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Polymorphism analysis of multi-parent advanced generation inter-cross (MAGIC) populations of upland cotton developed in China

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ABSTRACT. Upland cotton (*Gossypium hirsutum* L.) is an important cash crop that provides renewable natural fiber worldwide. Currently limited genetic base leads to a decrease in upland cotton genetic diversity. Multi-parent advance generation inter-cross (MAGIC) populations can be used to evaluate complex agronomic traits in crops. In this study, we developed an upland cotton MAGIC population. A total of 258 MAGIC population lines and their twelve founder lines were analyzed, using 432 pairs of simple sequence repeat (SSR) markers. Gene diversity indices and the polymorphism information content

were calculated using polymorphism analyses. Our genotype analysis showed that 258 inbred lines could be divided into 158 genotypes. Among these, we identified 17 pairs of specific SSR primers on the A chromosome subgroups and 24 pairs of specific SSR primers on the B chromosome subgroups of upland cotton. These were related to 77 and 128 genotypes, respectively. Our results suggest that the upland cotton MAGIC population contained abundant genetic diversity and may provide enormous resources for future genetic breeding.

Key words: Upland cotton; MAGIC; SSR; Genetic diversity

INTRODUCTION

Domesticated upland cotton (*Gossypium hirsutum* L.) is one of the world's leading cash crops. It constitutes the largest source of renewable natural textile fiber in China. Genetic improvement of fiber yield and quality are the primary objectives of cotton breeding programs (Pan, 1998). However, the genetic base of upland cotton cultivars is narrow (Iqbal et al., 1997; Tu et al., 2014), which leads to a decrease in upland cotton genetic diversity. In order to increase the intra-variety genetic diversity based on existing germplasm resources, multi-parent advance generation inter-cross (MAGIC), a new breeding method using multiple parents' convergent cross, has been widely used to improve the genetic diversity of crops (Li, 2014; Cavanagh et al., 2008).

Many major agronomic traits are controlled by multiple genetic loci, which makes it difficult to study multiple complicated agronomic traits in bi-parental or natural populations (Wang et al., 2016). MAGIC populations, including multiple parents and alleles, have been used for linkage mapping and association analyses without the limitations associated with structured populations (Sallam and Martsch, 2015). A MAGIC mapping population could enhance the mapping accuracy and resolution of any identified quantitative trait loci (QTLs) (Valdar et al., 2006). There is currently ample evidence supporting the value of MAGIC populations for gene mapping and crop breeding (Li et al., 2013; Wei and Xu, 2016). For example, MAGIC populations have been developed and used for construction of efficient genetic maps in wheat, *Arabidopsis thaliana*, and rice (Kover et al., 2009; Huang et al., 2012; Li et al., 2013).

Given the potential benefits of MAGIC populations, the first upland cotton MAGIC populations using pest-resistant upland cotton ecotypes could be developed to analyzed polymorphic simple sequence repeat (SSR) markers, which might provide germplasm resources for future upland cotton breeding.

MATERIAL AND METHODS

Parent materials and growing environment

The twelve founders included breeder-relevant germplasms with high yield and pest-, *Fusarium* wilt-, and *Verticillium* wilt-resistant varieties (Table 1). Detailed information about the parent materials is presented in Table 1. To construct the MAGIC population of upland cotton, the different parent material were planted at Yangtze University (112°E, 30°N). Common urea (N, 20 kg), calcium superphosphate [$Ca(H_2PO_4)_2$, 50 kg], and potassium sulfate (K_2SO_4, 15 kg) were applied as base fertilizers.

Table 1. List of parent materials of upland cotton (*Gossypium hirsutum*).

No.	Name	Material sources	Agronomic traits
P1	GY4	Agricultural College of Yangtze University	High yield
P2	GY5	Agricultural College of Yangtze University	High yield
P3	GY6	Agricultural College of Yangtze University	High yield
P4	GY7	Hebei Academy of Agriculture and Forestry	High yield
P5	GY2	Taicang City cotton seed farm in Jiangsu Province	High yield
P6	CQ9	Chinese Cotton Research Institute of Academy of Agricultural Sciences	Pest-resistant
P7	CQ13	Chinese Cotton Research Institute of Academy of Agricultural Sciences	Pest-resistant
P8	CQ2	Hubei province seed management station	Pest-resistant
P9	KC9	Hubei province seed management station	Pest-resistant
P10	GY8	Chinese Cotton Research Institute of Academy of Agricultural Sciences	*Fusarium* wilt-resistant and *Verticillium* wilt-resistant
P11	KB8	Chinese Cotton Research Institute of Academy of Agricultural Sciences	*Fusarium* wilt-resistant and *Verticillium* wilt-resistant
P12	KB10	Chinese Cotton Research Institute of Academy of Agricultural Sciences	*Fusarium* wilt-resistant and *Verticillium* wilt-resistant

Construction of the MAGIC population

According to a three-stage process including mixing, maintenance, and inbreeding (Churchill et al., 2004; Li, 2014), a MAGIC population of upland cotton was developed. As shown in Figure 1, a total of 12 inbred lines were intercrossed to develop the foundation population. Six F1 populations were produced using the following two-way crosses; A (P5 x P11), B (P7 x P2), C (P10 x P12), D (P8 x P9), E (P3 x P6), and F (P1 x P4), respectively. Subsequently, three four-way crosses; G (female group), H (male group 1), and I (male group 2), were identified (Figure 1). Plants from the four-cross group G were then used as females that were crossed with mixed pollen of each pair of the four-cross groups H and I. Following the method described above, 167 cotton hybrids were obtained. Each hybrid formed a family with 10 plants. Based on an F7 selfing population, 1500 recombinant inbred lines (RILs) were constructed using the single seed descent method (Li, 2014). Finally, 258 lines were selected as experimental materials used in this study.

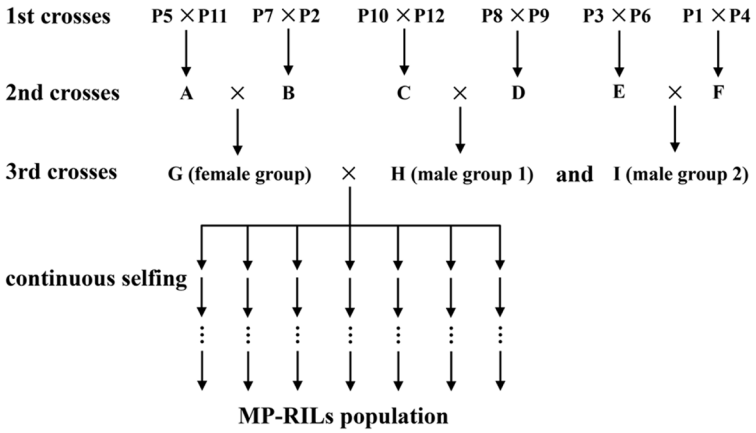

Figure 1. Graphical representation of the construction of the MAGIC population. Twelve founder accessions (P1 to P12) were mated pair-wise to produce six single crosses A, B, C, D, E, and F in the first season. Subsequently, three double-crosses (G, H, and I) were made in the second season. In the third season, multiple plants of G were used as females to cross with mixed pollens collected from each pair of H (male group 1) and I (male group 2) plants. The progeny of the complex crosses was then advanced by 7 generations of selfing.

SSR marker based genotyping of upland cotton

Total DNA was extracted from fresh leaves of different cotton varieties using the CTAB method (Song et al., 1998). Following the method of Chen and Du (2006), 436 primer pairs were distributed evenly among 26 linkage group in upland cotton. Among these primers, 42 polymorphic markers were selected for genotyping the 258 mid-parental-RILs and 12 founder lines (P1-P12) (Tables 2 and 3). The information for primer sequences were obtained from CottonGen (https://www.cottongen.org). Polymerase chain reaction (PCR) amplification was performed in a 10-μL total volume containing 2.5 μL template, 1 μL 10X PCR buffer (with Mg^{2+}), 0.15 μL Taq DNA polymerase, 10 mM dNTPs, 7.48 μL ddH_2O, and 0.16 μL each primer.

Table 2. Detailed information of 42 SSR markers used for genotyping the MAGIC population and 12 founders.

No.	Name	Chromosome locus	Allele frequency and No.	Gene diversity index	PIC value*
1	TMB1738	Chr.2	0.922 (3)	0.146	0.140
2	NAU3541	Chr.3	0.800 (4)	0.323	0.275
3	JESPR231	Chr.3	0.693 (5)	0.445	0.372
4	NAU4034	Chr.5	0.978 (3)	0.044	0.043
5	NAU3036	Chr.5	0.970 (4)	0.058	0.057
6	NAU5387	Chr.5	0.982 (4)	0.037	0.036
7	BNL1664	Chr.8	0.704 (3)	0.427	0.351
8	HAU0356	Chr.8	0.996 (2)	0.007	0.007
9	HAU1966	Chr.9	0.600 (2)	0.480	0.365
10	NAU859	Chr.9	0.619 (4)	0.505	0.420
11	BNL3031	Chr.9	0.900 (4)	0.183	0.171
12	JESPR29	Chr.9	0.459 (4)	0.594	0.510
13	NAU3390	Chr.11	0.985 (3)	0.029	0.029
14	Gh329	Chr.11	0.967 (4)	0.065	0.064
15	NAU1148	Chr.11	0.696 (3)	0.432	0.350
16	BNL598	Chr.12	0.978 (4)	0.044	0.043
17	Gh697	Chr.13	0.670 (3)	0.451	0.362
18	BNLl652	Chr.13	0.511 (4)	0.546	0.445
19	HAU1951	Chr.14	0.996 (2)	0.007	0.007
20	Gh471	Chr.14	0.622 (4)	0.503	0.417
21	JESPR156	Chr.14	0.989 (2)	0.022	0.022
22	TMB0071	Chr.14	0.956 (3)	0.086	0.084
23	HAU2489	Chr.15	0.948 (2)	0.098	0.094
24	TMB2295	Chr.18	0.600 (4)	0.527	0.445
25	NAU3943	Chr.18	0.993 (3)	0.015	0.015
26	MUSB1135	Chr.18	0.559 (3)	0.515	0.406
27	NAU3497	Chr.19	0.682 (4)	0.447	0.366
28	NAU3405	Chr.19	0.989 (2)	0.022	0.022
29	NAU2894	Chr.19	0.352 (7)	0.744	0.701
30	NAU5389	Chr.21	0.630 (4)	0.506	0.428
31	NAU2873	Chr.23	0.904 (3)	0.178	0.169
32	HAU3241	Chr.23	0.522 (3)	0.513	0.396
33	TMB0913	Chr.23	0.619 (3)	0.519	0.443
34	BNL3140	Chr.23	0.922 (4)	0.147	0.143
35	CIR388	Chr.24	0.585 (3)	0.492	0.378
36	NAU2773	Chr.25	0.596 (5)	0.548	0.478
37	NAU3502	Chr.25	0.982 (4)	0.037	0.036
38	CIR407	Chr.25	0.541 (4)	0.516	0.404
39	TMB2377	Chr.25	0.482 (3)	0.548	0.444
40	MGHES44	Chr.26	0.600 (3)	0.516	0.426
41	HAU1081	Chr.26	0.515 (3)	0.550	0.451
42	DPL0183b	Chr.26	0.944 (4)	0.107	0.104
Mean			0.761 (3)	0.309	0.260

*PIC = polymorphism information content.

The PCR cycle profile was as follows: 94°C for 3 min; 35 cycles of 94°C for 40 s, 50 to 62°C for 45 s, 72°C for 40 s, and 72°C for 5 min. PCR products were separated on a 6% denaturing polyacrylamide gel at 8 V/cm for 2 to 3 h and then silver stained as previously described (Xu et al., 2002). According to their sizes on the gel, the allelic bands amplified by each SSR primer pair were labeled A, B, C, etc.

Table 3. Primer sequences used for PCR analysis.

No.	Accession No.	Primer sequence (5'-3') and Tm* (°C)	Reverse primer sequence (5'-3') and Tm* (°C)
1	TMB1738	TTGTTAGCAATATGCAATATGAAC (55.3)	GGGTTTAGTTGAATGGGACC (56)
2	NAU3541	ATCCCACATTGCCTTCTTAT (53.8)	CTTTCCCCTCTCAAATACCC (55.5)
3	JESPR231	GCTGGTGGGATTCTCTG (51.6)	CTATGAACTGCTGGCTATGG (53.4)
4	NAU4034	CGACGGAAAGGGTTATCTTA (54.9)	ACGCCCTTCATTCAAACAC (54.1)
5	NAU3036	ATCTTGGGAATCTCAAATGG (54)	TGCTCCGATGAGTATTCAAA (54)
6	NAU5387	TTGGTCTTGCTCTCTTCCTC (54.2)	TCGTGTGAATGAAGCCTAAA (54.2)
7	BNL1664	ATTGCAAACGAGTGGAGAGC (57.7)	TGGATTCCAAGGCATTTTGT (57.9)
8	HAU0356	GATGCCCCTTCTCTAGCATA (54.6)	ACTCACGCAAAGCATACAAA (54.3)
9	HAU1966	CCCCCTTCAGATATCTTCATT (55.2)	CAGCCCAAAACAGCTAAAAT (55.2)
10	NAU859	CAGGCTTCATCTTTTTGGAC (54.8)	CATTGGATCCTAGTGGGAAG (54.4)
11	BNL3031	AGGCTGACCCTTTAAGGAGC (57.9)	AACCAACTTTTCCAACACCG (57.5)
12	JESPR29	CACCGTTTCCAAGTAAGATT (52.1)	GGTTAATCTTAGTTGAGGTC (45.6)
13	NAU3390	GAAAACAAGGCACTTGAACC (54.8)	AAACCGGTAACGACAACAAT (54.8)
14	Gh329	CAGCAGGCAGAAATCTTGTGATCG (66)	CTTAAATTTCTCTCCCTCAAAACCATC (60.8)
15	NAU1148	AGGGTTTGCAGTGGATTAAA (55)	CCAAAAGGATAGTAGCATGGA (54.9)
16	BNL598	TATCTCCTTCACGATTCCATCAT (58.1)	AAAAGAAAACAGGGTCAAAAGAA (57.5)
17	Gh697	TCCCTGAGCTCATATCTAACTTCC (59)	GACTTACTAAGCTATTCAAGCTTCC (56.2)
18	BNL1652	CACGGCTGAAAGGTGAAATT (57.8)	GCATGTACGCACTTGCACAG (58.2)
19	HAU1951	CAAACGCTGTCACTACCAAG (54)	TGACAAAATGTGCTTCCATC (53.8)
20	Gh471	CAGGCATCAACTAGCATTGAAAACG (65.2)	ATCTTCTGATCTCTATTAGCTACAACG (52.3)
21	JESPR156	GCCTTCAATCAATTCATACG (53.3)	GAAGGAGAAAGCAACGAATTAG (56)
22	TMB0071	GGCGGTCCCATGGTAGTAAT (58.9)	CGAACTATGACTCAATCCACC (54.5)
23	HAU2489	GGCACGAGGAGAAAATGAAAGA (61.1)	GATCGGATTCTGGGTCCCGC (66)
24	TMB2295	TGAGTTCATGTTCCCCACTG (56)	CTAAACATACTCTGTCAAACAC (47)
25	NAU3943	TTGGAGCAAACACAGACACT (53.3)	CCCTACGTCTCGAAAATACG (55.3)
26	MUSB1135	CCGCCGTCATTTCCATCACC (65.5)	TGGTACGGATCATGGGAATCCT (62.9)
27	NAU3497	TTGCTTAGCTGGTGAGACTG (53.7)	CCCTTCACCACCTCTTTCTA (54.2)
28	NAU3405	AATAGCAAAGCCTTCAGTGC (55.1)	GAAGTGCAAAAACCGTACCT (54.7)
29	NAU2894	GGCACGTTGCAAGTGTTAT (53.2)	AACCTTCCAGAGAAAGCAGA (54)
30	NAU5389	CCTCCATCGTCAACTCTTCT (53.8)	GCCTTGATCTTTGTCGTTCT (54.3)
31	NAU2873	TGTCCCCATGATTCTTCTTT (54.3)	TATTTTCAGCACCAAGAGCA (54.4)
32	HAU3241	AGGAATTCAAGGTTGAAGGAGGGT (63.7)	GGCTGCTGCTCAGTCTGCAT (61.1)
33	TMB0913	TAATTGCATGGCATCGAAAA (57.7)	GGGTGTGATCCAGACAGTCA (55.4)
34	BNL3140	CACCATTGTGGCAACTGAGT (56.1)	GGAAAAGGGAAAGCCATTGT (58.4)
35	CIR388	TTAAGCATCCAACAAGG (53.6)	TCCAACTTTTGGTCTATGT (48)
36	NAU2773	CCCTTTCAATCACAAAAACC (54.8)	CAAGGGCAGTCAATTTATCA (53.6)
37	NAU3502	AAGATCCACAAGAAACTGAAAC (53.1)	GGGGTTTTTCTTCTTTTCGT (55.6)
38	CIR407	GCACAGAACATCCATACA (42.4)	TCTCTCTCTCTTTCACACAC (45.8)
39	TMB2377	TAGTCCCTTACTTTCAATATTTTATA (52.3)	TGTTTTGGTGTAGTGATATAAACG (55)
40	MGHES44	ACCACTTGGGATTGGTTCAA (57.4)	GAGGCCACCACATATCGTTT (57.3)
41	HAU1081	TGTCTCCCGTACTCAGTGAA (53.2)	GGCTATGGGGTTACAATCAG (54.7)
42	DPL0183b	TTATTTCTTCCCTTGCGTTCTG (59.3)	AGAGGTGGCAATGGAGTTCTT (47.6)

*Tm = melting temperature.

Data analysis

The PIC value is an important index to evaluate genetic diversity in MAGIC populations (Agarwal et al., 2015). The polymorphism information content (PIC) was calculated using the following equation:

$$PIC_j = 1 - \sum P_{ij}^2 \quad \text{(Equation 1)}$$

in which P_{ij} is the frequency of allele j for marker i in the population. Gene diversity indices were then calculated based on the PIC values (Smith et al., 1997). The genetic similarity coefficient was obtained using the NTSYS-pc v. 2.1 software, and phylogenic trees were constructed using UPGMA and PowerMarker v. 3.25 software. Major allele frequencies and gene diversity indices were calculated using PowerMarker v. 3.25. The Jaccard coefficient was calculated using the following formula:

$$D_{ij} = 1 - (B_{ij} / M_{ij}) \quad \text{(Equation 2)}$$

in which D_{ij} is the genetic distance between genotypes i and j, B_{ij} is the number of amplified bands in the two genotypes i and j, and M_{ij} is the total number of amplified bands that were recorded in genotypes i and j.

RESULTS

Polymorphism analysis of the MAGIC population and its founders

To determine changes in genetic diversity, 42 primer pairs selected from 432 SSR markers were used. The results indicated amplification of a total of 145 polymorphic sites. The number of polymorphic sites per marker ranged from 2-7, with an average of 3.452 (Table 2). Interestingly, the number of polymorphic sites obtained from 20 primers higher than the average value. The results indicate that the best primers were screened as the primers to be used to analyze the genetic polymorphism in MAGIC population and its founders. The PIC ranged from 0.007 to 0.700. The highest PIC values were observed among 23 markers to compare with the average of SSR marker (about 0.260). This result indicated that the upland cotton core SSR primers were highly polymorphic. Compared with the founders, the amount of marker genotypes decreased in the MAGIC population. Increasing the number of markers might have contributed to the allelic variation in the MAGIC population (Li et al., 2013). As shown in Table 2, the percentage of new genotypes was 50%. These results suggest that these genotypes will provide abundant genetic variation for crop breeding as well as genes/QTLs for mapping upland cotton.

The genotype frequencies were dramatically different between the MAGIC population and the founder lines; the three genotypes, AA, AB, and BB, were detected by marker TMB1738 in both the MAGIC population and the founders (Table 4). In the founder lines, the highest genotype frequency observed was 50.0%, compared to 95.7% in the MAGIC population. The opposite was observed for the least frequent genotype, which was 16.7% in the founder lines and only 3.5% in the MAGIC population (Table 4). For marker NAU2894, four and six genotypes were identified in the founder lines and MAGIC population, respectively. Three genotypes were detected using marker NAU3390 in the founders, whereas only a single genotype was found in the MAGIC population.

Table 4. Changes in genotype frequency between the MAGIC population and the founder lines.

Genotype change	Marker name	Genotype*	Founder		MP-RILs	
			No.	Percent	No.	Percent
No change	TMB1738	01	2	**16.7**	9	**3.5**
		10	6	**50.0**	247	**95.7**
		11	4	33.3	2	0.8
Total			12	100	258	100
Increase	NAU2894	010	2	16.7	73	28.3
		011	8	66.7	87	33.7
		100	1	8.3	51	19.7
		111	1	8.3	10	3.9
		101	0	0	35	13.6
		110	0	0	2	0.8
Total			12	100	258	100
Decrease	NAU3390	101	8	66.6	258	100
		110	2	16.7	0	0
		111	2	16.7	0	0
Total			12	100	258	100
Substitution	BNL598	1010	3	12.5	102	39.5
		1111	8	66.7	137	53.1
		1101	1	8.3	0	0
		1110	0	0	19	7.4
Total			12	100	258	100

*The underlined genotypes appeared in both the MAGIC population and the founder lines, whereas the genotypes in bold were different.

To further investigate the genetic similarity in the MAGIC progeny population, the genetic similarity coefficient of the MAGIC population and founder lines was calculated using the Jaccard coefficient. Based on the phylogenic trees, the twelve founders clustered into five clades (Figure 2). The genetic similarity coefficient ranged from 0.524 to 1.000. When the genetic similarity coefficient was 0.721, the founders clustered into five clades, suggesting that the genetic similarity coefficient of the parental materials of the multi-genotype population was high but that the genetic diversity was narrow (Figure 2). Furthermore, the genetic similarity coefficient of the MAGIC population ranged from 0.515 to 1.000, with an average of 0.758. When the genetic similarity coefficient was 0.704, MAGIC population were clustered into four clades (Figure 3). These results indicate that the genetic diversity of the materials was narrow, and it is possible that many of the local materials were selected among the parental materials.

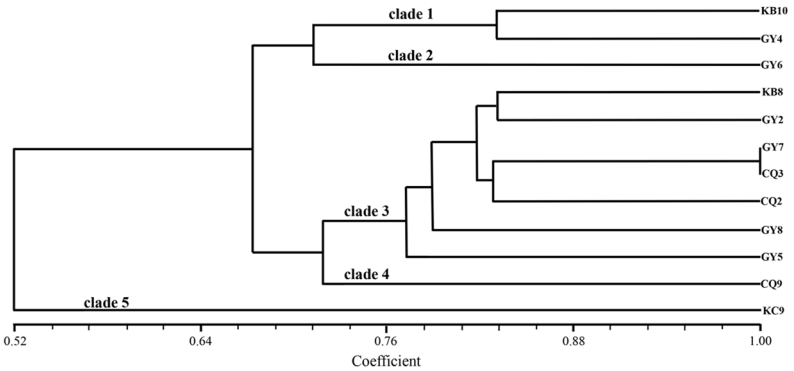

Figure 2. Genotype cluster analysis for the founders.

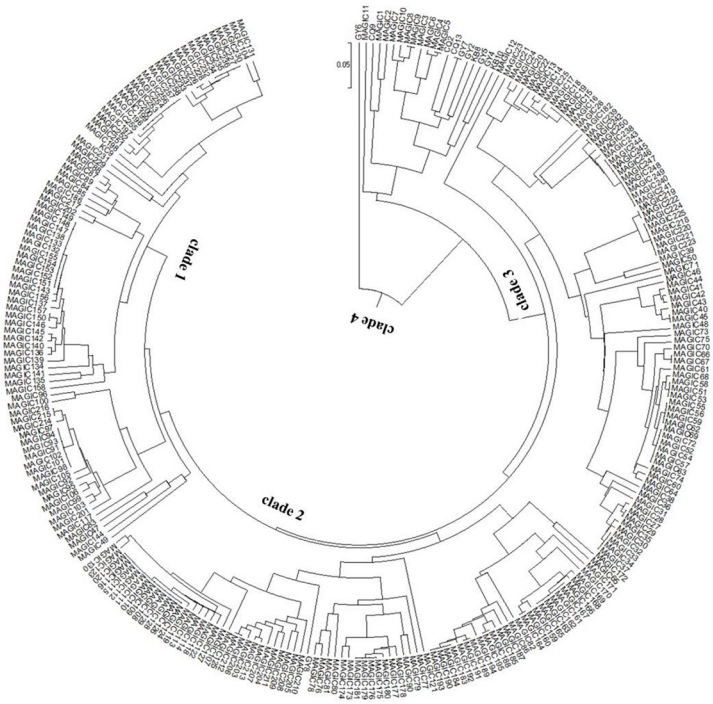

Figure 3. Genotype cluster analysis for the MAGIC population and the founders. The numbers represents different MAGIC population lines.

DISCUSSION

Upland cotton is mainly rooted in different upland cotton varieties, including Deltapine cotton, Stoneville, Delfos, Foster cotton, and King cotton, which are derived from twelve individual plants from one family in Mexico (Pan, 1998). This has led to a narrow genetic diversity of upland cotton varieties in China, and has resulted in barriers for breeding new cotton varieties (Pan, 1998). Therefore, it is necessary to improve the genetic diversity of the upland cotton population, e.g., by development of MAGIC populations. Previously, a new rice variety "Duo Ji Xin" has been developed using a MAGIC population (Li et al., 2013). "Duo Ji Xin" yields a comprehensive phenotype that has been approved by Hainan Province (Xu et al., 2014). The combined benefits of multi-genotype varieties for both yield and resistance improves their performance in favorable conditions, increases their stability under adverse conditions, and gives them an advantage over their component lines and even over mono-genotype varieties under cultivation (Li et al., 2013).

Each gene/QTL in a bi-parental population of RILs usually consists of two alleles and three genotypes. However, we detected more than two alleles in the MAGIC population derived from multiple parents. Kover et al. (2009) demonstrated that MAGIC populations enable the identification of novel QTLs with high precision in *A. thaliana*. Huang et al. (2011) used a public *A. thaliana* MAGIC population to test for complex traits, including QTL main effects and background interactions. Bi-parental RILs regularly display ordinary or bi-modal

distributions in the frequency of quantitative or qualitative traits, whereas MAGIC populations display an even and continuous distribution that tends to deviate from their founders due to unequal contribution. Elucidation of both the founder lines and the resulting MAGIC population enables linkage and association analysis of genes/QTLs, especially in allotetraploid upland cotton. Until now, only one MAGIC population with four wheat founders has been reported for allopolyploid plants (Huang et al., 2012). The average PIC of upland cotton from different parental origins, breeding periods, and ecologies has been calculated using polymorphism analysis (Chen and Du, 2006). Similarly, the average PIC value of 32 main cotton cultivars was obtained using 26 polymorphic SSR markers (Zhang et al., 2012). In our study, genotype frequency, as a key index, indicated significant changes between the MAGIC population and the founder lines, indicating that the MAGIC population possesses abundant genetic diversity (Huang et al., 2015).

Conflicts of interest

The authors declare no conflict of interest.

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