gossypium anomalum* and the cultivated Asiatic cottons. J. Genet. 42: 259–358.

Singh, R.J., K.P. Kollipara and T. Hymowitz (1998) Monosomic alien addition lines derived from *Glycine max* (L.) Merr. and *G. tomentella* Hayata: production, characterization, and breeding behavior. Crop Sci. 38: 1483–1489.

Sun, Y., X. Zhang, C. Huang, X. Guo and Y. Nie (2006) Somatic embryogenesis and plant regeneration from different wild diploid cotton (*Gossypium*) species. Plant Cell Rep. 25: 289–296.

Wang, X.X., Y.Y. Wang, C. Wang, Y. Chen, Y. Chen, S.L. Feng, T. Zhao and B.L. Zhou (2016) Characterization of eleven monosomic alien addition lines added from *Gossypium anomalum* to *Gossypium hirsutum* using improved GISH and SSR markers. BMC Plant Biol. 16: 218.

Wang, Y., K.F. Yu, Q. Xie, H.Y. Kang, L.J. Lin, X. Fan, L.N. Sha, H.Q. Zhang and Y.H. Zhou (2011) Cytogenetic, genomic *in situ* hybridization (GISH) and agronomic characterization of alien addition lines derived from wheat-*Psathyrostachys huashanica*. Afr. J. Biotechnol. 10: 2201–2211.

Wendel, J.F. (1989) New World tetraploid cottons contain Old World cytoplasm. Proc. Natl. Acad. Sci. USA 86: 4132–4136.

Zhai, C.J., P. Xu, X. Zhang, Q. Guo, X.G. Zhang, Z.Z. Xu and X.L. Shen (2015) Development of *Gossypium anomalum*-derived microsatellite markers and their use for genome-wide identification of recombination between the *G. anomalum* and *G. hirsutum* genomes. Theor. Appl. Genet. 128: 1531–1540.

Zhang, X., C. Zhai, L. He, Q. Guo, X. Zhang, P. Xu, H. Su, Y. Gong, W. Ni and X. Shen (2014) Morphological, cytological and molecular analyses of a synthetic hexaploid derived from an interspecific hybrid between *Gossypium hirsutum* and *Gossypium anomalum*. Crop J. 2: 272–277.

Zhou, Z.H., P. Yu, G.H. Liu, J.X. He, J.X. Chen and X.X. Zhang (2004) Morphological and molecular characterization of two *G. somalense* monosomic alien addition lines (MAALs). Chin. Sci. Bull. 49: 910–914.

Zhu, B., Q. Pan, O.G. Huo, P. Zeng, B.W. Cai, X.H. Ge and Z.Y. Li (2019) Transcriptional aneuploidy responses of *Brassica rapa-oleracea* monosomic alien addition lines (MAALs) derived from natural allopolyploid *B. napus*. Front. Genet. 10: 67.