Table S1. Primers used in the isolation of chromosome-specific markers.

Mega-scaffold	BACs/PCR products	scaffold-Gene-primer	Primer sequence	Product size (bp)
1	21A03	1-TFL2-F	TGAACAAGTGAAGCCAGACA	501
		1-TFL2-R	CCCCTGCCTAGATAATTGGT	
2	Sc-02	Lotus2-3F	GACTGCCAACCAACCGACACTCTGC	4561
		Lotus2-3R	AACACCCACAACCCATAACCCTCCC	
		Lotus2-4F	CCATATTTTTGTCTAAAGCAACCCA	5404
		Lotus2-4R	CTACAAGCCATCATTCCTAACCTATT	
		Lotus2-5F	CCATACCCGAGTTTAACCA	3009
		Lotus2-5R	AGAACTTAGCAGACAGAGGACG	
		Lotus2-6F	TTGTGGGGAGGGATACTACTTA	5030
		Lotus2-6R	ACTGCTCCAGAAAATGACACC	
3	10E06	3-VTC-F	CAAGTGGCTCAGTTTCTCGT	474
		3-VTC-R	ACTGCTTGGTGCTTAAGTGG	
4	06A17	4-PMI1-F	TGTTCAGACGGGTTTTCAAT	557
		4-PMI1-R	CAGAATACAACTGGCCCATC	
5	22O22	5-FY-F	CTAGGGGTGGGAAAAGACAT	537
		5-FY-R	TGGTAAGCACCCAACAAAAT	
6	02B06	6-WC-X-F	CCAAGGGTTAGATCCGAGTT	550
		6-WC-X-R	TAGGCAACTAGGCAACAAGG	
7	Sc-07	Lotus7-1F	TCATCCTTGAAACTTCCAAACGCTAC	4126
		Lotus7-1R	TTTTGACAGGTCAGGGTCCAATACA	
		Lotus7-2F	AGACGAAGGCAGAACAACCAGACG	6128
		Lotus7-2R	ACGAAATGAAGACAGAAACCGAAAG	
		Lotus7-4F	ATGACTAATGAGCAAGTAGCCCT	4745
		Lotus7-4R	ACAGGAGCAGAGTTACCAGACC	
		Lotus7-5F	CCTAGAGGCTTTACATACGTTGC	5161
		Lotus7-5R	CCCATTTCGGAGTCCTAACAA	
8	Sc-08	Lotus8-F	TGGAAGACACTGGCAAGATAGA	4613
		Lotus8-R	GCCTCACCCATAGTTGTCAAG	
		Lotus8-1-F	GTGGAACGTGGAAGACGATAG	5035
		Lotus8-1-R	ACAAGGTGACAAAAGTATCTAGGAC	
		Lotus8-2-F	CATGCAGCAATCTTCAACCCTA	5536
		Lotus8-2-R	GTCCCACCTAACCCAAAATACG	
9	Sc-09	Lotus9-1F	GAATAGGTTCCAGTTTGTGC	4392
		Lotus9-1R	CTCCCCATAGGTTGATAGAT	
		Lotus9-2F	GTCCGGCACAGTCATGTCTAC	3486
		Lotus9-2R	TTGCAGGATGAGTGGGTTTAA	
10	Sc-10	Lotus10-1F	ATTTCGGTCGGTTCAGTTTGCT	3317
		Lotus10-1R	TTTTAGGGAGGGACTCACCACC	
		Lotus10-2F	CACGCCTGACTGAAAACTTGA	3789
		Lotus10-2R	GCCTTGTTCGTTTATTGGTATG	
		Lotus10-3F	TCATCCTACTTATTTCTGAGACGCCTAC	3810
		Lotus10-3R	AAAACCACCCTTGCCCCACTAT	

Table S2. Karyotyping of the mitotic chromosomes in JianXuan 17.

Chr.	Short arm (μm)	Long arm (μm)	Arm ratio	Location of 45S DNA	Location of 5S DNA	Total Length (μm)	Relative length （%）
1	0.98±0.09	3.57±0.20	3.67±0.35			4.54±0.23	23.66±1.22
2	1.79±0.23	0.84±0.10	2.15±0.37	Short arm		2.62±0.25	13.67±1.32
3	0.69±0.07	1.92±0.11	2.81±0.30	Long arm		2.60±0.14	13.56±0.71
4	1.47±0.17	0.81±0.14	1.87±0.50	Short arm		2.28±0.20	11.87±1.06
5	0.95±0.07	1.35±0.09	1.43±0.13		Long arm	2.29±0.12	11.94±0.65
6	0.78±0.06	1.25±0.08	1.61±0.16	Long arm		2.03±0.11	10.63±0.60
7	0.65±0.06	0.86±0.06	1.34±0.16			1.51±0.08	7.87±0.43
8	0.56±0.07	0.76±0.12	1.37±0.21			1.32±0.16	6.85±0.85

Table S3. Karyotyping of the mitotic chromosomes in DFH.

Chr.	Short arm (μm)	Long arm (μm)	Arm ratio	Location of 45S DNA	Location of 5S DNA	Total Length (μm)	Relative length （%）
1	0.86±0.11	3.70±0.21	4.33±0.44			4.56±0.29	23.75±1.50
2	0.88±0.12	1.76±0.19	2.05±0.47	Short arm		2.65±0.17	13.78±0.87
3	0.78±0.08	1.85±0.17	2.39±0.32	Long arm		2.63±0.21	13.67±1.09
4	0.83±0.11	1.47±0.30	1.79±0.34	Short arm		2.30±0.36	11.97±1.87
5	0.80±0.13	1.42±0.18	1.79±0.27		Long arm	2.22±0.26	11.55±1.37
6	0.77±0.15	1.18±0.13	1.59±0.31	Long arm		1.95±0.23	10.46±1.35
7	0.60±0.08	0.93±0.15	1.58±0.23			1.53±0.19	7.96±1.01
8	0.57±0.11	0.81±0.14	1.44±0.22			1.38±0.23	7.18±1.21

Table S4. Karyotyping of the mitotic chromosomes in Thailand.

Chr.	Short arm (μm)	Long arm (μm)	Arm ratio	Location of 45S DNA	Location of 5S DNA	Total Length (μm)	Relative length （%）
1	0.82±0.10	3.54±0.27	4.36±0.40			4.36±0.33	23.42±1.78
2	0.81±0.13	1.81±0.17	2.28±0.41	Short arm		2.63±0.23	14.12±1.21
3	1.76±0.20	0.82±0.16	2.25±0.57	Long arm		2.58±0.22	13.88±1.19
4	0.75±0.13	1.50±0.25	2.04±0.38	Short arm		2.24±0.33	12.06±1.76
5	0.75±0.10	1.45±0.16	1.96±0.30		Long arm	2.20±0.21	11.84±1.14
6	0.69±0.09	1.27±0.16	1.85±0.32	Long arm		1.96±0.21	10.51±1.11
7	0.58±0.08	0.92±0.17	1.60±0.35			1.50±0.20	8.08±1.06
8	0.44±0.10	0.69±0.10	1.63±0.42			1.13±0.17	6.09±0.92

Table S5. The significant assays (*p* values) of 5S signal gray values among China Antique, JX17, DFH, and Thailand.

	China Antique	JX17	DFH	Thailand
China Antique	-	3.9E-02	2.2E-4	7.6E-09
JX17	-	-	2.9E-02	5.5E-07
DFH	-	-	-	2.1E-3