

Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

ScienceDirect



# Morphological, cytological and molecular analyses of a synthetic hexaploid derived from an interspecific hybrid between *Gossypium hirsutum* and *Gossypium anomalum*



Xia Zhang<sup>a,b</sup>, Caijiao Zhai<sup>a,b</sup>, Linchi He<sup>c</sup>, Qi Guo<sup>a,b</sup>, Xianggui Zhang<sup>a,b</sup>, Peng Xu<sup>a,b</sup>, Hongmei Su<sup>a,b</sup>, Yuanyong Gong<sup>a,b</sup>, Wanchao Ni<sup>a,b</sup>, Xinlian Shen<sup>a,b,\*</sup>

<sup>a</sup>Key Laboratory of Cotton and Rapeseed, Ministry of Agriculture, Nanjing, China

<sup>b</sup>The Institute of Industrial Crops, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China

<sup>c</sup>Jiangsu Yanjiang Institute of Agricultural Sciences, Nantong 226541, China

## ARTICLE INFO

### Article history:

Received 11 February 2014

Received in revised form

27 May 2014

Accepted 9 June 2014

Available online 12 July 2014

### Keywords:

Cotton cytogenetics

Polyploidy

SSR marker

*G. anomalum*

## ABSTRACT

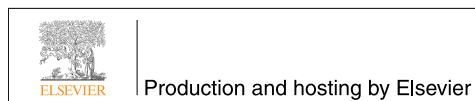
*Gossypium anomalum* represents an inestimable source of genes that could potentially be transferred into the gene pool of cultivated cotton. To resolve interspecific hybrid sterility problems, we previously treated triploid hybrids derived from a cross between *Gossypium hirsutum* and *G. anomalum* with 0.15% colchicine and obtained a putative fertile hexaploid. In this study, we performed morphological, molecular and cytological analyses to assess the hybridity and doubled status of putative interspecific hybrid plants. Most of the morphological characteristics of the putative hexaploid plants were intermediate between *G. hirsutum* and *G. anomalum*. Analysis of mitotic metaphase plates revealed 78 chromosomes, confirming the doubled hybrid status of the hexaploid. Genome-wide molecular analysis with different genome-derived SSR markers revealed a high level of polymorphism (96.6%) between *G. hirsutum* and *G. anomalum*. The marker transferability rate from other species to *G. anomalum* was as high as 98.0%. The high percentage of polymorphic markers with additive banding profiles in the hexaploid indicates the hybridity of the hexaploid on a genome-wide level. A-genome-derived markers were more powerful for distinguishing the genomic differences between *G. hirsutum* and *G. anomalum* than D-genome-derived markers. This study demonstrates the hybridity and chromosomally doubled status of the (*G. anomalum* × *G. hirsutum*)<sup>2</sup> hexaploid using morphological, cytological and molecular marker methods. The informative SSR markers screened in the study will be useful marker resources for tracking the flow of *G. anomalum* genetic material among progenies that may be produced by future backcrosses to *G. hirsutum*.

© 2014 Crop Science Society of China and Institute of Crop Science, CAAS. Production and hosting by Elsevier B.V. All rights reserved.

\* Corresponding author. Tel.: +86 25 84390291; fax: +86 25 84390366.

E-mail address: [xlshen68@126.com](mailto:xlshen68@126.com) (X. Shen).

Peer review under responsibility of Crop Science Society of China and Institute of Crop Science, CAAS.



## 1. Introduction

The genus *Gossypium* encompasses 50 species (45 diploids and five allopolyploids), which are distributed throughout most tropical and subtropical regions of the world [1]. Of the four cultivated species, *Gossypium hirsutum* L. ( $2n = 4x = 52$ ,  $A_1D_1$ ) is responsible for approximately 90% of the total cotton production worldwide. The other three principal cultivated species are the African diploid *Gossypium herbaceum* L. ( $2n = 2x = 26$ ,  $A_2$ ), the Asian and Indian diploid *Gossypium arboreum* L. ( $2n = 2x = 26$ ,  $A_1$ ), and the New World tetraploid *Gossypium barbadense* L. ( $2n = 4x = 52$ ,  $A_2D_2$ ). Diploid *Gossypium* species fall into eight cytological groups, designated A–G and K based on their chromosomal pairing relationships and geographical distribution [2,3]. Wild species of cotton represent a significant genetic repository for potential exploitation by cotton breeders, who have long recognized the beneficial effects of exotic genes [4]. The introduction of alien genetic variation into upland cotton from the chromosomes of wild species is a valuable and proven technique for cotton improvement. The most successful examples of the use of wild species during the history of cotton breeding history include *Gossypium harknessii* as a source of cytoplasmic male sterility [5] and *Gossypium thurberi* as a source of fiber quality [6,7]. More recently, other important traits, such as nematode resistance and the low- and high-gossypol plant traits, were successfully introduced from diploid species into upland cotton using various strategies [8,9]. Despite these successes, most of the genetic variation in wild *Gossypium* species remains to be exploited.

*G. anomalum* ( $2n = 2x = 26$ ,  $B_1$ ) is a wild species belonging to the  $B_1$  genome group. *G. anomalum* grows in Southwest Africa and along the southern fringes of the Sahara, almost from the Atlantic to the Red Sea [1]. As a member of subsection *Anomala* Todaro, *G. anomalum* possesses several desirable characters such as extremely fine fibers, good strength, low fiber weight, resistance to insect pests, immunity to the diseases black arm and bacterial blight and tolerance to water deficit, as this species is endemic to relatively dry areas [10]. Some efforts have been made to introduce desirable characters from *G. anomalum* to cultivated cotton [11,12].

*G. anomalum* represents an inestimable source of genes that can potentially be transferred to the cultivated cotton gene pool. However, the genomic differences between tetraploid cultivated cotton ( $A_1A_1D_1D_1$ ) and the diploid *G. anomalum* ( $B_1B_1$ ) represent serious interspecific reproductive barriers, which limit gene transfer between the species. In a previous study, we obtained triploid hybrids with the genome composition  $A_1D_1B_1$  by crossing *G. hirsutum* ( $A_1A_1D_1D_1$ ) with *G. anomalum* ( $B_1B_1$ ) [11]. Hybrid seedling plants were then treated with 0.15% colchicine and a putative fertile hexaploid ( $A_1A_1D_1D_1B_1B_1$ ) was obtained. This putative hexaploid produced flowers and set bolls normally. The objectives of this study were: (1) to confirm the hexaploid nature of the plants using morphological, cytological and molecular methods; (2) to compare EST-SSR transferability from other species to *G. anomalum*; and (3) to obtain a set of informative *G. anomalum*-specific SSR markers to monitor *G. anomalum*-specific chromosome segments.

## 2. Materials and methods

### 2.1. Experimental materials

Seedling plants of triploid hybrids from the cross between *G. hirsutum* ( $A_1A_1D_1D_1$ ) var. 86-1 and *G. anomalum* ( $B_1B_1$ ) were treated with 0.15% colchicine [11]. A putative fertile hexaploid ( $A_1A_1D_1D_1B_1B_1$ ) was selfed and the resulting hexaploid seeds were stored in a  $-20^\circ\text{C}$  freezer. In 2009, all experimental materials, including the putative hexaploid, *G. anomalum* and *G. hirsutum* var. 86-1, were planted in pots at the Lishui Experiment Station, Jiangsu Academy of Agricultural Science, and maintained in a greenhouse through the winter. Seeds were harvested in the greenhouse in the spring of 2010 and used in mitotic chromosome preparation.

### 2.2. Cytological analysis

To check the ploidy level of the putative hexaploid hybrid, mitotic chromosome preparations were carried out using root tips. Roots were excised from germinated seedlings on MS medium when they were approximately 3 cm long, pretreated with 0.025% (v/v) cycloheximide at room temperature for 2 h to accumulate metaphase cells, and fixed in Carnoy's solution (ethanol:acetic acid = 3:1, v/v). The root tips were macerated in 2% cellulose and 0.5% pectinase at  $37^\circ\text{C}$  for 40 min and squashed on slides in 60% acetic acid. All slides were stored at  $-70^\circ\text{C}$  overnight. The slides were then stained in 6-diamidino-2-phenylindole (Roche Diagnostics) for 3 min at room temperature and examined under an Olympus BX51 fluorescence microscope. Between 20 and 30 cells in each of the putative hexaploid hybrid plants were examined for chromosome number.

### 2.3. SSR analysis

Genomic DNA from four putative hexaploid plants and the parental accessions were extracted as described by Paterson et al. [13]. A total of 707 SSR primer pairs covering the whole cotton genome were selected based on the cotton reference map [14] and marker chromosome location information [15]. The sequences of these primers are available from the Cotton Marker Database (CMD) (<http://www.cottonmarker.org/>). SSR analysis was conducted according to Zhang et al. [16].

## 3. Results

### 3.1. Morphological observations

Most of morphological characteristics of the putative hexaploid plants were intermediate between *G. hirsutum* and *G. anomalum* (Fig. 1); for example the shapes and sizes of leaves, bolls and bracts of hexaploid plants. The hexaploid plants had large petal spots and intense hairiness inherited from *G. anomalum*. They exhibited prolific growth, and developed many bolls, with 5–13 seeds in every capsule. However, when they were used as male parents in backcrosses to *G. hirsutum*, seeds were rarely obtained.



**Fig. 1 – Morphological characteristics of *G. anomalum*, *G. hirsutum*, and the (*G. hirsutum* × *G. anomalum*)<sup>2</sup> hexaploid. a: *G. anomalum*; b: *G. hirsutum* var. 86-1; c: (*G. hirsutum* × *G. anomalum*)<sup>2</sup> hexaploid. I: adult plants; II: fully opened flowers; III: petals, pistil and bracts; IV: mature leaves; V: fuzzy seeds.**

### 3.2. Mitotic chromosome analysis

Mitotic metaphase counts revealed the presence of 78 chromosomes in all four plants of the (*G. hirsutum* × *G. anomalum*)<sup>2</sup> hexaploid (Fig. 2) confirming the amphiploid status of the material because it is in agreement with the number of chromosomes expected for a synthetic hexaploid ( $2n = 6x = 78$ ,  $A_1A_1D_1D_1B_1B_1$ ) resulting from a cross of *G. hirsutum* ( $2n = 4x = 52$ ,  $A_1A_1D_1D_1$ ) and *G. anomalum* ( $2n = 2x = 26$ ,  $B_1B_1$ ).

### 3.3. Analysis SSR band patterns in *G. hirsutum*, *G. anomalum*, and the hexaploid hybrid

A total of 707 SSR primer pairs covering the cotton genome were selected to amplify the two parents and four hexaploid hybrid plants. Among them, 94 were developed from *G. arboreum* EST sequences, 378 from *Gossypium raimondii* EST sequences and 235

from *G. hirsutum* EST sequences [14,15]. All 707 primer pairs yielded microsatellite products in *G. hirsutum* var. 86-1 and in the hexaploid hybrid plants; 683 produced polymorphic bands between *G. hirsutum* var. 86-1 and *G. anomalum*, 10 were monomorphic between the two parents and 14 failed to produce a PCR product in *G. anomalum*. A high level of polymorphism (96.6%) was observed between *G. hirsutum* and *G. anomalum*, confirming that these two species are genetically distant. Among the 683 polymorphic primer pairs, 674 (98.68%) had an additive banding pattern in the hexaploid, indicating the hybrid status of the hexaploid data genome-wide level; 9 SSR primer pairs failed to amplify *G. anomalum*-specific bands in the hexaploid plants.

Four hundred and twelve markers (58.3%) yielded easily distinguishable microsatellite products. The number of bands per SSR marker in *G. hirsutum* and *G. anomalum* was scored based on dominant scoring of the SSR bands, characterized by



**Fig. 2 – Mitotic metaphase in hexaploid plants, showing 78 chromosomes.**

the presence or absence of a particular band. In *G. hirsutum*, the 412 markers produced 1499 bands, averaging 3.87 bands per marker, whereas in *G. anomalum*, they produced 815 bands, averaging 2.2 bands per marker. There were 457 common bands between *G. hirsutum* and *G. anomalum*, averaging 1.22 common bands per marker. A-genome-derived markers produced more common bands (1.35 bands per markers) than D-genome-derived markers (1.18 bands per marker) (Table 1).

The polymorphisms of SSR loci between *G. hirsutum* and *G. anomalum* appeared as five types of basic banding patterns in the hexaploid hybrid. Of the 683 EST-SSRs that produced polymorphic amplifications, 333 (47.1%) displayed pattern A, where the polymorphic bands in the hexaploid hybrid were shared by both parents (codominant loci). A-genome-derived markers produced more codominant loci than D-genome-derived markers (Table 2). A total of 334 (47.24%) markers displayed pattern B, in which the polymorphic bands in the hexaploid hybrid were from *G. hirsutum* (dominant in *G. hirsutum*), whereas 16 markers displayed pattern C, in which the polymorphic bands in the hexaploid hybrid were from *G. anomalum* (dominant in *G. anomalum*) (Table 2). There were two other extreme instances of band pattern, including one instance in which *G. anomalum* produced no bands and one in which *G. anomalum*-specific bands were not amplified in the hexaploid hybrid plants. Among the 14 primer pairs that failed to produce a PCR product in *G. anomalum*, two were A-genome-derived, 10 were D-genome-derived, and two were AD-genome-derived, indicating that A-genome-derived SSR markers have a higher level of transferability than D-genome-derived SSR markers in *G. anomalum*. In addition, there were nine SSR primer pairs (NAU2139, NAU2169, NAU2182, NAU2954, NAU3119, NAU3317, NAU3480, NAU3489, and NAU5152) that produced no *G. anomalum*-specific bands in the hexaploid plants. As *G. hirsutum* will be used as the recurrent parent in backcrossing programs, those dominant loci in *G. hirsutum* cannot be used to monitor introgression of *G. anomalum*-specific segments during backcrossing. Therefore, a total of 349 informative SSR markers (333 codominant loci and 16 dominant loci in *G. anomalum*) can be used in future backcrossing programs.

#### 4. Discussion

EST-SSR markers derived from transcribed regions of DNA were shown to produce high rates of transferability in cotton species [17] and other related plant groups [18,19]. In this study, a total of 707 SSR markers developed from *G. arboreum* (A genome), *G. raimondii* (D genome), and *G. hirsutum* (AD genome) were chosen to amplify DNA from *G. hirsutum*, *G. anomalum*, and the synthetic. All 707 primer pairs yielded microsatellite

products in *G. hirsutum* and the synthetic, but 14 failed to produce a band in *G. anomalum*. However the transferability rate from the three species to *G. anomalum* was very high (98.0%). D-genome-derived SSR markers showed slightly lower rates of transferability than A- and AD-genome species-derived markers. Although all selected SSR markers expressed high levels of transferability and polymorphism, almost half of the markers (47.24%) were dominant in *G. hirsutum*. Since *G. hirsutum* will be used as the recurrent parent in future backcrossing programs, those dominant markers in *G. hirsutum* cannot be used to monitor the introgression of *G. anomalum*-specific segments in backcross populations. However, the A-genome-derived markers produced more codominant loci (56.38%) than the D-genome-derived markers (42.59%), indicating that the A-genome-derived markers were more powerful for distinguishing genomic differences between *G. hirsutum* and *G. anomalum*. In addition, the A-genome-derived SSR markers have a higher level of transferability between *G. hirsutum* and *G. anomalum* than the D-genome-derived SSR markers. These phenomena suggest that SSR markers developed from close relatives of the wild species are more applicable to the analysis of the transfer of chromosome segments from the wild species to cultivated cotton than other types of markers.

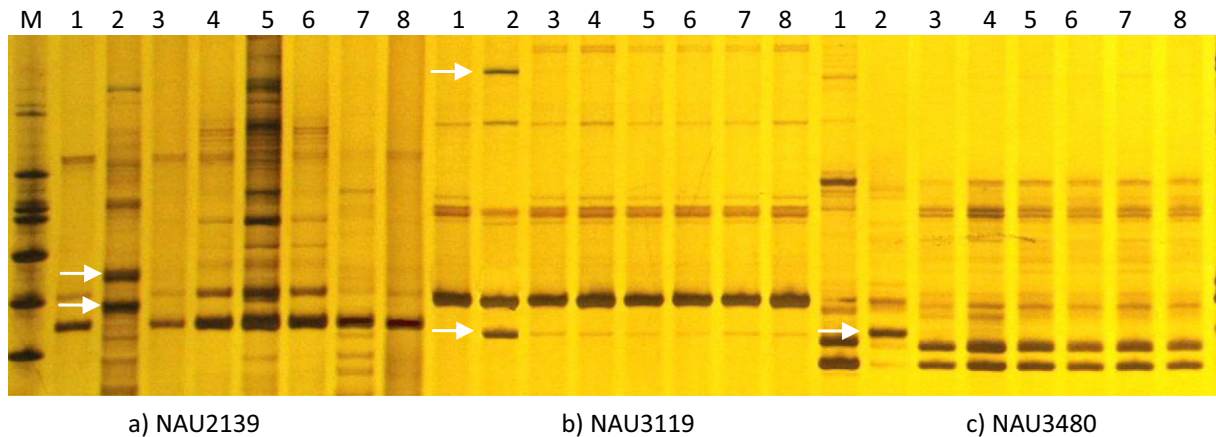
Although hybrids are expected to have additive banding profiles of the two parents, previous work has demonstrated that allopolyploid speciation in plants may be associated with non-Mendelian genomic changes in the early generations following polyploid synthesis in crops such as wheat [20,21] and rapeseed [22]. However, there is no evidence for structural genomic changes or *de novo* DNA methylation modifications in newly synthesized allopolyploid cotton [23]. In this study, 9 SSR primer pairs failed to amplify *G. anomalum*-specific bands in hexaploid plants. Possible explanations for this include chromosome loss, heterozygosity at some loci in a parental plant, chromosome rearrangement, and sequence discrepancy between the different species. Loss of chromosomes was not the causative factor because the synthetic plants had the expected chromosome number ( $2n = 78$ ). The presence of heterozygous loci in a parental plant was also unlikely since there was no evidence of variation among the tested parental plants. We also ruled out the possibility of chromosome rearrangement since one SSR marker (NAU2954) that failed to amplify *G. anomalum*-specific bands in the synthetic is closely linked to two markers (NAU2580 and NAU5373) on the Guo et al. [14] genetic map that did amplify as expected in the synthetic. Sequence discrepancy between the different species may be a likely reason for failure of expression of some SSR bands in the hexaploid hybrid. The SSR primers used were based on sequences of *G. arboreum* ( $A_1$  genome), *G. raimondii*

**Table 1 – Comparison of SSR alleles between *G. hirsutum* and *G. anomalum*.**

Genome-derived markers	No. of markers	Average alleles per SSR marker in <i>G. hirsutum</i>	Average alleles per SSR marker in <i>G. anomalum</i>	Common alleles per SSR marker
A genome	65	3.78	2.31	1.35
D genome	203	3.67	2.07	1.18
AD genome	144	4.19	2.36	1.22
Total	412	3.87	2.20	1.22

**Table 2 – Comparison of amplification patterns produced with different genome-derived SSR primers.**

Genome-derived primers	No. of primers	No. of codominant markers	No. of dominant markers in <i>G. hirsutum</i>	No. of dominant markers in <i>G. anomalum</i>	No. of monomorphic markers	No. of null markers in <i>G. anomalum</i>
A genome	94	53 (56.38%)	32 (34.04%)	3	4	2
D genome	378	161 (42.59%)	195 (51.59%)	9	3	10
AD genome	235	119 (50.64%)	107 (45.53%)	4	3	2
Total	707	333 (47.10%)	334 (47.24%)	16	10	14



**Fig. 3 – Amplification patterns of *G. anomalum*-specific bands for three SSR markers showing with amplification failure in the synthetic. M: marker; 1: *G. hirsutum* var. 86-1; 2: *G. anomalum*; 3–6: (*G. hirsutum* × *G. anomalum*)<sup>2</sup> synthetic; 7–8: DNA pool of *G. hirsutum* and *G. anomalum*. Arrows indicate bands that failed to amplify in the synthetic and in a DNA mixture from the parents.**

(D<sub>5</sub> genome) and *G. hirsutum* (A<sub>1</sub>D<sub>1</sub> genome). Compared to these species, many base substitutions may have occurred in the flanking sequences adjacent to the SSR loci in *G. anomalum* (B<sub>1</sub> genome); some of these substitutions may have been in the marker binding sites thus causing a preferential primer binding to genomic DNA from *G. hirsutum*. As a result, the specific SSR bands of *G. anomalum* would be undetectable in the hybrid plants. This explanation was confirmed by the observation that amplification of specific SSR bands of *G. anomalum* also failed when a DNA pool from *G. hirsutum* and *G. anomalum* was used instead of synthetic DNA as the template (Fig. 3).

The present results demonstrate the hybridity and doubled status of (*G. anomalum* × *G. hirsutum*) using morphological, cytological and molecular marker methods. These materials can be used as bridges for the transfer of useful agronomic traits from the wild species to cultivated varieties. The 349 informative SSR markers generated for the interspecific hybrids can be used to track the flow of genetic material from *G. anomalum* during backcrossing to *G. hirsutum*.

### Acknowledgments

This work was supported by the National Natural Science Foundation of China (31171595) and the Independent Innovation Funds for Agricultural Technology of Jiangsu Province, China [CX (12)5039].

### REFERENCES

- [1] P.A. Fryxell, A revised taxonomic interpretation of *Gossypium* L. (Malvaceae), *Rheedea* 2 (1992) 108–165.
- [2] J.F. Wendel, New World tetraploid cottons contain old world cytoplasm, *Proc. Natl. Acad. Sci. U. S. A.* 86 (1989) 4132–4136.
- [3] A.E. Percival, J.F. Wendel, J.M. Stewart, Taxonomy and germplasm resources, in: C.W. Smith, J. Cothren (Eds.), *Cotton: Origin, History, Technology, and Production*, John Wiley & Sons, Inc., NY, USA, 1999, pp. 33–63.
- [4] J.F. Wendel, C.L. Brubaker, T. Seelanan, Chapter 1: the origin and evolution of *Gossypium*, in: J.M. Stewart, D. Oosterhuis, J.J. Heitholt, J.R. Mauney (Eds.), *Physiology of Cotton*, Springer, Netherlands, 2010, pp. 1–18.
- [5] V.G. Meyer, Male sterility from *Gossypium harknessii*, *J. Hered.* 66 (1975) 23–27.
- [6] T.W. Culp, D.C. Harrell, Breeding methods for improving yield and fiber quality of upland cotton (*Gossypium hirsutum* L.), *Crop Sci.* 13 (1973) 686–689.
- [7] T.W. Culp, D.C. Harrell, T. Kerr, Some genetic implications in the transfer of high fiber strength genes to upland cotton, *Crop Sci.* 19 (1979) 481–484.
- [8] E.J. Sacks, A.F. Robinson, Introgression of resistance to reniform nematode (*Rotylenchulus reniformis*) into upland cotton (*Gossypium hirsutum*) from *Gossypium arboreum* and a *G. hirsutum*/*Gossypium aridum* bridging line, *Field Crops Res.* 112 (2009) 1–6.
- [9] H. Benbouza, J.M. Lacape, J.M. Jacquemin, B. Courtois, F.B.H. Diouf, D. Sarr, N. Konan, J.P. Baudoin, G. Mergeai, Introgression of the low-gossypol seed & high-gossypol plant trait in upland cotton: analysis of [(*Gossypium hirsutum* × *G.*

- raimondii*)<sup>2</sup> × *G. sturtianum*] trispecific hybrid and selected derivatives using mapped SSRs, *Mol. Breed.* 25 (2010) 273–286.
- [10] S.N. Ganesh, P.C. Vivek, S.M. Subhash, S.J. Ashok, Interspecific hybridization in *Gossypium* L.: characterization of progenies with different ploidy-confirmed multigenomic backgrounds, *Plant Breed.* 132 (2013) 211–216.
- [11] S.Y. Qian, J.Q. Huang, Y.J. Peng, B.L. Zhou, M.C. Ying, D.Z. Shen, G.L. Liu, T.X. Hu, Y.J. Xu, L.M. Gu, W.C. Ni, S. Cheng, Studies on the hybrid of *Gossypium hirsutum* L. and *G. anomalum* and application in breeding, *Sci. Agric. Sin.* 25 (1992) 44–51 (in Chinese with English abstract).
- [12] S.S. Mehetre, Wild *Gossypium anomalum*: a unique source of fibre fineness and strength-overview and achievements of pre-breeding efforts, *Curr. Sci.* 99 (2010) 58–71.
- [13] A.H. Paterson, E.S. Lander, J.D. Hewitt, S. Peterson, S.E. Lincoln, S.D. Tanksley, A rapid method for extraction of cotton (*Gossypium* spp.) genomic DNA suitable for RFLP or PCR analysis, *Plant Mol. Biol. Report.* 11 (1993) 122–127.
- [14] W.Z. Guo, C.P. Cai, C.B. Wang, Z.G. Han, X.L. Song, K. Wang, X. W. Niu, C. Wang, K.Y. Lu, B. Shi, T.Z. Zhang, A microsatellite-based, gene-rich linkage map reveals genome structure, function and evolution in *Gossypium*, *Genetics* 176 (2007) 527–541.
- [15] J. Xiao, K. Wu, D.D. Fang, D.M. Stelly, J. Yu, R.G. Cantrell, New SSR markers for use in cotton (*Gossypium* spp.) improvement, *J. Cotton Sci.* 13 (2009) 75–157.
- [16] J. Zhang, Y.T. Wu, W.Z. Guo, T.Z. Zhang, Fast screening of SSR markers in cotton with PAGE/silver staining, *Cotton Sci.* 12 (2000) 267–269 (in Chinese with English abstract).
- [17] W.Z. Guo, W. Wang, B.L. Zhou, T.Z. Zhang, Cross-species transferability of *G. arboreum*-derived EST-SSRs in the diploid species of *Gossypium*, *Theor. Appl. Genet.* 112 (2006) 1573–1581.
- [18] R. Bandopadhyay, S. Sharma, S. Rustgi, R. Singh, A. Kumar, H. S. Balyan, P.K. Gupta, DNA polymorphism among 18 species of *Triticum-Aegilops* complex using wheat EST-SSRs, *Plant Sci.* 166 (2004) 349–356.
- [19] M.C. Saha, M.A.R. Mian, I. Eujayl, J.C. Zwonitzer, L.J. Wang, G. D. Mayl, Tall fescue EST-SSR markers with transferability across several grass species, *Theor. Appl. Genet.* 109 (2004) 783–791.
- [20] M. Feldman, B. Liu, G. Segal, S. Abbo, A.A. Levy, J.M. Vega, Rapid elimination of low-copy DNA sequences in polyploid wheat: a possible mechanism for differentiation of homoeologous chromosomes, *Genetics* 147 (1997) 1381–1387.
- [21] K. Kashkush, M. Feldman, A.A. Levy, Gene loss, silencing and activation in a newly synthesized wheat allotetraploid, *Genetics* 160 (2002) 1651–1659.
- [22] K. Song, P. Lu, K. Tang, T.C. Osborn, Rapid genome change in synthetic polyploids of *Brassica* and its implications for polyploid evolution, *Proc. Natl. Acad. Sci. U. S. A.* 92 (1995) 7719–7723.
- [23] B. Liu, C.L. Brubaker, G. Mergeai, R.C. Cronn, J.F. Wendel, Polyploid formation in cotton is not accompanied by rapid genomic changes, *Genome* 44 (2001) 321–330.